Republic of Indonesia
Agency for the Assessment and Application of Technology
地球規模課題対応国際科学技術協力（SATREPS）

THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES IN THE REPUBLIC OF INDONESIA

Project Completion Report

March 2020

JAPAN INTERNATIONAL COOPERATION AGENCY (JICA)
UNIVERSITY OF TOKYO

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JAPAN'S TECHNICAL COOPERATION

Science and Technology Research Partnership for Sustainable Development

PROJECT COMPLETION REPORT

Title of Project:

THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES

Global Issue Focus: **INFECTIOUS DISEASES**

Indonesia side:

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Submitted: February 21, 2020

Project Completion Report

Project Title: The Project for Searching Lead Compounds Of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-Resources

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Submission Date : February 21st, 2020

I. Basic Information of The Project

1. Country
Indonesia

2. Title of the Project
The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-Resources

3. Duration of the Project
Planned : 5 years (April 1, 2015 – March 31, 2020)
Actual : 5 years (April 1, 2015 – March 31, 2020)

4. Background
Indonesia is the second largest biodiversity country in the world. The diversity and uniqueness of bioresources is an important capital for Indonesia to increase wealth and prosperity of the people. The Government of Indonesia (GOI) declared a strategic directive master plan for Indonesian economic development, through which GOI expects increase its economic competitiveness by transforming from bioresources-based comparative economic activities to innovation-based competitive economic activities. Indonesia Agency for the Assessment and Application of Technology (Badan

Pengkajian dan Penerapan Teknologi, BPPT) has been consistently developing innovation for utilization of Indonesian bioresources in order to support industries in Indonesia. One of the prioritized research topics is bio-prospecting for health by utilization of microbial and plant biodiversity. Many achievements have been obtained, however, very little of them had been practically used by industries or people. Thus, capacity building on utilization of bioresource for health, especially drug development, is very urgent.

Malaria and amebiasis are regarded as neglected diseases caused by parasites and a considerable concern in Indonesia which is needed to be overcome. Moreover, resistance of presently available drugs to the parasites is unavoidable, thus drug discovery for anti-malarial and anti-amebic compounds is urgently needed.

This technical cooperation is aimed to strengthen the capacity of Indonesian researchers and institutions in drug discovery against tropical infectious diseases including malaria and amebiasis using Indonesia bioresources.

GOI requested Japan International Cooperation Agency (JICA) to initiate the Indonesia-Japan collaborative project at BPPT and other associated institutions for conducting research on malaria and amebiasis. The joint research by the Indonesian institutes, BPPT and its collaborating institutes in Indonesia, the Indonesian Institute of Sciences (LIPI) and the Institute of Tropical Diseases, Airlangga University, and Japanese Institutions, The University of Tokyo, Kitasato University, Nagasaki University, and MicroBiopharm Japan, Co., Ltd., aim (i) strengthen capacity building for Indonesian researchers and institutions, (ii) to reinforce international research collaboration, and (iii) to increase added value of Indonesia bioresources especially for anti-malaria and anti-amebic drug discovery.

5. Overall Goal and Project Purpose

Overall Goal:

a. Strengthen capacity building for Indonesian researchers and institutions
b. To reinforce international research collaboration
c. To increase added value of Indonesia bioresources especially for anti-malaria and anti-amebic drug discovery

This overall goal was described in Record of Discussion document (ANNEX 4.B, in Appendix 1 section I. Background). During implementation of this

project, this overall goal was considered as the project overall goal by both Indonesia and Japan counterparts, and the goal was not changed until the end of the project.

Project Purpose:
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.

6. Implementing Agency

Indonesia side:
- Laboratory for Biotechnology, BPPT (BTC)
- Indonesia Culture Collection (InaCC), LIPI
- Institute for Tropical Disease, Airlangga University (AU)

Japan side:
- The University of Tokyo (UTo)
- Kitasato University (KU)
- Nagasaki University (NU)
- MicroBiopharm Japan, Co., Ltd. (MBJ)

In the 5th year of this project, during The 5th Joint Coordinating Committee (JCC) Meeting, Center for Primate Study, Bogor Agriculture University (IPB) and Brawijaya University (BU) were added as implementing agency of Indonesia side.

II. Results of the Project

1. Result of the Project

1-1. Input by the Japanese side

a. Amount of input by Japanese Side

Planned : JPY 300,000,000

Actual : JPY 302,859,448 (as per Feb 27th, 2020)

- Equipment provision = JPY 103,274,010
- Overseas activity strengthening expenses = JPY 22,186,602
- Total expert dispatched = 120 persons
- Total trainee received
  - Long-term trainee = 3 persons
  - Short-term trainee = 48 persons

b. Expert dispatched

Short-term dispatch=120 persons (as per Jan 20th, 2020)

(No long-term dispatch)

Major activities:

- Microbial isolation and identification
- Establishment of screening, assay and cell culture system
- Purification and structure purification and identification
- Scientific meeting, JCC Meeting

Experts from Japan were frequently dispatched to Indonesian counterparts to transfer techniques and technologies to be applied and to monitor, as well as to make sure, the techniques were implemented properly or not. Timing of dispatching the experts was arranged with the timing of dispatching Indonesian researchers to Japan for training, in order to maintain continuous techniques transfer activities and capacity building of Indonesian researchers.

c. Receipt of training participants

Long-term training : 3 persons (all are from Indonesia)

- BPPT : 1 person

- AU : 2 persons

Short-term training : 48 persons (all are from Indonesia)

- BPPT : 31 persons
- AU : 16 persons
- LIPI : 1 persons

There are 3 Indonesian researchers who are pursuing PhD course in Japanese universities as long-term training (2 persons in The University of Tokyo, and 1 in Kitasato University). Beside these, there are 7 persons funded by other resources, which are pursuing (or pursued) Master and PhD courses in Japanese universities and involving in this project as part of capacity building of Indonesian researchers. Detail of them is as follow.

- BPPT: 1 persons (PhD course, funded by Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), enrolling the course at Nagasaki University)
- AU: 3 persons (Master course 2 persons, PhD course 1 person, funded by Asian Development Bank and MEXT, all are enrolling the course at The University of Tokyo)
- LIPI: 2 persons (PhD 2 persons, funded by Indonesian government, one of them had graduated from Tsukuba University and enrolling post-doctoral fellowship at The University of Tokyo, while the other one is enrolling the course at the University of Tokyo).

Short-term training was aimed mainly to learn technology from Japanese counterparts to be applied in Indonesian counterparts, including accelerating progress of the research conducted in Indonesian counterparts. Major topics of the training as below:

- Microbial isolation and identification
- Establishing screening system
- Cell culture and assay
- Purification and structure identification
- Development of new screening system

1-2. Input by the Indonesian side

a. Counterpart assignment: Total 44 persons
   - BPPT: 28 persons
   - AU: 7 persons
   - LIPI: 6 persons
   - IPB: 2 persons
   - Brawijaya University (BU): 1 person

   List of researchers assigned by Indonesian counterparts into this project was revised every year in order to response the changing of technical strategies and to improve the progress of the project. As per January 9, 2020, Indonesian counterparts assigned 40 persons to be involved in this project.
   In addition to the above assignment, Indonesian side added Primate Research Center, Bogor Agriculture University (IPB) as member of Indonesian counterpart institute into this project in the 5th year. IPB involved in discussion of future direction of safety assessment of lead compounds in animal model (pre-clinical study). IPB assigned 2 persons to be involved in this project.
   Indonesian side also added Brawijaya University (BU) as member of Indonesian counterpart institute in this project in the 5th year. BU cooperated BPPT in assessing efficacy of an antimalarial compound in animal model in 2019. BU assigned 2 persons to be involved in this project. Total persons from Indonesian side are 44 persons.

b. Provision of office and other in-kinds
   - BPPT:
     - Office space for project coordinator
     - Laboratory spaces for microbial observation, preparation of microbial fermentation, culture collection and microbial fermentation, preparation of microbial extract, BSL-2 lab for parasite and mammalian cell culture, enzyme-based screening, preparation of target protein, purification of active compound purification, analysis of active compound, large-scale fermentation, sterilization of microbial medium, wastes, and apparatus, and consumables/reagents storage.

- Equipment for microbial incubation, handling and observation, microbial fermentation and sterilization, microbial extract production, and purification and chemical structure elucidation of active compound.
- Microbial collection composed from actinomycetes and fungi collected and preserved by BPPT before and during this project.

- AU
  - Laboratory spaces for parasite and mammalian cell culture (BSL-2 lab), purification of active compound purification
  - Equipment for handling and preservation of parasite and mammalian cells, screening of active compound, and purification of active compound.
- LIPI
  - Laboratory space for microbial handling.
  - Equipment of microbial preservation and handling.
  - Microbial collection (actinomycetes) collected and preserved by previous SATREPS project in LIPI.

c. Other items borne by the Government of Indonesia
Budget allocated by the Government of Indonesia through BPPT and AU for this project during the project term FY2015-2019 is as follow.

Table 1. Budget allocated by the Government of Indonesia for this project during the project term (FY2015-2019)

Expense Item	Amount (IDR)		Note
	BPPT	AU	
Salaries	797,403,000	558,963,650	Salaries for non-permanent researchers and technicians
Consumables/ reagents	1,037,336,000	224,403,740	Reagents and consumables for microbial isolation and

			identification, extract production, screening, and active compound purification
Travel	287,067,000	268,600,000	Presentation of research result in an scientific seminar and meeting of research initiation/progress with local counterparts
Meeting	132,991,000	0	JCC meetings, international symposiums
Equipment	213,800,000	52,000,000	Lab furniture (desks), refrigerators, air conditioners, electric stabilizers, pH meter, hotplate stirrer, etc.
TOTAL	2,468,597,000	1,103,967,390	
	3,572,564,390		

In addition, Indonesian counterparts purchased and installed equipment for this project as listed below.

Table 2. List of equipment purchased by Indonesian counterparts for supporting the project

Name	Installation place	Installation date	Purpose	Notes
Flash Chromatography	BTC-BPPT	6/30/2019	for active compound isolation and purification	Purchased by BPPT HQ

50 L jar fermentor	BTC-BPPT	7/11/2019	for mass production of microbial extract	Purchased by BPPT HQ
Laboratory furniture (lab desk)	BTC-BPPT	8/30/2018	for active compound isolation and purification	Purchased by BTC-BPPT
Laboratory furniture (lab desk)	BTC-BPPT	9/20/2019	for microbial observation	Purchased by BTC-BPPT
pH meter	BTC-BPPT	3/27/2018	for preparation of microbial medium	Purchased by BTC-BPPT
Electric stabilizer	BTC-BPPT	6/7/2018	for stabilizing electric input of deep freezer used for storage of microbial isolates	Purchased by BTC-BPPT
Hot plate stirrer	BTC-BPPT	3/27/2018	for analysis of active compound	Purchased by BTC-BPPT
Vortex mixer	BTC-BPPT	3/27/2018	for analysis of active compound	Purchased by BTC-BPPT
Showcase refrigerator 200 L	BTC-BPPT	3/27/2018	for storaging experimental samples	Purchased by BTC-BPPT
Showcase refrigerator 250 L	BTC-BPPT	6/7/2018	for storaging experimental samples	Purchased by BTC-BPPT
Air conditioner	BTC-BPPT	3/20/2018	for controlling temperature of microbial fermentation	Purchased by BTC-BPPT

Printer	BTC-BPPT	6/7/2018	for experimental data printing/recording	Purchased by BTC-BPPT
Exhaust fan	BTC-BPPT	4/19/2018	for improving air quality in shaker/incubation room	Purchased by BTC-BPPT
BSL-2 Room Renovation	BTC-BPPT	31/03/2015	for handling parasites and mammalian cells	Purchased by BTC-BPPT
Renovation and Preparation of Lab. anti amoeba	ITD-AU	12/01/2016	For the processing of antiamoeba research	Purchased by AU
Personal Computer set	ITD-AU	05/01/2016	For the processing and data recording of antiamoeba research	Purchased by ITD-AU
Router and Internet network	ITD-AU	12/01/2016	to improve internet connection in amoeba Lab.	Purchased by ITD-AU

1-3. Activities (Planned and Actual)

Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).

1.1 Primary screening for inhibitory activity of extract to the Plasmodium-derived recombinant enzyme

- Production system for 3 Plasmodium-derived recombinant enzymes (*Pf*DHODH, *Pf*MQO, *Pf*NDH2) for screening of active microbial extracts with inhibitory activity were established.
- Screening system targeting 3 Plasmodium-derived recombinant enzymes were established. Another screening system targeting a plasmodial enzyme is being established.
- More than 17,000 microbial extracts were objected to screening against the 3 target enzymes
- More than 100 microbial extracts showed inhibitory activity against the target enzymes

1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*

- Inhibitory activity against proliferation of *P.falciparum* of all extracts used for targeted screening was conducted.
- Secondary screening for selective inhibitor activity of the active extracts as the result from 1.1 to the proliferation of *P.falciparum* was not continued according to change of screening strategy as suggested by the experts in the 3rd year of the project. Inhibitory activity result from screening for selective inhibitory activity of extracts to the proliferation of *P.falciparum* was regarded as result of secondary screening, since all extracts objected to enzyme-based screening were also objected to cell-based screening as described in 1.3 below.
- More than 25 microbial extracts showed double inhibitory activity to the target enzyme activity and proliferation of *P.falciparum* cell

1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1 and 1-2

- More than 12,000 extracts were screened for inhibitory activity of

proliferation of *P.falciparum*

- More than 100 active extracts with inhibitory activity of proliferation of *P.falciparum* were obtained
- Several dereplication

1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against Plasmodium

- More than 80 active extracts with anti-malarial activity were objected for active compound isolation and purification
- Several dereplication methods were applied to avoid frequently obtained hit (dereplication of free fatty acids by complexation with α-cyclodextrin, dereplication of known compound based on HPLC profile of active extracts, dereplication of polyethers and peptaibols by antibiotics activity (against gram-positive bacteria) assay, and choosing of uncommon microbial producers).
- 10 anti-malarial active compounds were obtained, 1 of them was presumed as a novel compound. Two other active compounds are being purified

1.5 Establishment of mass production system of the lead compound candidates

- Large-scale fermentation (up to 5 L) was established using shaking-flask method
- Large-scale fermentation using jar fermenter (up to 30 L) was conducted
- Large-scale lead compound isolation and purification system was established using chromatography-based method, including flash chromatography

1.6 Determination of chemical structures of the lead compound candidates

- Chemical structure of 10 active compounds with anti-malarial activity were elucidated
- In addition to this, there were several other compounds which the chemical structure were elucidated, including linoleic acid as inhibitor for *Pf*MQO homogentisic acid as inhibitor of *Pf*DHODH, NC3B (1,3-dihydro-7-methyl-4,5,6-iso- benzofurantriol) and its derivative as a compound that is responsible for false positive result in *Pf*MQO assay. Linoleic acid is a ubiquitous compound in microbes, and homogentisic

acid is a derivative of previously isolated gentisyl alcohol.

1.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing

- Toxicity of all isolated and purified active compounds with antimalarial activity was tested *in-vitro* in mammalian cell.
- Efficacy of 1 anti-malarial active compound (gentisyl alcohol) was tested in animal model

1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment

- Planning of derivatization and pre-clinical testing of antimalarial active compound (particularly borrelidin) was discussed during scientific meeting held on October 10, 2019. The meeting was also attended by researchers from Nagoya Institute of Technology and University of Malaya, two prospective counterparts from Japan and Malaysia, respectively, for chemical structure modification, and IPB for pre-clinical testing.
- A joint proposal concerning on development of structure modification and pre-clinical assessment system for development of anti-infection agents (including antimalaria and antiamebic agents) was submitted on October 2, 2019 to Japanese Government through JICA-AMED SATREPS-scheme 2nd phase project. The proposal was submitted by BPPT in collaboration with LIPI and IPB from Indonesian side, The University of Tokyo, Bozo Research Institute, Kitasato University, and Nagoya Institute of Technology from Japan side, and University of Malaya, Universiti Teknologi Mara, and Universiti Putra Malaysia from Malaysia side.

Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)

2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica*-derived site-specific recombinant enzyme

- Production system for 4 Entamoeba-derived recombinant enzymes (*Eh*CS3, *Eh*SAT1, *Eh*NADK, *Eh*NO1) for screening of active microbial

extracts with inhibitory activity were established

- Three screening system targeting 4 Entamoeba-derived recombinant enzymes were established (*Eh*CS3, *Eh*SAT1/CS3, *Eh*NADK/NO1)
- More than 9,700 microbial extracts were objected to screening against the 4 target enzymes.
- More than 40 microbial extracts showed inhibitory activity against the target enzymes

2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*

- Inhibitory activity against proliferation of *E.histolytica* of all extracts used for targeted screening was conducted.
- Seven microbial extracts were proposed for active compound isolation and purification

2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2

- More than 16,000 extracts were screened for inhibitory activity of proliferation of *E.histolytica*
- More than 40 active extracts with inhibitory activity of proliferation of *E.histolytica* were obtained

2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*

- More than 20 extracts with antiamebic activity were objected for active compound isolation and purification
- Several dereplication methods were applied to avoid frequently obtained hit (dereplication of known compound based on HPLC profile of active extracts, and choosing of uncommon microbial producers).
- Two antiamebic active compounds were obtained, and another 1 active compound is being purified.

2.5 Establishment of mass production system of the lead compound candidates

- Large-scale fermentation (up to 5 L) was established using shaking-flask method
- Large-scale lead compound isolation and purification system was

established using chromatography-based method, including flash chromatography

2.6 Determination of chemical structures of the lead compound candidates

- Chemical structure of 2 active compounds with anti-amebic activity were elucidated

2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing

- Toxicity of all isolated and purified active compounds with anti-amebic activity was tested *in-vitro* in mammalian cell.
- Efficacy of 1 anti-amebic active compound (ovalicin) was tested in animal model.

2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment

- Planning of derivatization and pre-clinical testing of antiamebic active compound (particularly ovalicin) was discussed during scientific meeting held on October 10, 2019. The meeting was also attended by researchers from Nagoya Institute of Technology and University of Malaya, two prospective counterparts from Japan and Malaysia, respectively, for chemical structure modification, and IPB for pre-clinical testing.
- A joint proposal concerning on development of structure modification and pre-clinical assessment system for development of anti-infection agents (including antimalaria and antiamebic agents) was submitted on October 2, 2019 to Japanese Government through JICA-AMED SATREPS-scheme 2nd phase project. The proposal was submitted by BPPT in collaboration with LIPI and IPB from Indonesian side, The University of Tokyo, Bozo Research Institute, Kitasato University, and Nagoya Institute of Technology from Japan side, and University of Malaya, Universiti Teknologi Mara, and Universiti Putra Malaysia from Malaysia side.

Output 3: Technologies and research system for drug discovery using biological resources are established at the Indonesian research institutes.

3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library

- More than 3,500 microbial isolates were newly isolated and registered into microbial collection database in BPPT
- More than 20,000 microbial extracts were produced and registered in microbial extract collection database in BPPT
- A novel fungus species was isolated and identified
- New microbial isolation technique for improving diversity of microbial isolates in the culture collection was established in BPPT
- Number of unidentified microbes was decreased, indicated that the capability in morphology-based microbial identification of Indonesian researchers was improved.
- A system for producing microbial extract, including extract for first screening, reconfirmation, pre-scale up, and purification of active compound was established
- Microbial core library composed from 1000 diverse microbial isolates is being constructed. Currently, 150 isolates had been registered into the core library.

3.2 Establishment of screening systems

- Three anti-malarial enzyme-based and 1 cell-based high-throughput screening system were established
- Three anti-amebic enzyme-based and 1 cell-based high-throughput screening system were established
- In addition to anti-malarial and anti-amebic screening system, a cell-based and an enzyme-based screening system for searching anti-tuberculosis agents from microbial resources were established
- A system to determine active extract from high-throughput screening result based on high-throughput assay parameter (z-factor, signal-to-background ration) and counter assay result (against corresponding mammalia-originated target enzyme or mammalian cell) was established
- Several dereplication methods (for avoiding frequent hits and obtaining novel compound) based on selection of the producer, HPLC profile of active extract, antibiotic activity, and precipitation of specific commonly

obtained active compound, were established

- A system for selecting active extracts to be prioritized for further active compound isolation and purification based on the producer, selectivity (against inhibitory activity of proliferation of mammalian cell), HPLC profile of the extract (chemical properties of the estimated active compound), multiple activity (showing inhibitory activity against both target enzyme and proliferation of the parasite cell) was established.

3.3 Establishment of culture and evaluation systems

- Parasite cell culture system (*P.falciparum* and *E.histolytica*) was established and maintained
- Several mammalian cells (DLD1, HepG2, MCF-7, T47D, Vero, Huh7) culture system was established and maintained
- Evaluation system of inhibitory activity against proliferation of *P.falciparum* (based on LDH assay) and *E.histolytica* (based on WST-1 assay) was established
- Counter assay using mammalian cell (DLD1, HepG2, MCF-7, T47D, Vero, Huh7) for *in vitro* toxicity evaluation (based on WST-7 assay) was established

3.4 Introduction of technologies of isolation and purification

- Pre-extraction Test (PET) method for estimating the properties of active compound in the extract was introduced and implemented. Such information related to the properties of the compound is required for successful and efficient isolation and purification of active compound.
- Isolation method (including liquid-liquid extraction and maceration method) and purification method (including open column chromatography, HPLC (analytical, semi-preparative, recycle HPLC), and TLC (analytical, semi-preparative)) were introduced and implemented.
- Several TLC visualization methods (using polymolibdic acid (PMA) and EE reagent for mostly detecting reducing compounds, sulfuric acid for detecting polyethers, iodine, anisaldehyde, ninhidrine, and water) were introduced and implemented.

3.5 Introduction of technologies of chemical structure elucidation

- A method for estimating molecular weight of the target compound based on HPLC spectrum and LC-MS analysis was established and implemented
- A method for estimating chemical structure of target compound based on its UV spectrum using Natural Product Dictionary was introduced and implemented.
- A method for elucidating the structure of the target compound based on NMR analysis result was introduced and implemented.

3.6 Establishment and enhancement of a research network in Indonesia

- International symposium on natural resources-based drug development was held twice (2017 and 2019) in Indonesia (Jakarta). Each symposium was attended by more than 120 participants (researchers, government officials, pharmaceutical companies, from Asian Countries, mainly from Indonesia and Japan). The first symposium (held in 2017) gained an attention from 2015 Nobel Laurette in field of Medicine and Physiology, Prof. Satoshi Omura (Kitasato University), by sending a public letter to BPPT Chairperson to express his appreciation and support to BPPT and other Indonesian counterparts for their efforts in discovering lead compound for development of anti-malarial and anti-amebic drug from Indonesian biological resources, especially from Indonesia-originated microbes.
- Another international symposium was jointly held with AMED (Asia Infectious Disease Project Joint Symposium – Toward the Social Implementation of Health Technology through the Asian Research Network) on 2019 in Indonesia (Jakarta). The symposium presented invited speakers from Asian Countries (Indonesia, Japan, Malaysia, Singapore, Philippine, Thailand, Vietnam, India), and participated by researchers from universities and research institues, health affairs-related ministries from Asian Countries, as well as pharmaceutical industries. A panel discussion was held to formulate strategies for social implementation of health-research achievements, especially those are funded by AMED (including project under scheme of SATREPS, e-ASIA, and J-GRID).
- Research collaboration on development of anti-toxoplasmosis drug from Indonesian microbial resources was established between BPPT and

Obihiro University of Agriculture and Veterinary Medicine (OUAVM). An Material Transfer Agreement between the two parties was signed in 2017.

- Research collaboration on efficacy test of anti-malarial active compound in animal model was established between BPPT and Brawijaya University (BU) in 2019. A collaboration agreement was signed in 2019. Efficacy of an antimalarial active compound (gentisyl alcohol) in inhibiting proliferation of *P.berghei* in mice was examined *(in-vivo)* in BU.
- Research collaboration on development of anti-tuberculosis agents was established between BPPT, Airlangga University and The University of Tokyo in 2018. This collaboration was supported by US-based The Global Alliance for TB Drug Development, Inc. (TB Alliance). An MoU concerning this collaboration was signed in 2019.
- Research collaboration on development of anti-cancer agents was initialized between BPPT and Gadjah Mada University (UGM) in 2018. BPPT transferred a set of microbial extracts to UGM to be examined their inhibitory activity against cancer cell lines. A screening system for searching anti-cancer agents from microbial extracts is expected as an output from this collaborative work.
- Research collaboration between BPPT and UGM was initiated on topic of anti-malarial activity assay of active compound from algae in 2018. BPPT provided anti-malarial activity assay of active compound from algae prepared by UGM.
- Research collaboration on development of antimalarial agents from local plants was conducted between BPPT and Islamic State University Syarif Hidayatullah (UIN) in 2018. BPPT received and supervised 2 students from UIN on evaluating, isolating, and purifiying anti-malarial compound from Indonesia-originated plant. Result of this collaborative works will be jointly published in a scientific journal.
- Research collaboration between BPPT and Bandung Institute of Technology (ITB) was conducted with topic of anti-malarial activity assay of active compound from plant and development of anti-malarial drug delivery system using nano particles in 2019. BPPT provided supervision on anti-malarial activity assay to ITB. Result of this collaborative works will be jointly published in a scientific journal.
- Research collaboration between BPPT and Eijkman Institute was

conducted with topic of anti-malarial activity assay of active compound from plant in 2017. BPPT supervised anti-malarial activity assay to Eijkman Institute.

- Research collaboration between Indonesia (BPPT, IPB, LIPI), Japan (The University of Tokyo, Kitasato University, Bozo Research Institute, Nagoya Institute of Technology) and Malaysia (University of Malaya, Universiti Putra Malaysia, Universiti Teknologi Mara) concerning on development of structure modification and pre-clinical assessment system for development of anti-infection agents was initiated. A Letter of Intent (LoI) between representative institute from each country (BPPT, The University of Tokyo, and University of Malaya) was signed in 2019.

2. Achievements of the Project

2-1. Outputs and Indicators

Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological resources (microorganism, plants, etc.).

1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.
This indicator was 100% achieved in 1st year (level of achievement=high).

- Ten antimalarial compounds were isolated and purified. The first antimalarial compound was isolated and purified in the 1st year of this project. These compounds were isolated from microbial extracts showing anti-malarial activity as the result of screening from more than 17,000 extracts.
- Two other active compounds are being purified.

1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation.
This indicator was 100% achieved in 1st year (level of achievement=high).

- Chemical structure of 10 isolated and purified antimalarial compounds were elucidated. The 1st structure was elucidated in the 1st year.
- One of these structure elucidated compounds was presumed as a novel

compound.

- Currently, there is another 1 purified compound that the chemical structure is being elucidated.

1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.
This indicator was 100% achieved in the 5th year (level of achievement=high).

- One of isolated, purified and structure elucidated antimalarial active compound (gentisyl alcohol) was tested its efficacy in animal model. Efficacy test was conducted under collaborative work between BPPT and Brawijaya University.
- The result showed that the compound was effective in killing the malarial parasite *in-vivo*.
- Chemical structure of another antimalarial active compound (borrelidin) is currently being modified. The derivatives will be objected for activity assay, including efficacy test using animal model.

Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)

2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.
This indicator was 100% achieved in the 4th year of the project (level of achievement=high).

- Two antiamebic compounds were isolated and purified. These compounds were isolated from microbial extracts showing anti-amebic activity as the result of screening from more than 16,000 extracts.
- Another 1 active compound is being purified.

2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.
This indicator was 100% achieved in the 5th year of the project (level of achievement=high).

- Chemical structure of 2 isolated and purified antiamebic compunds were elucidated.
- Chemical structure elucidation of other isolated and purified compounds

were also conducted, but resulted in frequently obtained compounds.

2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.
This indicator was 100% achieved in the 5th year of the project (level of achievement=high).

- One of isolated, purified and structure elucidated antiamebic active compound (ovalicin) was tested its efficacy in animal model. Efficacy test was conducted at National Institute for Infectious Diseases Japan.
- The result showed that the compound was effective in killing the amebic parasite *in-vivo.*
- Chemical structure of another antiamebic active compound (ovalicin) is currently being modified. The derivatives will be objected for activity assay, including efficacy test using animal model.

Output 3: Technologies and research system for drug discovery using biological resources are established at the Indonesian research institutes.

3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project.
This indicator was 100% achieved in the 3th year of the project (level of achievement=high).

- Biological resource library in BPPT is composed from microbial isolate library and microbial extract library. At the beginning of the project, there are about 23,500 microbial isolates, and none of microbial/plant extracts were registered in biological resource library.
- During the project, more than 3,500 newly obtained microorganisms are registered and added into microbial collection in BPPT by the end of the project (1,900 of which was obtained by the 3rd year of the project). Together with pre-existing microorganisms, total number of microbial isolates in the microbial library in BPPT by the end of the project reached 27,000 isolates.
- Beside registration of microbial isolates into microbial library, more than 20,000 microbial extracts and 300 plant extracts were prepared and registered into extract library in BPPT (more than 13,000 of them were produced by the 3rd year of the project).

- A microbial core library composed from 1000 diverse microbial isolates is being constructed. Currently, 150 isolates had been registered into the core library.
- Diversity of microbial isolated used for screening of lead compounds is one of important factor in drug discovery from biological resources. To increase diversity level of microbial library, method for isolation of microbial isolate that is implemented in this project was directed to obtain rare microbes. Among them, a novel fungus species was isolated and identified.
- New microbial isolation technique for improving diversity of microbial isolates in the culture collection was established in BPPT
- Number of unidentified microbes was decreased, indicated that the capability in morphology-based microbial identification of Indonesian researchers was improved.
- A system for producing microbial extract, including extract for first screening, reconfirmation, pre-scale up, and purification of active compound was established

3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project.

This indicator was 100% achieved in the 2nd year of the project (level of achievement=high).

- Three anti-malarial enzyme-based and 1 cell-based high-throughput screening system were established
- Three anti-amebic enzyme-based and 1 cell-based high-throughput screening system were established
- By using knowledge and experience on establishment of anti-malarial and anti-amebic screening system, a cell-based and an enzyme-based screening system for searching anti-tuberculosis agents from microbial resources were also established
- A system to determine active extract from high-throughput screening result based on high-throughput assay parameter (z-factor, signal-to-background ration) and counter assay result (against corresponding mammalia-originated target enzyme or mammalian cell) was established
- One key for successful drug discovery is whether a novel compound can be obtained as an output from screening process or not. To achieve this,

several dereplication methods (for avoiding frequent hits and obtaining novel compound) based on selection of the producer, HPLC profile of active extract, antibiotic activity, and precipitation of specific commonly obtained active compound, were established

- Since output of screening process is active extract (which is composed from numerous compounds), further process for isolating and identifying the active compound from the extract is required. This process, so called isolation and purification process, is a time consuming process and requires a lot of efforts and expertise. While the number of active extracts is high (in this project, it exceeded more than 200 active extracts), suitable prioritization system for selecting active extracts to be proceeded into the next purification process is another key for successful drug discovery from natural resources. A system for selecting active extracts to be prioritized for further active compound isolation and purification based on selection of its producer, degree of selectivity (comparison of its activity against pathogen to its toxicity against mammalian cell), HPLC profile of the extract (chemical properties of the estimated active compound to determine whether the active compound is a known or novel compound), multiple activity (showing inhibitory activity against both target enzyme and proliferation of the parasite cell) was established.

3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project.

This indicator was 100% achieved in the 3rd year of the Project (level of achievement=high).

- Parasite cell culture system (*P.falciparum* and *E.histolytica*) system was established and maintained
- Several mammalian cells (DLD1, HepG2, MCF-7, T47D, Vero, Huh7) culture system was established and maintained
- Evaluation system of inhibitory activity against proliferation of *P.falciparum* (based on LDH assay) and *E.histolytica* (based on WST-1 assay) was established
- Counter assay using mammalian cell (DLD1, HepG2, MCF-7, T47D, Vero, Huh7) for in vitro toxicity evaluation (based on WST-7 assay) was established

3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.
This indicator was 100% achieved in the 3rd year of the project (level of achievement=high).

- Equipment needed for isolation and purification of compounds were installed in August 2016.
- Pre-extraction Test (PET) method for estimating the properties of active compound in the extract was introduced and implemented. Such information related to the properties of the compound is required for successful and efficient isolation and purification of active compound.
- Isolation method (including liquid-liquid extraction and maceration method) and purification method (including open column chromatography, HPLC (analytical, semi-preparative, recycle HPLC), and TLC (analytical, semi-preparative)) were introduced and implemented.
- Several TLC visualization methods (using polymolibdic acid (PMA) and EE reagent for mostly detecting reducing compounds, sulfuric acid for detecting polyethers, iodine, anisaldehyde, ninhidrine, and water) were introduced and implemented.
- Dereplication method for avoiding obtaining of fatty acids as active compound with PfMQO inhibitory activity was introduced.
- Dereplication method for avoiding obtaining frequent hit produced by fungi and actinomycetes by examining extract activity against gram positif bacteria was introduced.
- Dereplication method for avoiding obtaining frequent hit with antiamebic activity by excluding *Aspergillus fumigatus* from the list of the producer of those hits.
- A new dereplication method based on HPLC profile of extracts was introduced in BTC.

3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.
This indicator was 100% achieved in the 5th year of the project (level of achievement=high).

- A method for estimating molecular weight of the target compound based on HPLC spectrum and LC-MS analysis was established and implemented

- A method for estimating chemical structure of target compound based on its UV spectrum using Natural Product Dictionary was introduced and implemented.
- A method for elucidating the structure of the target compound based on NMR analysis result was introduced and implemented.

3-6. International symposiums are held for drug discovery for two (2) times at least.
This indicator has been 100% achieved in the 5th year of the project (level of achievement=high).

- The 1st international symposium on natural resources based drug development was held in August 21-22, 2017 in Jakarta. Total number of participants was 116 persons.
- The 2nd international symposium on natural resources based drug development was held in October 9, 2019 in Jakarta. Total number of participants was 120 persons.
- A joint symposium between BPPT and AMED with title "Asia Infectious Disease Project Joint Symposium - Toward The Social Implementation Of Health Technology Through The Asian Research Network" was held in October 8, 2019 in Jakarta. Total number of participants was 93 persons.

2-2. Project Purpose and Indicators

Project purpose: Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.

1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy.
This indicator is 100% achieved in the 5th year of the project (level of achievement=high)

- Ten compounds with anti-malarial activity were isolated and their chemical structures were elucidated. One of them was a novel compound.
- One of isolated compound with anti-malarial activity (gentisyl alcohol) was objected for efficacy test using animal model. The compound showed

efficacy against malarial parasites *in-vivo*.

- Chemical structure of another one isolated compound with anti-malarial activity (borrelidin) is being modified. Derivatives of this compound will be objected for activity test *in-vitro* and *in-vivo*.

2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy.
This indicator is 100% achieved in the 5th year of the project (level of achievement=high)

- Two active compounds with anti-amebic acitivity were isolated and their chemical structures were elucidated.
- One of isolated compound with anti-amebic activity (ovalicin) was objected for efficacy test using animal model. This compound showed efficacy against amebic parasites *in-vivo*.
- The chemical structure of an active compound with anti-amebic activity (ovalicin) is being modified. Derivatives of this compound will be objected for activity test *in-vitro* and *in-vivo*.

3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.
This indicator is 100% achieved in the 4th year of the project (level of achievement=high)

- Four scientific papers in which first author are an Indonesian researcher had been published in peer-reviewed journals.
  - Hartuti ED., Inaoka DK, Komatsuya K, Miyazaki Y, Miller RJ, Xinying W, Sadikin M, Prabandari EE, Waluyo D, Kuroda M, Amalia E, Matsuo Y, Nugroho NB, Saimoto H, Pramisandi A, Watanabe Y, Mori M, Shiomi K, Balogun EO, Shiba T, Harada S, Nozaki T, Kita K (2018). Biochemical studies of membrane bound *Plasmodium falciparum* mitochondrial L-malate:quinone oxidoreductase, a potential drug target. BBA Bioenergetics 1859(3):191-200.
  - Pramisandi A, Dobashi K, Mori M, Nonaka K, Matsumoto A, Tokiwa T, Higo M, Kristiningrum, Amalia E, Nurkanto A, Inaoka DK, Waluyo D, Kita K, Nozaki T, Omura S, Shiomi K (2020). Microbial inhibitors active against *Plasmodium falciparum* dihydroorotate dehydrogenase derived

from an Indonesian soil fungus *Talaromyces pinophilus* BioMCC-f.T.3979. J Gen Appl Microbiol (*in-press*).

- Nurkanto A, Jeelani G, Yamamoto T, Naito Y, Hishiki T, Mori M, Suematsu M, Shiomi K, Hashimoto T, Nozaki T (2018). Characterization and validation of *Entamoeba histolytica* pantothenate kinase as a novel anti-amebic drug target. IJP: Drugs and Drug Resistance 8:125:136.
- Nurkanto A, Jeelani G, Yamamoto T, Hishiki T, Naito Y, Suematsu M, Hashimoto T, Nozaki T (2018). Biochemical, metabolomics, and genetic analyses of desphospho coenzyme A kinase involved in coenzyme A biosynthesis in human enteric parasite *Entamoeba histolytica*. Frontier in Microbiol. 9:2902.

- Two other related research papers were published.
  - Mori M, Jeelani G, Masuda Y, Sakai K, Tsukui K, Waluyo D, Tarwadi, Watanabe Y, Nonaka K, Matsumoto A, Omura S, Nozaki T, Shiomi K (2015). Identification of natural inhibitors of *Entamoeba histolytica* cysteine synthase from microbial secondary metabolites. Front Microbiol. 6:962.
  - Setyowati EA, Isnansetyo A, Djohan TS, Nurcahyo RW, Prabandari EE (2019). Antimalarial activity of microalgae extracts based on inhibition of PfMQO, a mitochondrial *Plasmodium falciparum* enzyme. Pharcogn J. 11(6)Suppl: 1477-1482.
- Results of this project were also published in scientific conferences.
  - Mahsunah AH, Kurnia K, Siska E, Nurlaila, Pramisandi A, Dewi D, Prabandari EE, Nugroho NB, Waluyo D, Wibowo AE, Mori M, Dobashi K, Shiomi K, Yamashita M, Nozaki T (2017). Purification and Identification of Antimalarial Compounds from Soil Fungus BioMCC-f.T.7495 as Inhibitors of *Plasmodium falciparum* Dihydroorotate Dehydrogenase. The 9th International Seminar of Indonesian Society for Microbiology, Palembang, Indonesia, November 14-15, 2017 (oral presentation).
  - Hidayati DN, Dewi D, Kristiningrum, Mintarsih H, Sari NR, Nastiti SK, Prabandari EE, Waluyo D, Nugroho NB, Watanabe Y, Wibowo AE (2017). The Genus Diversity of Fungal Isolates in Biotechnology Microbial Culture Collection (BioMCC), BPPT. The 9th International Seminar of Indonesian Society for Microbiology, Palembang, Indonesia, November 14-15, 2017 (oral presentation).
  - Waluyo D, Prabandari EE, Nugroho NB, Tarwadi, Chaidir, Dewi D,

Hidayati DN, Suryani, Kristiningrum, Afrianti KR, Mahsunah AH, Puspitasari DJ, Putri TZ, Adipratiwi N, Fuad A, Adianti M, Inaoka DK, Miyazaki Y, Nurlaila, Siska E, Kurnia K, Mori M, Dobashi K, Shiomi K, Nozaki T (2017). Searching Lead Compound of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources. The 9th International Seminar of Indonesian Society for Microbiology, Palembang, Indonesia, November 14-15, 2017 (oral presentation).

- Erwahyuni EE, Hartuti ED, Waluyo D, Adipratiwi N, Putri TZ, Nugroho NB, Inaoka DK, Nozaki T. (2017). Expression and characterization of Plasmodium falciparum Malate:Quinone Oxidoreductase (PfMQO) and establishment of screening system for searching PfMQO inhibitor. The 9th International Seminar of Indonesian Society for Microbiology, Palembang, Indonesia, November 14-15, 2017 (oral presentation).
- Kristiningrum, Hidayati DN, Nonaka K, Suryani, Dewi D, Tokiwa T, Mori M, Prabandari EE, Waluyo D, Wibowo AE, Shiomi AE, Nozaki T (2019). A new *Aureobasidium* species from fallen leaves in Kupang, Indonesia. Asian Mycological Congress 2019, Mie, October 1-4, 2019 (poster presentation).
- Pramisandi A, Dobashi K, Mori M, Inaoka DK, Nozaki T, Omura S, Shiomi K (2018). Fast and effective dereplication of free fatty acids from microbial extracts in *Plasmodium falciparum* Malate:Quinone-oxidoreductase inhibitor screening: The effect of α-cyclodextrin complexation. 2018 Annual meeting of the Japan society for bioscience, biotechnology, and agrochemistry, Nagoya, March 15-18, 2018 (oral presentation)
- Hartuti ED, Inaoka DK, Komatsuya K, Miyazaki Y, Miller RJ, Xinying W, Prabandari EE, Waluyo D, Matsuo Y, Nugroho NB, Saimoto H, Pramisandi A, Watanabe Y, Mori M, Shiomi K, Balogun EO, Shiba T, Harada S, Nozaki T, Kita K (2018). Biochemical studies of membrane bound Plasmodium falciparum mitochondrial L-malate: quinone oxidoreductase, a potential drug target. The U.S.-Japan Cooperative Medical Science Program. The 48th Joint Conference on Parasitic Diseases. Nagasaki, February 16, 2018 (oral presentation).
- Hartuti ED, Inaoka DK, Prabandari EE, Putri TZ, Waluyo D, Adipratiwi N, Puspitasari DJ, Miyazaki Y, Mori M, Shiomi K, Nozaki T, Kita K. (2018) High throughput screening of butanolic extracts from Indonesian natural

products against Plasmodium falciparum dihydroorotate dehydrogenase and malate:quinone oxidoreductase. The 87th Annual meeting of Japan Society for Parasitology, Nagasaki, March 17-18, 2018 (oral presentation)

- Hartuti ED, Inaoka DK, Komatsuya K, Miyazaki Y, Miller RJ, Xinying W, Prabandari EE, Waluyo D, Matsuo Y, Nugroho NB, Saimoto H, Pramisandi A, Watanabe Y, Mori M, Shiomi K, Balogun EO, Shiba T, Harada S, Nozaki T, Kita K (2018). Biochemical studies of membrane bound Plasmodium falciparum mitochondrial L-malate: quinone oxidoreductase and identification of potent inhibitor. The 14th International Congress of Parasitology, Daegu, South Korea, August 19-24, 2018 (poster presentation)
- Hartuti ED, Inaoka DK, Komatsuya K, Miyazaki Y, Miller RJ, Xinying W, Prabandari EE, Waluyo D, Matsuo Y, Nugroho NB, Saimoto H, Pramisandi A, Watanabe Y, Mori M, Shiomi K, Balogun EO, Shiba T, Harada S, Nozaki T, Kita K (2018). Characterization of membrane bound Plasmodium falciparum mitochondrial L-malate: quinone oxidoreductase as potential target for antimalarial treatment. The 91st Annual meeting of Japanese Biochemical Society, Kyoto, September 24-26, 2018 (poster and oral presentation)
- Hartuti ED, Inaoka DK, Komatsuya K, Miyazaki Y, Miller RJ, Xinying W, Prabandari EE, Waluyo D, Matsuo Y, Nugroho NB, Saimoto H, Pramisandi A, Watanabe Y, Mori M, Shiomi K, Balogun EO, Shiba T, Harada S, Nozaki T, Kita K (2018). Biochemical studies of membrane bound Plasmodium falciparum mitochondrial L-malate: quinone oxidoreductase and identification of potent inhibitor. The 59th Annual Meeting for the Japanese Society of Tropical Medicine, Nagasaki, September 9-11, 2018 (poster presentation)
- Hartuti ED, Inaoka DK, Komatsuya K, Miyazaki Y, Miller RJ, Xinying W, Prabandari EE, Waluyo D, Matsuo Y, Nugroho NB, Saimoto H, Pramisandi A, Watanabe Y, Mori M, Shiomi K, Balogun EO, Shiba T, Harada S, Nozaki T, Kita K (2019). Biochemical studies of membrane bound Plasmodium falciparum mitochondrial L-malate: quinone oxidoreductase and identification of potent inhibitor. WISE Program Kickoff Meeting-Symposium, Nagasaki, March 9-10, 2019 (poster presentation).

- Hartuti DH, Inaoka DK, Sakura T, Wang X, Mochizuki K, Acharjee R, Matsuo Y, Mori M, Shiomi K, Nozaki T, Hamano S and Kita K (2019). Novel inhibitors of Plasmodium falciparum mitochondrial malate:quinone oxidoreductase. The 88th Annual Meeting of Japanese Society of Parasitology, Nagasaki, March 15-16, 2019 (oral presentation)
- Hartuti ED, Sakura T, Wang X, Mochizuki K, Acharjee R, Matsuo Y, Mori M, Shiomi K, Nozaki T, Hamano S, Kita K and Inaoka DK (2019). Target-based screening against dihydroorotate dehydrogenase in *Plasmodium falciparum*. Molecular Parasitology Workshop, Ehime, August 31, 2019 (oral presentation)
- Hartuti ED, Sakura T, Wang X, Mochizuki K, Acharjee R, Matsuo Y, Mori M, Shiomi K, Nozaki T, Hamano S, Kita K and Inaoka DK (2019). Identification of the *Plasmodium falciparum* mitochondrial malate:quinone oxidoreductase (PfMQO) and dihydroorotate dehydrogenase (PfDHODH) inhibitors as antimalarial drug candidates. The 92nd annual meeting of The Japanese Biochemical Society, Yokohama, September 18-20, 2019 (oral and poster presentation).
- Chrisnayanti E, Siska, E, Nurlaila, Bernawati P, Melinda, Mahsunah AH, Dobashi K, Yamashita M, Waluyo D, Prabandari EE, Nugroho NB, Wibowo AE, Nozaki T, Shiomi K (2019). Purification of antimalarial compounds from Indonesian natural resources. Asia Infectious Disease Project Joint Symposium-Toward the Social Implementation of Health Technology through the Asian Research Network, Jakarta, October 8, 2019 (poster presentation)
- Waluyo D, Puspitasari DJ, Adipratiwi N, Sakura T, Inaoka DK, Dobashi K, Wibowo AE, Nozaki T (2019). Development of microbial resources-based antimalarial phenotypic screening system. Asia Infectious Disease Project Joint Symposium-Toward the Social Implementation of Health Technology through the Asian Research Network, Jakarta, October 8, 2019 (poster presentation).
- Dewi D, Suryani, Hidayati DN, Afriyani KR, Kristiningrum, Octaviani AN, Waluyo D, Prabandari EE, Mahsunah AH, Chaidir, Wibowo AE, Mori M, Shiomi K, Nozaki T (2019). Production of active compounds extracts from Indonesian microbial resources. Asia Infectious Disease Project Joint Symposium-Toward the Social Implementation of Health Technology through the Asian Research Network, Jakarta, October 8,

2019 (poster presentation)

- Prabandari EE, Hartuti ED, Inaoka DK, Ariyani T, Nugroho NB, Mahsunah AH, Wibowo AE, Waluyo D, Nozaki T (2019). Screening of *Plasmodium falciparum* mitochondrial malate:quinone oxidoreductase inhibitors from microbial extracts. Asia Infectious Disease Project Joint Symposium-Toward the Social Implementation of Health Technology through the Asian Research Network, Jakarta, October 8, 2019. (poster presentation)
- Hidayati DN, Kristiningrum, Octaviani AN, Dewi D, Suryani, Afriani KR, Chaidir, Mori M, Prabandari EE, Waluyo D, Wibowo AE, Shiomi K, Nozaki T (2019). Isolation and morphological based identification of microbes from Indonesian resources. Asia Infectious Disease Project Joint Symposium-Toward the Social Implementation of Health Technology through the Asian Research Network, Jakarta, October 8, 2019 (poster presentation).
- Waluyo D (2017). The project for searching lead compound of anti-malarial and anti-amebic agents by utilizing diversity of Indonesian bio-resources. The 1st international symposium on natural resources-based drug development, Jakarta, August 21-22, 2017 (oral presentation).
- Waluyo D (2019). Utilization of Indonesian microbial resources for anti-malarial drug discovery. Asia Infectious Disease Project Joint Symposium-Toward the Social Implementation of Health Technology through the Asian Research Network, Jakarta, October 8, 2019 (oral presentation)
- Pramisandi A, Mahsunah AH, Takemoto D, Dewi D, Inaoka DK, Waluyo D, Omura S, Shiomi K (2017). 4-Quinolone Isomers Isolated from *Penicillium chrysogenum* BioMCC-f.T.6691 Targeting *Plasmodium falciparum* Dihydroorotate Dehydrogenase. The 30th Kitasato University Bioscience Forum, Kitasato University, Towada (oral presentation).
- Pramisandi A, Dobashi K, Mori M, Inaoka DK, Nozaki T, Omura S, Shiomi K (2017). Fast and Effective Dereplication of Free Fatty Acids from Microbial Extracts in *Plasmodium falciparum* Malate:Quinone-Oxidoreductase Inhibitor Screening: The Effect of Alpha-Cyclodextrin Complexation. All Kitasato Project Study The 10th Kitasato Chemistry Symposium, Kitasato University, Sagamihara (oral presentation).

- Pramisandi A, Dobashi K, Mori M, Inaoka DK, Omura S, Nozaki T, Shiomi K (2018). Searching for Microbial Metabolites Targeting *Plasmodium falciparum* L-Malate:Quinone Oxidoreductase as Antimalarial Lead Compounds. The 31st Kitasato University Bioscience Forum, Kitasato University, Sagamihara (oral presentation)
- Pramisandi A (2018). Searching for Microbial Metabolites Targeting *Plasmodium falciparum* L-Malate:Quinone Oxidoreductase as Antimalarial Lead Compounds. Chemical Biology Seminar, Keio University, Hiyoshi (oral presentation)
- Pramisandi A, Chrisnayanti E, Kurnia K, Hasegawa J, Hontoku M, Kristiningrum, Dobashi K, Mori M, Inaoka DK, Nozaki T, Omura S, Shiomi K (2019). Searching for Microbial Metabolites Active Against Plasmodium falciparum Dihydroorotate Dehydrogenase as Antimalarial Lead Compounds. The 2019 Annual Meetings of The Japan Society of Bioscience, Biotechnology and Agrochemistry, Tokyo University of Agriculture, Tokyo. (oral presentation)
- Pramisandi A, Dobashi K, Mori M, Inaoka DK, Omura S, Nozaki T, Shiomi K (2019). Search for Microbial Metabolites Active Against *Plasmodium falciparum* Dihydroorotate Dehydrogenase as Antimalarial Lead Compounds. The 32nd Kitasato University Bioscience Forum, Kitasato University, Tokyo (oral presentation)
- Pramisandi A, Dobashi K, Mori M, Nonaka K, Inaoka DK, Omura S, Nozaki T, Shiomi K (2019). Search for Microbial Metabolites Active Against *Plasmodium falciparum* Dihydroorotate Dehydrogenase as Antimalarial Lead Compounds. The Joint Symposium of "10th Korea-Japan Chemical Biology Symposium" and "30th Meeting for New Drug Discovery", Kanazawa (oral presentation).
- Pramisandi A, Dobashi K, Mori M, Nonaka K, Inaoka DK, Omura S, Nozaki T, Shiomi K (2019). Search for Microbial Metabolites Active Against *Plasmodium falciparum* Dihydroorotate Dehydrogenase as Antimalarial Lead Compounds. Chemical Biology Seminar, Kitasato University, Tokyo (oral presentation).
- Pramisandi A, Dobashi K, Mori M, Nonaka K, Inaoka DK, Omura S, Nozaki T, Shiomi K (2019). Search for Microbial Metabolites Active Against *Plasmodium falciparum* Dihydroorotate Dehydrogenase as Antimalarial Lead Compounds. All Kitasato Project Study The 11th

Kitasato Chemistry Symposium, Kitasato University, Tokyo (oral presentation).

- At least 2 more scientific papers are being prepared.
- A patent document related to isolated novel compound is being prepared.

3. History of PDM Modification

Project Design Matrix (PDM) was modified several times during the project term.

3-1. Change of counterpart

a. Main counterpart institute from Indonesia was changed from Biotech Center of BPPT to Center for Pharmaceutical and Medical Technology of BPPT, due to reorganization in BPPT. The change was recorded in Minutes of Meeting of JCC Meeting in 2016.
b. Unit of LIPI that is involved in this project was changed from Research Center for Biotechnology of LIPI to Indonesia Culture Collection (InaCC) of LIPI, due to appropriateness for the project. The change was recorded in Minutes of Meeting of JCC Meeting in 2016.
c. Main counterpart institute from Japan was changed from Tsukuba University to The University of Tokyo, due to position movement of Chief Advisor. The change was recorded in Minutes of Meeting of JCC Meeting in 2017.
d. Main counterpart institute from Indonesia was changed from Center for Pharmaceutical and Medical Technology of BPPT to Laboratory for Biotechnology of BPPT, due to reorganization in BPPT. The change was recorded in Minutes of Meeting of JCC Meeting in 2017.
e. Brawijaya University and Center for Primate Study of Bogor Agriculture University were added as member of counterpart institute from Indonesia side. The change was recorded in Minutes of Meeting of JCC Meeting in 2020.

3-2. Project member list

There were several changes in project member list. All changes were reported in JCC Meeting and recorded in the Minutes of Meeting.

4. Others

4-1. Results of Environmental and Social Considerations

(Not applicable)

4-2. Results of Considerations on Gender/Peace Building/Poverty Reduction

(Not applicable)

III. Results of Joint Review

1. Results of Review based on DAC Evaluation Criteria

1-1. **Relevance** (Consistency of this project with development policies, high-level plans and needs etc. to the partner country)

Review result: **HIGH**

Drug raw material is considered as materials required for formulating a drug, including active ingredients (so called Active Pharmaceutical Ingredient, API) and excipients (substance formulated alongside the active ingredient of a medication, including substance for stabilize the API, increase solubility or adsorption of the API, etc). These two materials are used for formulating/manufacturing a drug in a pharmaceutical company.

Currently, Indonesia is importing more than 95% of drug raw material from abroad. These materials are used by more than 200 pharmaceuticals companies in Indonesia for formulating/manufacturing drugs for supplying more than 90% drugs in Indonesia. According to Indonesia Long-term National Development Plan 2005-2025, health and drug is assigned as a field in National Prime Research Program. The program is directed to develop and implement technology for drug raw material production for import product substitution. In 2016, the government released a Presidential Instruction no.6 to related ministries in order accelerate the development of local pharmaceutical and health devices industry. Ministry of Research Technology and Higher Education was instructed to coordinate research and development of pharmaceutical products in Indonesia. This SATREPS project is aimed to develop the capacity of Indonesian institutes and researchers on developing drugs. Through this project, Indonesia is expected to be able to produce the necessary drug by themselves with their own capacity. Thus, this project is strongly in line with the current policy.

In Mid-term National Development Plan 2015-2019, controlling malaria was one of the government priority in field of health and infectious disease control. This priority still remains in Mid-term National Development Plan 2020-2024. Not only in national level, malaria has become one topic in Sustainable Development Goals organized by the United Nations (SDG 3: Ensure healthy lives and promote wellbeing for all

at all ages), suggested the importance of control of malaria for world people. This SATREPS Project focused on development of anti-malarial drug, as well as anti-amebic drug, which is highly beneficial for that purpose.

Recently, a new law concerning on National System of Science and Technology was established (Constitution no.11, 2019). According to this law, advancement of science and technology in Indonesia should be based on utilization of local bioresources. During this project, development of drug was conducted by utilizing Indonesian biological resources, including microbial and plant resources. Thus, this project is also in line with the policy.

1-2. **Effectiveness** (Achievement level of the project purpose, influence of impediments, relations between outputs and project purposes, etc.)

Review result: **HIGH**

Project purpose of this project was determined in the Record of Discussion Document. Indicators for evaluating the level of achievement of the project purpose were also defined at the starting point of this project. As already described in Section II of this Report (Result of the Project), all of these indicators have been achieved successfully by the Project.

Input from Government of Japan (GOJ) is described in Section II.1 (Result of the Project). GOI through Indonesian institutes also contributed significant input to this project, including budget (more than IDR 3.5 billion or approx. JPY 28.6 million) and other *in-kind* such as precious Indonesia-originated biological resources (microbes and plants), researchers, lab and office spaces, and equipment. This project also utilized microbial collection from InaCC-LIPI, which is established through the previous SATREPS Project. Additionally, some of microbes showing anti-malarial and anti-amebic activities are being deposited in InaCC-LIPI (currently under process). This indicated that the project utilized input from both Japan and Indonesia side, as well as output of previous SATREPS Project, in order to achieve Project Purpose.

The purpose of this project is to enhance research capacity of Indonesian research institute for the development of anti-malarial and anti-amebic agents by utilizing Indonesia biological diversity through

collaborative research activities with Japanese research institutes. During this project, not only active compounds with anti-malarial and anti-amebic activities were isolated and identified successfully, but also research network between research institute in Japan and Indonesia was enhanced. Numerous research collaborations in field of drug discovery were established among Indonesian institutes, as well as among Indonesia, Japan, and other Asian Countries. This indicated that capacity of Indonesian researchers and institutes on drug discovery is improved.

1-3. **Efficiency** (Relations with the achievement level of inputs and outputs, etc.)

Review result: **HIGH**

In Record of Discussion Document, both GOJ and GOI will contribute inputs to this project, including budget, experts, personnel, lab and office spaces, equipment, and biological resources. As described in Section II (Results of the Project), both side had realized their inputs for this project. Moreover, during the project both Indonesia and Japan side actively seek external funding for initiating a new field of drug discovery, including searching for active compound with anti-tuberculosis. All of these inputs were utilized by the project to achieve all indicators for project outputs, outcomes, and project purpose as described in the same Section of this report. Thus, efficiency of this project is regarded as high.

1-4. **Impact** (Contribution to the achievement level of the overall goal, level of contribution to policies and communities, contribution to other projects, etc.)

Review result: **HIGH**

As described in Section III.1-1 (Result of Joint Review: Relevance) above, the government promotes the development of science and technology for drug raw material production in order to realize national sovereign in drug raw material. Starting from 2020, budget for research and development from the government to govermental research institutes (including BPPT and LIPI) are managed to be allocated for national level flagship program. With long experience and significant achievement in drug raw material research, including the current achievement from this SATREPS project, BPPT was mandated by the government to conduct a

flagship program related to development of technology for drug raw material production.

BPPT received technical supports from Japanese counterparts through this SATREPS project, particularly in term of microbial isolation and identification, microbial extract preparation, anti-malarial assay, and purification of active compound. These significantly enhanced the capacity of BPPT on developing drug from biological resources, especially anti-malarial drug. Althogh the research was just started from past 5 years, BPPT had been recognized by other local and international institute for its capability in drug development, particularly anti-malarial drug. As described in Section II.1-3 (Result of the Project: Activities, Output 3, 3.6), numerous research collaborations between BPPT and local, as well as international institutes, were initiated and implemented.

BPPT is mandated by the government to conduct assessment and application of technology that is required for the people of Indonesia. This assignment is implemented through several roles, including providing technology service for the community. Laboratory for Biotechnology-BPPT provides various technology services, including services for chemical and genetic analysis. Some of the services were already accreditated with ISO/IEC 17025:2018, so analysis results generated in BPPT are highly assured and recognized worldwide. With enhanced capacity in anti-malarial assay gained during the project, BPPT is preparing to include anti-malarial assay/anti-amebic assay into its service scope. During the project, BPPT already provided such services to other research institutes and researchers, but this was conducted on research collaboration basis, due to some administrative preparation should be done before BPPT is able to provide such service (according to status of BPPT as a governmental body).

This project focused on development of anti-malarial and anti-amebic drug. Capacity of Indonesian institutes and researchers on this field was significantly increased. By using knowledge and experience on establishment of anti-malarial and anti-amebic screening system, a cell-based and an enzyme-based screening system for searching anti-tuberculosis agents from microbial resources were also established. This indicated that achievements of this project also contributes to solve other health issue in Indonesia, particularly tuberculosis, an infectious

disease that Indonesia and other Asian countries still suffer on it.

1-5. **Sustainability** (Likely continuation from the aspects of policy, technology, organization, finance, etc.)

Review result: **HIGH**

As described in 1-1 and 1-4 above, BPPT is mandated to conduct a flagship program related to development of drug raw material by the government in order to reduce dependency of imported products. Through the program, technology development of production of drug, including discovery of lead compounds as drug candidate, will be the main activities. BPPT is also committed to continue searching of lead compounds with anti-infection activity from Indonesia bioresources, and allocating budget as much as IDR 400 million in FY 2020. BPPT will also assign researchers, mainly those who previously involved in this SATREPS project, for this activity. This is also in line with the policy from the government as mentioned in 1-1 above.

BPPT signed a Letter of Intent concerning a joint research on development of drug candidate for infectious diseases with local partners including LIPI and IPB, and international partners including The University of Tokyo and Malaya University in October 2019. This document is the basis for international joint research between research institutes in Indonesia, Japan and Malaysia to continue the development of drug from Indonesian bioresources which had been initiated by this SATREPS Project. Lead modification and pre-clinical study of promising lead compounds obtained from this project will become the focus for the next joint research.

To realize the collaboration, BPPT, The University of Tokyo, and University of Malaya jointly proposed a research proposal to GOJ for seek a research grant from SATREPS scheme (SATREPS 2nd phase project). The proposal had been submitted in October 2019 through JICA, as well as AMED for Japanese counterpart. Along with this, BPPT also seek external funding by submitting a grant proposal to Ministry of Research and Technology. LIPI and BPPT also submitted a grant proposal to JSPS with topic of development of anti-tuberculosis from Indonesian bioresources. The proposal was granted and will be funded from FY 2020.

As microbial resources are the key for successful lead discovery, BPPT committed to continue establishing a core microbial library composed from highly diverse Indonesian microbial isolates in FY 2020. Identification of uncommon microbes based on molecular approach will be conducted. Purification of anti-malarial active compound will also be continued based on screening result during the project term. In addition to this, anti-tuberculosis screening system will also be established in BPPT. As described in 1-4 above, BPPT is also preparing to include anti-malarial assay/anti-amebic assay into its service scope for the community.

2. Key Factors Affecting Implementation and Outcomes

a. Biosafety and biosecurity system

Development of drug, particularly drug for infectious diseases, needs a proper environment, in order to ensure safety of researcher involved and the materials being used (pathogens, biohazard materials, etc.). Such circumstances should be established under an appropriate biosafety and biosecurity system.

b. Regulations related to importation

Not all necessary equipment, reagents and consumables for this project are available in Indonesia. Importing items from abroad is unavoidable to ensure the project runs smoothly. Complicated importing procedure due to lack of coordination between related ministries resulted in difficulties on importation of equipment/reagents/supplies for conducting research in Indonesia. Such occasion may stop or delay the progress of the project.

c. Material transfer

In this project, Indonesian institute learned technologies developed in Japan. Some of these technologies need to be verified using real sample that will be used for the project. This verification is very important to make sure the technologies are ready to be applied in Indonesia for building the capacity of Indonesian institutes and researchers. Thus, a system to transfer materials from Indonesia to Japan and vice versa is necessary. This material transfer should be conducted in line with related regulations

in both countries.

d. Task distribution

One of successful key of capacity building is to properly distribute the tasks of the project based on potency and capability of each team/personnel. Good communication and mutual understanding between all involved institutes and researchers are indispensable for task arrangement.

3. Evaluation on the results of the Project Risk Management

3-1. Risk Management Results

a. Biosafety and biosecurity system

- Establishment of BSL-2 laboratory

Since this project deals with non-aerosol pathogens (malaria and amebiasis parasites) and mammalian cells, a BSL-2 laboratory was established in BPPT and AU. Appropriate system to ensure the safety of researcher, such as obligation to wear specific lab coat and use mask and glove during experiment, was applied to the lab. All involved researchers were obligated to attend lab safety training. Parasite cells, as well as mammalian cells, were handled only in specified Biological Safety Cabinet Level 2 (BSC 2) by only trained researchers.

Surrounded with lab space for handling microbes, this lab is susceptible from contamination, which will potentially harmful for the project. To anticipate this, the lab was equipped with UV lamp that will sterilize the entire room before and after experiment. Water sink in the room was closed and relocated to other room. Access to the room was also restricted only for the person who will work in the room. Special lab coat and sandal to be used only in the room was also prepared. Such anticipation seemed to be effective to prevent the spread of contamination.

Malaria cell culture uses human red blood, which is regarded as biohazard. Lab applied a system to ensure safety disposal of these wastes. All pathogen-contained wastes, as well as microbe-contained wastes, will be sterilized by autoclave (121°C,

15 min) before dispose them as lab waste. This waste will be managed by trained researchers until being sterilized.

- Establishment of Standard Operating Procedure (SOPs)

  In order to maintain safety in the lab, as well as to obtain a reliable data during experiment, solid and obeyable SOPs are crucial. As advised by Japanese experts, BPPT established written SOPs for major experiments, especially experiments that involve pathogens and biohazards. These SOPs were shared and communicated to all researchers and stored in a way so it can be easily accessed by all researchers. The SOPs were reviewed periodically by related research team member. These SOPs were also useful as reference for discussion among researchers when the experiment was not properly conducted.

b. Regulations related to importation

- Understanding current regulation

  Regulation related to importing items from abroad are vary depend on many parameters. Legal procedures should be taken to import any items required for this project. Complicated procedures were often the obstacle to clearance the items before entering Indonesia. At this point, understanding current regulation by researchers is unavoidable.

  In Record of Discussion document, it was mentioned that GOI will take measure to exempt any importation-related charges on equipment, machinery, and other material necessary for the implementation of the project. BPPT supported JICA for tax exemption application to GOI, and most of them were granted by GOI.

  However, there was equipment that could not be imported through this scheme, which was freezer. It could not be imported, not because of listed as non-tax exempted item, but there was regulation that Indonesia had closed importing freezer from abroad, and all imported freezer should follow very strict condition, including submission of certificate of compliance from local trusted testing body, which will rise additional cost and potentially delay

the delivery time, though. While tax exemption process required BPPT to fulfill all of these requirements, both Indonesian and Japan side agreed to regard this item as a special one and excluded from the equipment list to avoid delivery delay, and treated it as a special case. The project then asked a private company to handle the importation process without tax exemption, so time-consuming tax exemption process could be avoided. Finally, the freezer could be delivered to Indonesian institutes without significant delay (only about 2 weeks behind). Such countermeasure was able to speed up the delivery of equipment imported from Japan effectively.

- Selecting local prominent vendor

  Most of reagents and consumables necessary for the project are imported from outside of Indonesia. Thus, delivery time of ordered items usually takes several months (6~12 weeks). This will potentially harmful for the project.

  Although difficult to shorten this delivery time, even by the vendor, any countermeasure should be taken to, at least, ensure the promised delivery time. This is important because of complicated importing procedures that may prolong the delivery time. Selecting local prominent vendor with excellent performance (in term of delivery) was effective to overcome this problem. Gathering information about the items, including stock condition in vendor's warehouse and possible substituted items was also effective way to ensure the availability of items in the laboratory.

  Moreover, knowing the turnover time of these items in the laboratory was also very crucial. An in-house inventory system for consumables and reagents was established and implemented. Timing for re-purchasing the item, especially for fast-moving items, was determined and communicated with all researchers. This countermeasure was also very effective to run the project smoothly.

c. Material transfer

- Exchanging Material Transfer Agreement (MTA) between involved

counterparts

According to Nagoya Protocol, Indonesia has sovereign right on its biological resources, and utilization of this resource should be based on mutual beneficial. Although Indonesia had ratified this protocol since 2013, there is no specific and clear regulation regarding to this issue. Thus, in this project, as stipulated in Memorandum of Understanding (MoU) and Implementation Agreement (IA) between BPPT and The University of Tokyo, all material transfer between parties involved in this project should be done under a MTA and signed by the parties. Term "material" refers to any living biological material (such as microbial isolates) and any non-living biological resources-derived material (such as microbial extracts).

Procedures for material transfer and all necessary documents were clearly stipulated in IA. All material transfer was done based on request. Any counterpart could use these documents for requesting a material transfer from other institute. Material transfer can only be done after the owner of the requested material agreed to the request and MTA was signed by both parties and endorsed by both BPPT and the University of Tokyo as the main counterpart from Indonesia and Japan, respectively. The requester should follow all terms stipulated in MTA when use the material.

- Monitoring the implementation of material transfer

  Material transfer was one of key factors for running this project successfully. To ensure that such material transfer could enhance the progress of the project, monitoring of the implementation of material transfer was conducted by both Indonesia and Japan counterparts. As stipulated in MTA, the requester should report the use of the transferred material to the provider. Monitoring was conducted during scientific meeting. Impact of the transferred material to the experiment was also evaluated and discussed in scientific meeting or other meeting.

d. Task distribution

- Determination of bottle neck of the process

Lead discovery process employs many complicated steps which involves many researchers. To keep the progress of the project smoothly, a meeting was held every week (so called weekly meeting) in BPPT. Each team responsible for specific task reported the progress of the project under its scope. Any problem arise from each team was discussed together with other team, so the solution could be proposed from comprehensive view of angle. Impact of this solution on solving the problem was evaluated and monitored in the following weekly meeting. Presentation material in this weekly meeting was stored in an easy-to-access location, so all researchers could review and monitor the progress of the project.

A scientific meeting was also held occasionally, typically 4~6 times a year. This meeting was attended by both Indonesia and Japan side and discussed progress and issues of the project. Bottle neck of the process was determined during the meeting, and some of countermeasures were discussed, in order to run the project smoothly. Similar with weekly meeting, all presentation material in this meeting was stored in the same way.

- Re-distribution of tasks to potential and capable counterparts

This project uses the advantages of biological diversity of microbes for searching lead compounds with antimalarial and antiamebic activity. In particular, microbial isolates from BPPT's microbial collection were used as the main resources for this project. Consequently, all microbial extracts were prepared by BPPT for all counterparts in this project.

At the same time, BPPT was also responsible for screening, as well as purification of active compound. Numbers of active extracts were proposed as the result of screening activity. With limiting number of researchers, however, it is difficult to follow up all of this screening result in purification step. This will significantly slow down the progress of the project.

During scientific meeting, countermeasures for overcoming this issue were discussed. One of them was to re-distribute the responsible tasks that were predetermined to each institute.

Initially, purification of active compound will be done in BPPT. During the meeting, this task would be also done in other institute. This, of course, would also consider the capability and potentiality of the institutes. Some consequences, including equipment and training issues, were also discussed. This countermeasure was shown to effectively improve the progress of the project.

3-2. Results of the Use of Lessons Learnt

There were 3 Indonesian institutes and 4 Japanese institutes involved in this project. Role of each institute was determined since the beginning of this project (e.g. described in R/D document). The project progress done by each institute was reviewed and evaluated in JCC Meeting and Scientific Meeting, which is attended by researchers (or representatives) from each institute. During project term, JCC meeting was held annually (usually in January or February), and scientific meeting was held more frequently, 4-6 times a year. Especially in scientific meeting, many issues were discussed to find solution for overcoming the issues. This meeting was so effective to keep running the project as planned. Moreover, the meeting also successfully promoted good communication and mutual understanding between all involved institutes, which is the key for successful collaborative research work

In addition to this, BPPT held a weekly meeting since 2016. In this meeting, progress of each research group was presented every week. Countermeasures for any issues from the team were also discussed. Such meeting was very effective for the project to maintain the progress of the project, so all of target stipulated in PDM could be achieved smoothly. This meeting also encouraged all of team members to train them self in term of presentation skill and ability, especially in English.

Similar to previous SATREPS project "Identification of Anti-hepatitis C Virus (HCV) Substances and Development of HCV and Dengue Vaccines" (project period February 2010 to February 2014), this project utilized Indonesian biological resources as sources for screening of active compounds for controlling infectious diseases. Learning from that, the project also managed biological resources used by the project so it could be protected and fully utilized. Material transfer between involved institutes was done under MTA. This project also learned from the

previous project that biorisk management was indispensable issue when handling pathogenic cells. To ensure the safety of the researchers, biosafety and biosecurity system was established and communicated to all team members. Establishment of SOPs in the lab helped the project to avoid contamination and experimental failure, which may lead to delay of the time for target achievement and unnecessary effort. SOPs were also useful for the project to obtaining reliable and traceable experimental data.

4. Lessons Learnt

a. Biosafety and biosecurity system is indispensable for ensuring the safety of researchers in the laboratory and for avoiding unnecessary contamination problem.
b. Solid and obeyable SOP is the key for obtaining trustworthy data during research.
c. Understanding related regulations may accelerate the achievement of the target.
d. Careful selection of local prominent vendor for delivering spec-matched items required for the project will also accelerate the achievement of the target.
e. Advancement of technology is part of successful drug development activities
f. Biological resources can be protected and fully utilized through MTA.
g. More efforts for improving efficiency are needed when working with limited resources.
h. Good communication and mutual understanding between all involved institutes is the key for successful collaborative research work.

IV. For the Achievement of Overall Goals after the Project Completion

1. Prospects to Achieve Overall Goal

Overall Goal:

a. Strengthen capacity building for Indonesian researchers and institutions
b. To reinforce international research collaboration
c. To increase added value of Indonesia bioresources especially for anti-malaria and anti-amebic drug discovery

This overall goal was described in Record of Discussion document (ANNEX 4.B, in Appendix 1 section I. Background). During implementation of this project, this overall goal was considered as the project overall goal by both Indonesia and Japan counterparts, and the goal was not changed until the end of the project.

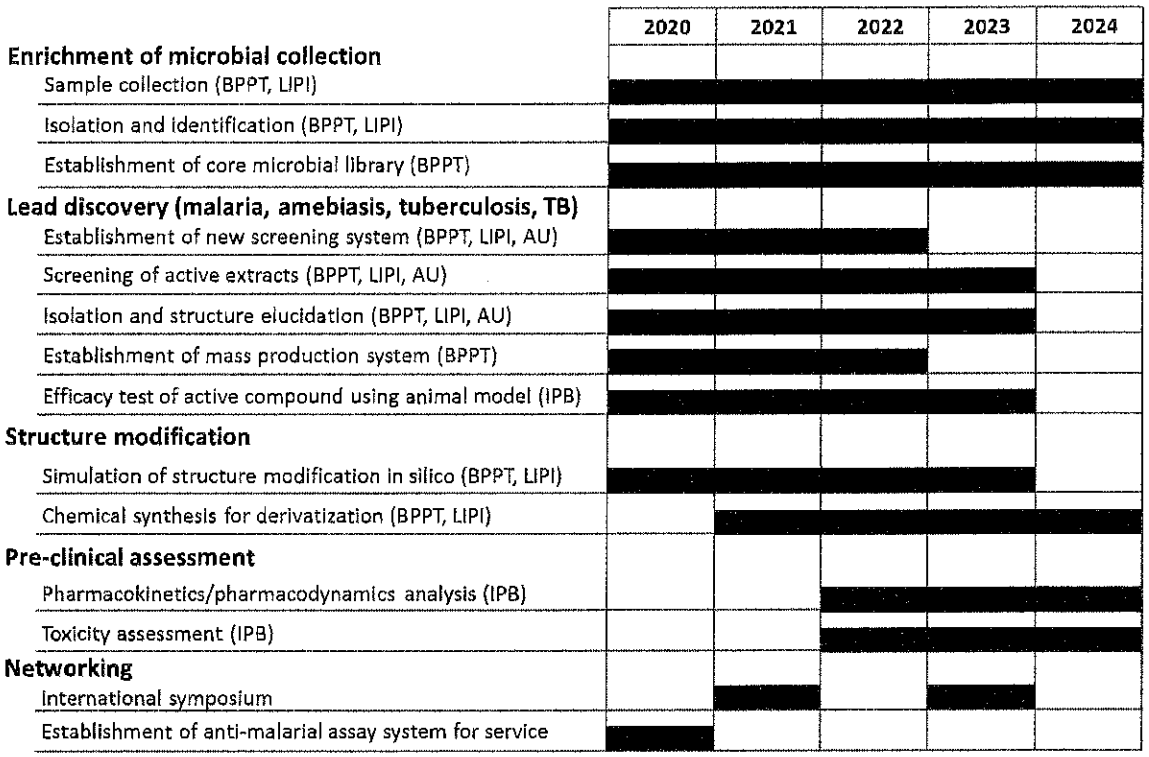
Prospects

There are several prospects for achieving overall goal as below.

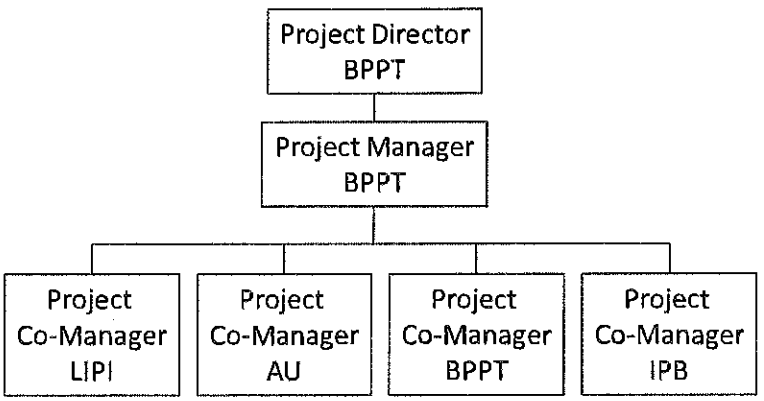
a. Capacity on isolation and identification of microbial isolates is improved
   This prospect will be realized through establishment of core microbial library composed from highly diverse microbial isolates.
b. Screening and assay system is implemented in Indonesian research institutes
   This prospect will be realized through development and implementation of new screening and assay system.
c. Potential active compounds are isolated and identified
   This prospect will be realized through structure modification for lowering toxicity level of the active compounds, and pre-clinical assessment of promising lead compounds
d. Research networks between research institutes in Indonesia and Japan is built and maintained
   This prospect will be realized through research collaboration with local and international research institutes.

2. Plan of Operation and Implementation Structure of the Indonesia Side to Achieve Overall Goal

Plan of Operation



Implementation Structure



3. Recommendations for the Indonesia Side

Based on experiences gained from the current SATREPS Project, there are several recommendations to be addressed to Indonesia Side for achieving of overall goals after project completion.

a. Microbial collection is a precious capital for Indonesia. It should be well

managed and fully utilized for the sake of wealth and prosperity of the people.

b. Since drug development requires time, sustainability is a key factor for successful drug development. Continuous support from top management and promoting drug development research activities in Indonesia is very important to sustain the program.
c. Strong research networks in drug development field among research institutes will accelerate the achievement of target. This could be realized by promoting natural resources based drug discovery research activities, promoting competency-based research network in Indonesia, and promoting Academic-Business-Government (A-B-G) networks for social implementation of research outputs.
d. Enhancing research environment in Indonesia will improve the quality of the achievement. This could be realized through maintaining and improving the quality of scientific discussion among researchers/ institutes.

4. Monitoring Plan from the End of the Project to Ex-post Evaluation

a. Core library construction
   Indicator: A core library composed from at least 1000 highly diversed microbial isolates is established.
   Plan:
   - Identification of 1000 microbial isolates is conducted
   - A system for managing core microbial library is established

b. Establishment of new screening system
   Indicator: At least 1 screening system for obtaining anti-infectious diseases (malaria/amebiasis/dengue/tuberculosis) is developed and implemented
   Plan:
   - A new screening system for obtaining anti-infectious diseases is developed
   - Screening for selective inhibitory activity of the extracts is conducted

c. Obtaining active compound with antimalaria/antiamebiasis/dengue/ tuberculosis activities
   Indicator: At least 1 active compound with antimalarial/antiamebiasis/anti

dengue/antituberculosis is obtained and the chemical structure is elucidated

Plan:

- Isolation and purification of active compound with inhibitory activity against target enzyme/proliferation of target pathogen are conducted

d. International symposium on drug development

Indicator: At least 1 international symposium is held.

Plan:

- An international symposium is arranged and held

ANNEX

ANNEX 1: Result of the Project

A. List of Dispatched Experts
B. List of Counterparts
C. List of Training

ANNEX 2: List of Products Produced by the Project

A. Standard Operating Procedures
B. Presentation Material in Scientific Meeting
C. Presentation Material in the JCC Meeting
D. Program Book of The 1st International Symposium on Natural Resources-based Drug Development
E. Program Book of The 2nd International Symposium on Natural Resources-based Drug Development

ANNEX 3: Project Design Matrix

A. Project Design Matrix version 0
B. Project Design Matrix version 1
C. Project Design Matrix version 2
D. Project Design Matrix version 3
E. Project Design Matrix version 4
F. Project Design Matrix version 5
G. Project Design Matrix version 7
H. Project Design Matrix version 8

ANNEX 4: Minutes of Meeting, Record of Discussion, Minutes of JCC Meeting

A. Minutes of Meeting
B. Record of Discussion
C. Minutes of The 1st JCC Meeting
D. Minutes of The 2nd JCC Meeting
E. Minutes of The 3rd JCC Meeting
F. Minutes of The 4th JCC Meeting
G. Minutes of The 5th JCC Meeting

ANNEX 5: Project Monitoring Sheet

A. Project Monitoring Sheet version 1
B. Project Monitoring Sheet version 2
C. Project Monitoring Sheet version 3
D. Project Monitoring Sheet version 4
E. Project Monitoring Sheet version 5
F. Project Monitoring Sheet version 6
G. Project Monitoring Sheet version 7
H. Project Monitoring Sheet version 8

ANNEX 1～5

ANNEX 1: Result of the Project

A. List of Dispatched Experts

No	Name	Affiliation	Period		Duration
			From	To	Days
1	Tomoyoshi NOZAKI	Tsukuba U	2015/7/25	2015/8/4	10
2	Kazuro SHIOMI	Kitasato U	2015/7/25	2015/7/31	6
3	Kenichi NONAKA	Kitasato U	2015/7/25	2015/8/7	13
4	Tomoyoshi NOZAKI	Tsukuba U	2015/12/14	2015/12/22	8
5	Daisaku TAKEMOTO	Kitasato U	2015/12/14	2015/12/22	8
6	Daniel INAOKA	Tokyo U	2015/12/14	2015/12/18	4
7	Tomoyoshi NOZAKI	Tsukuba U	2016/1/31	2016/2/3	3
8	Kazuro SHIOMI	Kitasato U	2016/1/31	2016/2/4	4
9	Atsuko MATSUMOTO	Kitasato U	2016/1/31	2016/2/17	17
10	Daniel INAOKA	Tokyo U	2016/1/25	2016/3/4	39
11	Azuma WATANABE	MicroBiopharm Japan	2016/1/31	2016/2/5	5
12	Tomoyoshi NOZAKI	Tsukuba U	2016/5/22	2016/5/26	4
13	Daisaku TAKEMOTO	Kitasato U	2016/4/18	2016/6/16	59
14	Tomoyoshi NOZAKI	Tsukuba U	2016/8/7	2016/8/13	6
15	Yukiko MIYAZAKI	Nagasaki U	2016/8/6	2016/9/10	35
16	Daniel INAOKA	Nagasaki U	2016/7/28	2016/9/12	46
17	Mihoko MORI	Kitasato U	2016/9/5	2016/9/26	21
18	Tomoyoshi NOZAKI	Tsukuba U	2016/11/14	2016/11/22	8
19	Daniel INAOKA	Nagasaki U	2017/1/12	2017/2/19	38
20	Yukiko MIYAZAKI	Nagasaki U	2017/1/14	2017/3/11	56
21	Tomoyoshi NOZAKI	Tsukuba U	2017/1/24	2017/1/31	7
22	Ratna Wahyuni	Tsukuba U	2017/1/24	2017/1/29	5
23	Kazuro SHIOMI	Kitasato U	2017/1/24	2017/1/28	4
24	Mihoko MORI	Kitasato U	2017/1/11	2017/1/29	18
25	Azuma WATANABE	MicroBiopharm Japan	2017/1/24	2017/1/28	4

26	Tomoyoshi NOZAKI	Tsukuba U	2017/3/14	2017/3/19	5
27	Mihoko MORI	Kitasato U	2017/3/22	2017/3/31	9
28	Mihoko MORI	Kitasato U	2017/5/9	2017/5/27	18
29	Toshiyuki TOKIWA	Kitasato U	2017/5/9	2017/5/14	5
30	Tomoyoshi NOZAKI	Tokyo U	2017/5/16	2017/5/23	7
31	Kazuyuki DOBASHI	Kitasato U	2017/5/21	2017/5/25	4
32	Michio YAMASHITA	Tokyo U	2017/5/21	2017/5/25	4
33	Kazuyuki DOBASHI	Kitasato U	2017/7/31	2017/8/25	25
34	Daniel INAOKA	Nagasaki U	2017/8/7	2017/8/26	19
35	Michio YAMASHITA	Tokyo U	2017/8/13	2017/9/9	27
36	Tomoyoshi NOZAKI	Tokyo U	2017/8/14	2017/8/24	10
37	Yukiko MIYAZAKI	Nagasaki U	2017/8/14	2017/8/23	9
38	Kazuro SHIOMI	Kitasato U	2017/8/20	2017/8/23	3
39	Azuma WATANABE	MicroBiopharm Japan	2017/8/20	2017/8/27	7
40	Tomoyoshi NOZAKI	Tokyo U	2017/10/10	2017/10/18	8
41	Daniel INAOKA	Nagasaki U	2017/11/10	2017/11/22	12
42	Takaya SAKURA	Nagasaki U	2017/11/10	2017/12/7	27
43	Kazuyuki DOBASHI	Kitasato U	2017/11/12	2017/12/8	26
44	Tomoyoshi NOZAKI	Tokyo U	2017/12/21	2017/12/29	8
45	Kazuyuki DOBASHI	Kitasato U	2018/1/8	2018/2/2	25
46	Mihoko MORI	Kitasato U	2018/1/23	2018/2/10	18
47	Tomoyoshi NOZAKI	Tokyo U	2018/1/25	2018/2/6	12
48	Daniel INAOKA	Nagasaki U	2018/1/27	2018/2/3	7
49	Takaya SAKURA	Nagasaki U	2018/1/27	2018/2/3	7
50	Kazuro SHIOMI	Kitasato U	2018/1/28	2018/2/2	5
51	Michio YAMASHITA	Tokyo U	2018/1/28	2018/2/24	27
52	Azuma WATANABE	MicroBiopharm Japan	2018/1/30	2018/2/4	5
53	Tomoyoshi NOZAKI	Tokyo U	2018/3/6	2018/3/15	9
54	Kazuyuki DOBASHI	Kitasato U	2018/4/18	2018/5/16	28
55	Takaya SAKURA	Nagasaki U	2018/5/6	2018/5/19	13

56	Mihoko MORI	Kitasato U	2018/5/7	2018/5/19	12
57	Katsuhiko ANDO	National Institute of Technology and Evaluation	2018/5/7	2018/5/18	11
58	Tomoyoshi NOZAKI	Tokyo U	2018/5/8	2018/5/16	8
59	Toru OKUDA	Hypha Genesis	2018/5/14	2018/5/19	5
60	Michio YAMASHITA	Tokyo U	2018/6/24	2018/7/21	27
61	Tomoyoshi NOZAKI	Tokyo U	2018/6/27	2018/7/4	7
62	Daniel INAOKA	Nagasaki U	2018/7/2	2018/7/14	12
63	Takaya SAKURA	Nagasaki U	2018/7/2	2018/7/14	12
64	Kazuyuki DOBASHI	Kitasato U	2018/7/25	2018/8/17	23
65	Mihoko MORI	Kitasato U	2018/8/22	2018/9/7	16
66	Katsuhiko ANDO	National Institute of Technology and Evaluation	2018/8/26	2018/9/6	11
67	Toru OKUDA	Hypha Genesis	2018/8/27	2018/9/1	5
68	Toshiyuki TOKIWA	Kitasato U	2018/8/28	2018/9/1	4
69	Tomoyoshi NOZAKI	Tokyo U	2018/9/9	2018/9/13	4
70	Tomoyoshi NOZAKI	Tokyo U	2018/9/26	2018/10/5	9
71	Kazuyuki DOBASHI	Kitasato U	2018/11/20	2018/12/13	23
72	Tomoyoshi NOZAKI	Tokyo U	2018/11/27	2018/12/7	10
73	Tomoyoshi NOZAKI	Tokyo U	2018/12/19	2018/12/25	6
74	Michio YAMASHITA	Tokyo U	2019/1/13	2019/2/10	28
75	Takaya SAKURA	Nagasaki U	2019/1/22	2019/2/1	10
76	Tomoyoshi NOZAKI	Tokyo U	2019/1/27	2019/2/5	9
77	Kazuro SHIOMI	Kitasato U	2019/1/28	2019/2/1	4
78	Azuma WATANABE	MicroBiopharm Japan	2019/1/28	2019/2/1	4
79	Mihoko MORI	Kitasato U	2019/1/28	2019/2/1	4

80	Kazuyuki DOBASHI	Kitasato U	2019/1/28	2019/2/16	19
81	Mihoko MORI	Kitasato U	2019/3/3	2019/3/9	6
82	Katsuhiko ANDO	National Institute of Technology and Evaluation	2019/3/3	2019/3/9	6
83	Tomoyoshi NOZAKI	Tokyo U	2019/3/27	2019/4/3	7
84	Mihoko MORI	Kitasato U	2019/4/20	2019/4/30	10
85	Katsuhiko ANDO	National Institute of Technology and Evaluation	2019/4/21	2019/4/30	9
86	Tomoyoshi NOZAKI	Tokyo U	2019/4/21	2019/5/1	10
87	Kazuyuki DOBASHI	Kitasato U	2019/6/9	2019/6/29	20
88	Michio YAMASHITA	Tokyo U	2019/6/16	2019/7/6	20
89	Mihoko MORI	Kitasato U	2019/7/2	2019/7/12	10
90	Katsuhiko ANDO	Tokyo U	2019/7/7	2019/7/12	5
91	Toru OKUDA	Hypha Genesis	2019/7/7	2019/7/12	5
92	Tomoyoshi NOZAKI	Tokyo U	2019/8/22	2019/8/29	7
93	Mihoko MORI	Kitasato U	2019/8/22	2019/8/31	9
94	Tetsuo SHIBATA	Nagoya Institute of Technology	2019/8/22	2019/8/25	3
95	Katsuhiko ANDO	Tokyo U	2019/8/26	2019/8/31	5
96	Toru OKUDA	Hypha Genesis	2019/8/26	2019/8/31	5
97	Daniel INAOKA	Nagasaki U	2019/10/6	2019/10/12	6
98	Takaya SAKURA	Nagasaki U	2019/10/6	2019/10/12	6
99	Tomoyoshi NOZAKI	Tokyo U	2019/10/7	2019/10/11	4
100	Kazuro SHIOMI	Kitasato U	2019/10/7	2019/10/11	4
101	Azuma WATANABE	MicroBiopharm Japan	2019/10/7	2019/10/15	8

102	Mihoko MORI	Kitasato U	2019/10/7	2019/10/11	4
103	Kazuyuki DOBASHI	Kitasato U	2019/10/7	2019/11/2	26
104	Michio YAMASHITA	Tokyo U	2019/10/7	2019/11/2	26
105	Tetsuo SHIBATA	Nagoya Engineering U	2019/10/7	2019/10/11	4
106	Kenichiro SUZUKI	Tokyo Agriculture U	2019/10/7	2019/10/11	4
107	Hiroyuki OSADA	Institute of Physical and Chemical Research	2019/10/7	2019/10/11	4
108	Choo Yeun Mun	Malaya U	2019/10/7	2019/10/11	4
109	Kei KATSUNO	GHIT Fund	2019/10/7	2019/10/9	2
110	Arif Nulkant	Tokyo U	2019/10/7	2019/10/14	7
111	Tomoyoshi NOZAKI	Tokyo U	2019/12/8	2019/12/11	3
112	Tomoyoshi NOZAKI	Tokyo U	2019/12/24	2019/12/26	2
113	Mihoko MORI	Kitasato U	2020/1/3	2020/1/12	9
114	Azuma WATANABE	MicroBiopharm Japan	2020/1/6	2020/1/10	4
115	Daniel INAOKA	Nagasaki U	2020/1/6	2020/1/11	5
116	Takaya SAKURA	Nagasaki U	2020/1/6	2020/1/11	5
117	Kazuyuki DOBASHI	Kitasato U	2020/1/6	2020/1/11	5
118	Michio YAMASHITA	Tokyo U	2020/1/7	2020/1/11	4
119	Tomoyoshi NOZAKI	Tokyo U	2020/1/7	2020/1/10	3
120	Kazuro SHIOMI	Kitasato U	2020/1/7	2020/1/10	3

ANNEX 1: Result of the Project

B. List of Counterparts

No	Name	Affiliation	Position
1	Soni Solistia Wirawan	BPPT	Deputy Chairperson, Project Director
2	Agung Eru Wibowo	BPPT	Director, Project Manager
3	Agus Supriyono	BPPT	Researcher
4	Amila Pramisandi	BPPT	Researcher
5	Anis Herliyanti Mahsunah	BPPT	Researcher
6	Anna Safarrida	BPPT	Researcher
7	Avi Nurul Oktaviani	BPPT	Researcher
8	Chaidir	BPPT	Researcher
9	Danang Waluyo	BPPT	Program Head, Project Co-manager
10	Dian Japany Puspitasari	BPPT	Researcher
11	Diana Dewi	BPPT	Researcher
12	Dyah Noor Hidayati	BPPT	Researcher
13	Eka Siska	BPPT	Researcher
14	Endah Dwi Hartuti	BPPT	Researcher
15	Erwahyuni E. Prabandari	BPPT	Researcher
16	Evita Chrisnayanti	BPPT	Researcher
17	Kiki Rizkia Afrianti	BPPT	Researcher
18	Kristiningrum	BPPT	Researcher
19	Kurnia Agustini	BPPT	Researcher
20	Nadia Adipratiwi	BPPT	Researcher
21	Nuki Bambang Nugroho	BPPT	Researcher
22	Nurlaila	BPPT	Researcher
23	Qarii Ainaya	BPPT	Researcher
24	Sasmito Wulyoadi	BPPT	Researcher
25	Suryani	BPPT	Researcher
26	Suyanto	BPPT	Researcher
27	Tarwadi	BPPT	Researcher

28	Titin Ariyani	BPPT	Researcher
29	Maria Inge Lusida	AU	Director, Project Co-manager
30	Achmad Fuad Hafid	AU	Researcher
31	Aty Widyawaruyanti	AU	Researcher
32	Dwi Peni Kartikasari	AU	Researcher
33	Lidya Tumewu	AU	Researcher
34	Myrna Adianti	AU	Researcher
35	Ratna Wahyuni	AU	Researcher
36	Ade Lia Putri	LIPI	Researcher
37	Arif Nurkanto	LIPI	Researcher
38	Atit Kanti	LIPI	Head of InaCC, Project Co-manager
39	Muhammad Ilyas	LIPI	Researcher
40	Puspita Lisdiyanti	LIPI	Researcher
41	Dewi Wulansari	LIPI	Researcher
42	Huda Shalahudin Darusman	IPB	Director, Researcher
43	Suryo Saputro	IPB	Researcher
44	Loeki Enggar Fitri	BU	Researcher
45	Rivo Yudhinata Brian Nugraha	BU	Researcher
46	Tomoyoshi Nozaki	UTokyo	Professor, Chief Advisor
47	Michio Yamashita	UTokyo	Researcher
48	Ghulam Jeelani	UTokyo	Researcher
49	Katsuhiko Ando	UTokyo	Researcher
50	Toru Okuda	UTokyo	Researcher
51	Daniel Ken Inaoka	NagasakiU	Associate Professor, Researcher
52	Wang Xinying	NagasakiU	Researcher
53	Youichi Matsuo	NagasakiU	Researcher
54	Kota Mochizuki	NagasakiU	Researcher
55	Takaya Sakura	NagasakiU	Researcher
56	Yukiko Miyazaki	NagasakiU	Researcher
57	Kazuro Shiomi	KitasatoU	Professor, Researcher

58	Mihoko Mori	KitasatoU	Researcher
59	Kazuyuki Dobashi	KitasatoU	Researcher
60	Atsuko Matsumoto	KitasatoU	Researcher
61	Kenichi Nonaka	KitasatoU	Researcher
62	Toshiyuki Tokiwa	KitasatoU	Researcher
63	Azuma Watanabe	MBJ	Researcher
64	Kumiko Tsukui	NIID	Researcher
65	Herbert Santos	NIID	Researcher

ANNEX 1: Result of the Project

C. List of Tranings

No	Name	Affiliation	Destination	Period of Training		Topic	Length (day)
				From	To		
1	AMILA PRAMISANDI	Biotech Center	Kitasato U	2015/5/9	2015/6/10	Purification :Basic Method	32
2	MYRNA ADIANTI SUBIANTO	Airlangga U	Natinal Institute of Infectious Diseases	2015/5/9	2015/7/10	Enzymatic Assay for Ameba: Basic Method	62
3	RATNA WAHYUNI ZAINURI	Airlangga U	Natinal Institute of Infectious Diseases	2015/5/9	2015/7/10	Culture for Ameba: Basic Method	62
4	ASTUTIATI NURHASANAH	Biotech Center	Tokyo U	2015/5/9	2015/7/10	Culture for Malaria: Basic Method	62
5	SISKA ANDRINA KUSUMATUTI	Biotech Center	Tokyo U	2015/5/9	2015/7/10	Enzymatic Assay for Malaria: Basic Method	62
6	ENDAH DWI HARTUTI	Biotech Center	Tokyo U	2015/5/9	2015/6/10	Purification :Basic Method	32
7	AMILA	Biotech	Kitasato U	2015/6/15	2015/7/16	Purification :Advanced	31

	PRAMISANDI	Center				Method	
8	ASTUTIATI NURHASANAH	Biotech Center	Tokyo U	2015/9/23	2015/10/23	Culture for Malaria: Advanced Method	30
9	PRABANDARI ERWAHYUNI ENDANG	Biotech Center	Tokyo U	2015/9/23	2015/10/23	Enzymatic Assay for Malaria: Advanced Method	30
10	MAHSUNAH ANIS HERLIYATI	Biotech Center	Kitasato U	2015/9/23	2015/10/23	Purification :Advanced Method/Specific Method	30
11	KARTIKASARI DWI PENI	Airlangga U	Natinal Institute of Infectious Diseases	2015/9/26	2015/11/26	Screening Technique	61
12	RATNA WAHYUNI ZAINURI	Airlangga U	Natinal Institute of Infectious Diseases	2016/1/16	2016/3/17	Amebic Assay general	61
13	KARTIKASARI DWI PENI	Airlangga U	Natinal Institute of Infectious Diseases	2016/5/8	2016/6/18	Screening Technique	41
14	DIANA DEWI	Biotech Center	Kitasato U	2016/10/01	2016/10/29		28

15	EKA SISKA	Biotech Center	Kitasato U	2016/10/01	2016/10/29		28
16	DANANG WALUYO	Biotech Center	Natinal Institute of Infectious Diseases	2016/11/05	2016/12/17		42
17	PRABANDARI ERWAHYUNI ENDANG	Biotech Center	Natinal Institute of Infectious Diseases	2016/11/05	2016/12/17		42
18	NURLAILA	Biotech Center	Kitasato U	2016/11/06	2016/12/03		27
19	MAHSUNAH ANIS HERLIYATI	Biotech Center	Kitasato U	2016/11/06	2016/12/03		27
20	KARTIKASARI DWI PENI	Biotech Center	Natinal Institute of Infectious Diseases	2017/01/02	2017/02/11		40
21	AMILA PRAMISANDI	Biotech Center	Kitasato U	2016/10/22	2016/11/05		14
22	ENDAH DWI HARTUTI	Biotech Center	Nagasaki U	2017/6/11	2017/7/14	Expression of enzyme, Purification, Activity measurement	33

						of enzyme of plasmodium falciparum	
23	PRABANDARI ERWAHYUNI ENDANG	Biotech Center	Tokyo U	2017/9/17	2017/10/14	Production of enzyme for screening of antiparasitic active compounds	27
24	NURLAILA	Biotech Center	Kitasato U	2017/9/17	2017/10/14	Purification of active compounds	27
25	EKA SISKA	Biotech Center	Kitasato U	2017/10/8	2017/12/2	Structure elucidation of active compounds	55
26	NADIA ADIPRATIWI	Biotech Center	Nagasaki u/Tokyo U	2017/10/29	2017/12/23	Training on Amebic/MRC-5 cell Culture and Cell screening technique for Malaria	55
27	KRISRININGRUM	Biotech Center	Tokyo U	2017/10/29	2017/12/23	Isolation and Identification of microbes	55
28	MYRNA ADIANTI SUBIANTO	Airlangga U	Tokyo U	2018/1/7	2018/1/29	Cell Toxicity assay and new enzyme assays for antiamebic coumpound discovery	22

29	DANANG WALUYO	Biotech Center	Tokyo U	2018/2/27	2018/3/24	Cell Toxicity test of active compounds/ in vivo assay of actibve compounds	25
30	EKA SISKA	Biotech Center	Kitasato U	2018/9/1	2018/9/29	Structure elucidation of active compound for antiamebic coumpound discovery	28
31	LYDIA TUMEWU	Airlangga U	Tokyo U	2018/9/1	2018/9/30	Structure elucidation of active compound	29
32	AVI NURUL OCTAVIANI	Biotech Center	Kitasato U	2018/9/1	2018/12/22	Identification of Microbes	112
33	EVITA CHRISNAYANTI	Biotech Center	Kitasato U	2018/9/23	2018/10/20	Purification of Anti Parastic Agent	27
34	KRISRININGRUM	Biotech Center	Kitasato U	2018/10/30	2018/12/1	Isolation and Identification of Microbes	32
35	HIKATUL ILMI	Airlangga U	Nagasaki U	2018/11/3	2018/12/2	Cell toxicity assay and new enzyme assays for anti-Malaria discovery	29
36	DANANG WALUYO	Biotech Center	Tokyo U	2018/11/10	2018/12/8	Cell toxicity test of active compounds/in	28

						vivo assay of active compounds	
37	FARIDA IFADOTUNNIKMAH	Airlangga U	Tokyo U	2019/2/9	2019/3/11	Purification of anti-amebic compounds	30
38	MELINDA LAURENSIA	Biotech Center	Kitasato U	2019/6/29	2019/7/27	Purification of antimalarial active compound	28
39	DANANG WALUYO	Biotech Center	Tokyo U	2019/7/15	2019/8/11	Development of target for screening of anti-tuberculosis agents	27
40	FARIDA IFADOTUNNIKMAH	Airlangga U	Tokyo U	2019/7/6	2019/8/3	Purification of anti-amebic compounds	28
41	DEWI WULANSARI	LIPI	Tokyo U	2019/7/6	2019/7/20	Drug Discovery of Anti Parasite	14
42	TITIN ARIYANI	Biotech Center	Nagasaki U	2019/8/30	2019/9/28	Isolation of P.falciparum mitochondria and measurement of respiration chain reaction activity	29

43	DEFI KARTIKA SARI	Airlangga U	Tokyo U	2019/11/4	2019/12/14	Establishment of enzyme screening protocols	40
44	KRISRININGRUM	Biotech Center	Kitasato U	2019/9/23	2019/10/5	Isolation and Identification of microbes	12
45	LYDIA TUMEWU	Airlangga U	Tokyo U	2019/11/16	2019/12/14	Structure elucidation of active compound	28
46	PRABANDARI ERWAHYUNI ENDANG	Biotech Center	Tokyo U	2019/10/26	2019/11/23	Screening of inhibitors from microbial extracts for PfDCPK	28
47	SURYANI	Biotech Center	Kitasato U	2019/9/28	2019/10/5	Identfication of interesting actinomycetes	7
48	PUTRI BERNAWATI	Biotech Center	Kitasato U	2019/11/3	2019/11/30	Purification of antimalarial active compound	27
49	HUAD SHALAHUDIN DARUSMAN	IPB	Tokyo U	25/02/2020	14/03/2020	Drug safety and toxicty Examinationon Laboratory Animals	18
50	SURYO SAPUTRO	IPB	Tokyo U	25/02/2020	14/03/2020	Drug safety and toxicty Examinationon Laboratory Animals	18

51	RATNA WAHYUNI ZAINURI	Airlangga U	Tokyo U	2016/4/1	2020/3/31	PHD Course	1460
52	AMILA PRAMISANDI	Biotech Center	Kitasato U	2017/4/1	2020/3/31	PHD Course	1095
53	KARTIKASARI DWI PENI	Airlangga U	Tokyo U	2017/4/1	2020/3/31	PHD Course	1095

BalaiBioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

MINI SCALEEXTRACTS PRODUCTION (ACTINOMYCETES)

Reference :

Reviewed and approved by :

Date last modification

MINI SCALE EXTRACTS PRODUCTION (ACTINOMYCETES)

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for mini scale actinomycetesextract production byfermentation. The extractsareprepared for first screening purpose.One piece of agar culture disk (taken from agar culture or frozen stock) is inoculated to 30 mL sterile main culture medium (**C**, **A9, A14 and A21** medium) in 250 ml Erlenmeyer flask, then incubate it in a shaker incubator at 28 ^{o}C, agitation 220 rpm for 7 days (harvest time). Six mL of fermentation broth is transferredinto a 15 mL centrifuge tube, then extracted by 6 mL butanol. The tube is shakenfor 15 minutes, 300 strokes/minutes,then centrifugeat 3000 rpm for 10 minutes. One mL of supernatant is transferred into a 96-deep well plate. The extract is driedusing vacuum concentrator for 8-16 h at 45 ^{o}C. The extracts are kept at 4-8^{o}C.

B. METHOD

Required media and solutions (see Appendix):

- Actinomycetes cultures on ISP2 agar
- C medium
- A9 medium
- A14 medium
- A21 medium
- Butanol

Required equipment:

- 15 mL centrifuge tubes
- 250 mL Erlenmeyer flask
- 1 mL micropipette
- 96 deep-well plate
- Shaker incubator
- Vacuum concentrator
- Laboratory centrifuge
- 4^{o}C refrigerator

Procedure:

1. Take one piece of agar disk (Ø ± 7 mm) from a reviving cultureor frozen stock vial using a steriletoothpick andtransferinto two 250 mL Erlenmeyer flask, each containing **C, A9, A14** and **A2**1 medium. Do this step aseptically in a biosafety cabinet.
2. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 7 days (harvest time).
3. Transfer 6 mL broth into 15 mL centrifuge tube.
4. Add 6 mL of butane into the tube.

5. Mix the tube by a reciprocal shaker for 15 minutes, 300 stroke/minutes
6. Centrifuge the tube at 3000 rpm, 10 minutes, r.t.
7. Transfer 1 mL of the supernatant into a 96-deep well plate (5 replication). Be careful to put the extract in the right well.
8. Dry the extract using vacuum concentrator for 8-16 h at 45 ^{o}C.
9. Cover the plate using plate mat and plastic cover.
10. Put label on the cover and side of the plate. Be careful to put the right label.
11. Store the plate at 4-8^{o}C.

Appendix :

A. Preparation of C medium (composition in 1000 mL):

Rice powder	20 g (Rose Brand)
Glucose	20 g
Soybean meal	20 g
Yeast extract	5 g
NaCl	2,5g
$CaCO_3$*	3,2g
Mineral solution*	2 mL
pH	7,4

Mineral solution composition (in 500 mL)

$CuSO_4.5H_2O$	1,25 g
$MnCl_2.4H_2O$	1,25 g
$ZnSO_4.7H_2O$	1,25 g

* $CaCO_3$and mineral solution must be added after pH adjustment

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients except $CaCO_3$ and mineral solution, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.4 using NaOH 5 M or HCl 2 N.
4. Add $CaCO_3$ and mineral solution into the medium.
5. Add distilled water up to 1000 mL using a measuring cylinder.
6. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL.Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
7. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
8. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

B. Preparation of A21 medium (composition in 1000 mL):

Glucose	5 g
Tryptone	2 g
Calcium carbonate	4 g
Sodium chloride	2 g
Monobasic potassium phosphate	0,5 g
pH 7.0	

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.0 using NaOH 5 M or HCl 2 N.

4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL.Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

C. Preparation of A9 medium (composition in 1000 mL):

Tomato paste	24 g
Dextrin	24 g
$CoCL_2.6H_2O$	0.006 g
Yeast extract	12 g
pH 7.0	

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.0 using NaOH 5 M or HCl 2 N.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

D. Preparation of A14 medium (composition in 1000 mL)

Glucose	4 g
Yeast extract	4g
Malt extract	4 g
pH 7.8	

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.0 using NaOH 5 M or HCl 2 N.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL.Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature.
8. Note: Prepare the medium 1 day before using.

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

MINI SCALE EXTRACTS PRODUCTION (FUNGI)

Reference :

Reviewed and approved by :

Date last modification

MINI SCALE EXTRACTS PRODUCTION (FUNGI)

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for mini scale fungi extract production by fermentation. The extracts are prepared for first screening purpose. One piece of agar culture disk (taken from agar culture frozen stock) is inoculated to 30 mL sterile main culture medium (F and F15 medium) in 250 mL Erlenmeyer flask, then incubate it in a shaker incubator at 25 ^{o}C, agitation 220 rpm for 7 days (harvest time). Six 6 mL of broth fermentation is transferred into 15 mL centrifuge tube, then extracted by 6 mL butanol. The tube is shaken for 15 minutes; 300 stroke/minutes then centrifuge at 3000 rpm for 10 minutes. One mL of supernatants is transferred into a 96-deep well plate. The extract is dried using vacuum concentrator for 8-16 h at 45 ^{o}C. The extracts are kept at 4-8 ^{o}C.

B. METHOD

Required media and solutions (see Appendix):

- Fungi culture on PDA medium
- F medium
- F15 medium
- Butanol

Required equipment:

- 15 mL centrifuge tubes
- 250 mL Erlenmeyer flask
- 1 mL micropipette
- 96 deep-well plate
- Shaker incubator
- Vacuum concentrator
- Laboratory centrifuge
- 4^{o}C refrigerator

Procedure:

1. Take one piece of agar disk (Ø ± 7 mm) from a reviving culture or frozen stock vial using a sterile toothpick and transfer into two 250 mL Erlenmeyer flask, each containing **F** and **F15** medium in working volume 30 mL. Do this step aseptically in a biosafety cabinet.
2. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 7 days (harvest time).
3. Transfer 6 mL broth into 15 mL centrifuge tube.
4. Add 6 mL of butanol into the tube.
5. Mix the tube by a reciprocal shaker for 15 minutes, 300 stroke/minutes
6. Centrifuge the tube at 3000 rpm, 10 minutes, r.t.
7. Transfer 1 mL of the supernatant into a 96-deep well plate (5 replication). Be careful to put the extract in the right well.

8. Dry the extract using vacuum concentrator for 8-16 h at 45 ^{o}C.
9. Cover the plate using plate mat and plastic cover.
10. Put label on the cover and side of the plate. Be careful to put the right label.
11. Store the plate at 4-8 ^{o}C.

Appendix :

Preparation of F medium (composition in 1000 mL):

Rice powder	20 g (Rose Brand)
Glucose	10 g
Soybean meal	20 g
KH2PO4	1 g
MgSO4.7H2O	0,5 g
Distilled water	1000 mL

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients, then dissolve in distilled water using a stirrer.
3. No need adjust pH.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature

Note: Prepare the medium 1 day before using.

Preparation of F15 medium (composition in 1000 mL):

Glucose	30 g
Glycerol	20 g
Dextrin	10 g
Malt extract	10 g
Yeast extract	20 g
Tryptone	1 g
NH_4NO_3	1 g
KH_2PO_4	1 g
Distilled water	1000 mL
pH	6,5

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients except $CaCO_3$ and mineral solution, then dissolve in distilled water using a stirrer.
3. Adjust pH to 6.5 using NaOH 5 M or HCl 2 N.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

MEDIUM SCALE EXTRACTS PRODUCTION (ACTINOMYCETES)

Reference :

Reviewed and approved by :

Date last modification

MEDIUM SCALE EXTRACTS PRODUCTION (ACTINOMYCETES)

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for medium scale actinomycetes extract production by fermentation. The extracts are prepared for second screening purpose. One piece of agar culture disk (taken from agar culture or frozen stock) is inoculated to 30 mL sterile main culture medium (**C** and **A21** medium) in 250 ml Erlenmeyer flask, then incubate it in a shaker incubator at 28 ^{o}C, agitation 220 rpm for 7 days (harvest time). Six mL of fermentation broth is transferred into a 15 mL centrifuge tube, then extracted by 6 mL butanol. The tube is shaken for 15 minutes, 300 strokes/minutes, then centrifuge at 3000 rpm for 10 minutes. One mL of supernatant is transferred into a 96-deep well plate. The extract is dried using vacuum concentrator for 8-16 h at 45 ^{o}C. The extracts are kept at 4-8 ^{o}C.

B. METHOD

Required media and solutions (see Appendix):

- Actinomycetes cultures on ISP2 agar
- C medium
- A21 medium
- Butanol

Required equipment:

- 15 mL centrifuge tubes
- 250 mL Erlenmeyer flask
- 1 mL micropipette
- 96 deep-well plate
- Shaker incubator
- Vacuum concentrator
- Laboratory centrifuge
- 4^{o}C refrigerator

Procedure:

1. Take one piece of agar disk (Ø ± 7 mm) from a reviving culture or frozen stock vial using a sterile toothpick and transfer into two 250 mL Erlenmeyer flask, each containing **C** and **A2**1 medium. Do this step aseptically in a biosafety cabinet.
2. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 7 days (harvest time).
3. Transfer 6 mL broth into 15 mL centrifuge tube.
4. Add 6 mL of butanol into the tube.
5. Mix the tube by a reciprocal shaker for 15 minutes, 300 stroke/minutes
6. Centrifuge the tube at 3000 rpm, 10 minutes, r.t.
7. Transfer 1 mL of the supernatant into a 96-deep well plate (5 replication). Be careful to put the extract in the right well.

8. Dry the extract using vacuum concentrator for 8-16 h at 45 ^{o}C.
9. Cover the plate using plate mat and plastic cover.
10. Put label on the cover and side of the plate. Be careful to put the right label.
11. Store the plate at 4-8 ^{o}C.

Appendix :

Preparation of C medium (composition in 1000 mL):

Rice powder	20 g (Rose Brand)
Glucose	20 g
Soybean meal	20 g
Yeast extract	5 g
NaCl	2,5 g
$CaCO_3$*	3,2 g
Mineral solution*	2 mL
pH	7,4

Mineral solution composition (in 500 mL)

$CuSO_4.5H_2O$	1,25 g
$MnCl_2.4H_2O$	1,25 g
$ZnSO_4.7H_2O$	1,25 g

* $CaCO_3$ and mineral solution must be added after pH adjustment

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients except $CaCO_3$ and mineral solution, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.4 using NaOH 5 M or HCl 2 N.
4. Add $CaCO_3$ and mineral solution into the medium.
5. Add distilled water up to 1000 mL using a measuring cylinder.
6. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
7. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
8. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

Preparation of A21 medium (composition in 1000 mL):

Glucose	5 g
Tryptone	2 g
Calcium carbonate	4 g
Sodium chloride	2 g
Monobasic potassium phosphate	0,5 g
pH 7.0	

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.0 using NaOH 5 M or HCl 2 N.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.

7. Use the medium after cooled down at room temperature.
Note: Prepare the medium 1 day before using.

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

MEDIUM SCALE EXTRACTS PRODUCTION (FUNGI)

Reference :

Reviewed and approved by :

Date last modification

MEDIUM SCALE EXTRACTS PRODUCTION (FUNGI)

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for medium scale fungi extract production by fermentation. The extracts are prepared for second screening purpose. One piece of agar culture disk (taken from agar culture or frozen stock) is inoculated to 30 mL sterile main culture medium (**F** and **F15** medium) in 250 ml Erlenmeyer flask, then incubate it in a shaker incubator at 28 ^{o}C, agitation 220 rpm for 7 days (harvest time). Six mL of fermentation broth is transferred into a 15 mL centrifuge tube, then extracted by 6 mL butanol. The tube is shaken for 15 minutes, 300 strokes/minutes, then centrifuge at 3000 rpm for 10 minutes. One mL of supernatant is transferred into a 96-deep well plate. The extract is dried using vacuum concentrator for 8-16 h at 45 ^{o}C. The extracts are kept at 4-8 ^{o}C.

B. METHOD

Required media and solutions (see Appendix):

- Fungi culture on PDA medium
- F medium
- F15 medium
- Butanol

Required equipment:

- 15 mL centrifuge tubes
- 250 mL Erlenmeyer flask
- 1 mL micropipette
- 96 deep-well plate
- Shaker incubator
- Vacuum concentrator
- Laboratory centrifuge
- 4^{o}C refrigerator

Procedure:

1. Take one piece of agar disk (Ø ± 7 mm) from a reviving culture or frozen stock vial using a sterile toothpick and transfer into two 250 mL Erlenmeyer flask, each containing **F** and **F15** medium. Do this step aseptically in a biosafety cabinet.
2. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 7 days (harvest time).
3. Transfer 6 mL broth into 15 mL centrifuge tube.
4. Add 6 mL of butanol into the tube.
5. Mix the tube by a reciprocal shaker for 15 minutes, 300 stroke/minutes
6. Centrifuge the tube at 3000 rpm, 10 minutes, r.t.

7. Transfer 1 mL of the supernatant into a 96-deep well plate (5 replication). Be careful to put the extract in the right well.
8. Dry the extract using vacuum concentrator for 8-16 h at 45 ^{o}C.
9. Cover the plate using plate mat and plastic cover.
10. Put label on the cover and side of the plate. Be careful to put the right label.
11. Store the plate at 4-8 ^{o}C.

Appendix :

Preparation of F medium (composition in 1000 mL):

Rice powder	20	g (Rose Brand)
Glucose	10	g
Soybean meal	20	g
KH2PO4	1	g
MgSO4.7H2O	0,5	g
Distilled water	1000	mL

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients, then dissolve in distilled water using a stirrer.
3. No need adjust pH.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature

Note: Prepare the medium 1 day before using.

Preparation of F15 medium (composition in 1000 mL):

Glucose	30	g
Glycerol	20	g
Dextrin	10	g
Malt extract	10	g
Yeast extract	20	g
Tryptone	1	g
NH_4NO_3	1	g
KH_2PO_4	1	g
Distilled water	1000	mL
pH	6,5	

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients except $CaCO_3$ and mineral solution, then dissolve in distilled water using a stirrer.
3. Adjust pH to 6.5 using NaOH 5 M or HCl 2 N.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

LARGE SCALE EXTRACTS PRODUCTION (ACTINOMYCETES)

Reference :

Reviewed and approved by :

Date last modification

LARGE SCALE EXTRACTS PRODUCTION (ACTINOMYCETES)

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for large scale actinomycetes extract production by fermentation. The extracts are prepared for purification purpose. One piece of agar culture disk (taken from agar culture or frozen stock) is inoculated to 30 mL sterile vegetative culture medium (**C** medium) in 250 ml Erlenmeyer flask, then incubate it in a shaker incubator at 28 ^{o}C, agitation 220 rpm for 3 days. Two percent of inoculum vegetative are transferred to main culture medium (**C** and **A21** medium), then incubate it in a shaker incubator at 28 ^{o}C, agitation 220 rpm for 4 days (harvest time). All of fermentation broth (5000 mL) is transferred into a 15000 mL pan, then extracted by 5000 mL butanol. Stire the solution for 1 hours, agitation 500 rpm with mixer. Then centrifuge the moixer at 3000 rpm for 10 minutes. Transfer the supernatant into rotary rotavavor flask and then evavorate its with rotavavor.

B. METHOD

Required media and solutions :

- Actinomycetes cultures on ISP2 agar plate
- C medium
- A21 medium
- Butanol

Required equipment:

- 15 mL centrifuge tubes
- 250 mL Erlenmeyer flask
- 1 mL micropipette
- 96 deep-well plate
- Shaker incubator
- Rotavavor
- Mixer

Procedure:

1. Take one piece of agar disk (Ø ± 7 mm) from a reviving culture or frozen stock vial using a sterile toothpick and transfer into two 250 mL Erlenmeyer flask, containing sterile **C** medium. Do this step aseptically in a biosafety cabinet.
2. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 3 days.
3. Transfer 2% v/v inoculum vegetative to main sterile culture medium (C and A21 medium).
4. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 4 days (harvest time).
5. Pour the broth fermentation to 15000 mL pan and then
6. Add 5000 mL of butanol into the pan.
7. Strire the solution with mixer for 60 minutes, agitation 500 rpm.

8. Centrifuge the mixer at 3000 rpm, 10 minutes.
9. Transfer the supernatant into rotary evavorator flask.
10. Evavorate the supernatant.

Appendix :

Preparation of C medium (composition in 1000 mL):

Rice powder	20 g (Rose Brand)
Glucose	20 g
Soybean meal	20 g
Yeast extract	5 g
NaCl	2,5 g
$CaCO_3$*	3,2 g
Mineral solution*	2 mL
pH	7,4

Mineral solution composition (in 500 mL)

$CuSO_4.5H_2O$	1,25 g
$MnCl_2.4H_2O$	1,25 g
$ZnSO_4.7H_2O$	1,25 g

* $CaCO_3$ and mineral solution must be added after pH adjustment

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients except $CaCO_3$ and mineral solution, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.4 using NaOH 5 M or HCl 2 N.
4. Add $CaCO_3$ and mineral solution into the medium.
5. Add distilled water up to 1000 mL using a measuring cylinder.
6. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
7. Sterilize the medium using autoclave at 121 ^oC for 15 minutes.
8. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

Preparation of A21 medium (composition in 1000 mL):

Glucose	5 g
Tryptone	2 g
Calcium carbonate	4 g
Sodium chloride	2 g
Monobasic potassium phosphate	0,5 g
pH 7.0	

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients, then dissolve in distilled water using a stirrer.
3. Adjust pH to 7.0 using NaOH 5 M or HCl 2 N.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^oC for 15 minutes.

7. Use the medium after cooled down at room temperature.
Note: Prepare the medium 1 day before using.

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

LARGE SCALE EXTRACTS PRODUCTION (FUNGI)

Reference :

Reviewed and approved by :

Date last modification

LARGE SCALE EXTRACTS PRODUCTION (FUNGI)

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for large scale fungi extract production by fermentation. The extracts are prepared for purification purpose. One piece of agar culture disk (taken from agar culture or frozen stock) is inoculated to 30 mL sterile vegetative culture medium (F medium) in 250 ml Erlenmeyer flask, then incubate it in a shaker incubator at 28 ^{o}C, agitation 220 rpm for 3 days. Two percent of inoculum vegetative are transferred to main culture medium (**F** and F15 medium), then incubate it in a shaker incubator at 28 ^{o}C, agitation 220 rpm for 4 days (harvest time). All of fermentation broth (5000 mL) is transferred into a 15000 mL pan, then extracted by 5000 mL butanol. Stire the mixer for 1 hours, agitation 500 rpm with mixer. Then centrifuge at 3000 rpm for 10 minutes. Transfer the supernatant into rotary rotavavor flask and then evavorate its with rotavavor.

B. METHOD

Required media and solutions :

- Fungi culture on PDA medium
- F medium
- F15 medium
- Butanol

Required equipment:

- 15 mL centrifuge tubes
- 250 mL Erlenmeyer flask
- 1 mL micropipette
- 96 deep-well plate
- Shaker incubator
- Rotavavor
- Mixer

Procedure:

1. Take one piece of agar disk (Ø ± 7 mm) from a reviving culture or frozen stock vial using a sterile toothpick and transfer into two 250 mL Erlenmeyer flask, containing sterile **C** medium. Do this step aseptically in a biosafety cabinet.
2. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 3 days.
3. Transfer 2% v/v inoculum vegetative to main sterile culture medium (C and A21 medium).
4. Incubate using shaker incubator at 28 ^{o}C, 220 rpm for 4 days (harvest time).
5. Pour the broth fermentation to 15000 mL pan and then
6. Add 5000 mL of butanol into the pan.
7. Strire the solution with mixer for 60 minutes, agitation 500 rpm.

8. Centrifuge the mixer at 3000 rpm, 10 minutes.
9. Transfer the supernatant into rotary evavorator flask.
10. Evavorate the supernatant.

Appendix :

Preparation of PDA medium :

Potatoes Dextrose Agar 39 g,
Demineral water 1000 mL
Mixed well PDA and demineral water in Erlenmeyer flask. Medium are autoclaved at 121 ^{o}C, 15 minutes.

Preparation of F medium (composition in 1000 mL):

Rice powder 20 g
Glucose 10 g
Soybean meal 20 g
KH_2PO_4 1 g
$MgSO_4.7H_2O$ 0,5 g
Distilled water 1000 mL

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients except $CaCO_3$ and mineral solution, then dissolve in distilled water using a stirrer.
3. No need adjust pH.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 250 mL Erlenmeyer flask, each 30 mL. (for vegetative medium) / 500 mL Erlenmeyer flask, each 100 mL.(for main medium) Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.
7. Use the medium after cooled down at room temperature.

Note: Prepare the medium 1 day before using.

Preparation of F15 medium (composition in 1000 mL):

Glucose 30 g
Glycerol 20 g
Dextrin 10 g
Malt extract 10 g
Yeast extract 20 g
Tryptone 1 g
NH_4NO_3 1 g
KH_2PO_4 1 g
Distilled water 1000 mL
pH 6,5

1. Put 800 mL distilled water into a beaker glass.
2. Weigh all ingredients except $CaCO_3$ and mineral solution, then dissolve in distilled water using a stirrer.
3. Adjust pH. To 6.5 using NaOH 5 M or HCl 2 N.
4. Add distilled water up to 1000 mL using a measuring cylinder.
5. Distribute the medium into 500 mL Erlenmeyer flask, each 100 mL.(for main medium) Plug with cotton plug, and cover the plug using 100 mL plastic beaker.
6. Sterilize the medium using autoclave at 121 ^{o}C for 15 minutes.

7. Use the medium after cooled down at room temperature.
Note: Prepare the medium 1 day before using.

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

EXTRACTION OF BROTH FERMENTATION

Reference :

Reviewed and approved by :

Date last modification

EXTRACTION OF BROTH FERMENTATION

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure of extraction of broth fermentation. The broth fermentation is 5000 mL. Solvent for extraction is buthanol. Add 5000 mL broth with 5000 mL Buthanol (1:1). Stire the solution for 1 hours, 500 rpm. Centrifuge 6000 rpm, 10 minutes. Take a supernatant and dried its.

B. METHOD

Required solutions (see Appendix):
A. Broth fermentation
B. Organic solvent (Buthanol)

Required equipment:

- Stirer
- Big Pan
- Evavoprator
- Centrifuge
- Nalgen botlles

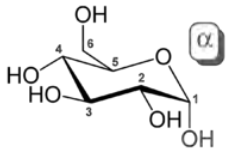
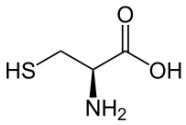
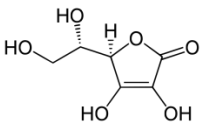
Procedure:

1. Take 5000 mL broth fermentation and put into big pan
2. Add 5000 mL a buthanol
3. Stire at 500 rpm, 1 hours.
4. Pour the solution to nalgen bottles and
5. Centrifuge at 6000 rpm, 10 minutes.
6. Take a supernatant and dried its.

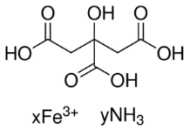
Standard Operating Procedure **Preparation of BI and BIS Medium for *Entamoeba histolytica* Cell Culture**	**No:**	**Eh-1 (Rev. 1)**
	Page:	**1/4**

- **General precaution**
  1. Don't share reagents
  2. Always check the storage time for each solutions
  3. Aliquot reagents
  4. Put label vial, tube, bottle individually (name (preparation, open), date, content)

- **Composition of BI Medium (in 880 mL or 440 mL DW)**

Reagent	Description	Amount (g) (for 880 mL)	Amount (g) (for 440 mL)	Storage	Brand
Biosate	Animal-origin, mixed hydrolysate comprised of 65% pancreatic digest of casein and 35% yeast extract	30.0	15.0	4°C	BD
D-glucose		10.0	5.0	RT	Nacalai
Sodium chloride	NaCl	2.0	1.0	RT	Wako
Potassium phosphate	KH_2PO_4	0.6	0.3	RT	Sigma
Dipotassium hydrogenphosphate	K_2HPO_4	1.0	0.5	RT	TCI
L-cysteine		1.3	0.65	RT	Sigma
L-ascorbic acid		0.2	0.1	4°C	Wako

Prepared by	Verified by	Date of use
Danang W		June 26th, 2019

Ferric ammonium citrate		0.0228	0.0114	RT	Sigma

Store BI medium in -30°C

- **Composition of BIS Medium**

Composition	Volume (mL)	Remark
BI medium	88 mL	
ABS (adult bovine serum, heat-inactivated)	15 mL	Inactivate ABS (500 mL) by heating up at 56°C for 3 h with occasional mixing by inverting the bottle, aliquot 45 mL into 50 mL tube, then store at -30°C. Thaw ABS prior to use into 4°C, and store the thawed ABS in 4°C.
Vitamin solution	2 mL	Refer to SOP "Preparation of Vitamin Solution for *Entamoeba histolytica* cell culture". Protect the solution from light by covering the tube using aluminum foil in closed refrigerator.

Store BIS medium in 4°C

- **Preparation of BI medium**

1. Take out Biosate and Ascorbic acid from 4°C storage
2. Add about 800 mL (or 350 mL) DW and a stir bar into 1 L beaker.
3. Weight all reagents above one by one and dissolve by stirring. If it is difficult to weigh Ferric ammonium citrate due to small amount, weigh 10x amount in 1.5 mL tube, add 1 mL DW, then add 100 μL into medium.
4. Adjust pH to 6.8 by 5 N of NaOH solution
5. Filtrate the medium by kimwipe/kimtowel or filtrating paper, then transfer into 1 L measuring cylinder.
6. Add DW up to the desired total volume (880 mL or 440 mL). Dispense 88 mL into 100 mL bottle.
7. Write "BI" and date of preparation on the bottle label, then sterilize by autoclave immediately (121°C, 20 min).
8. After cooled down, store the medium at -30°C
9. Use the frozen medium up to 6 months.

- **Preparation of BIS medium**

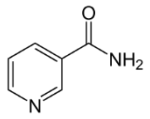
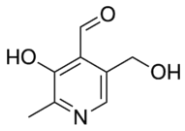
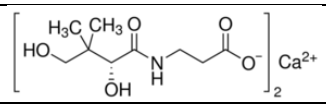
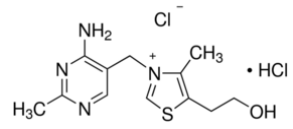
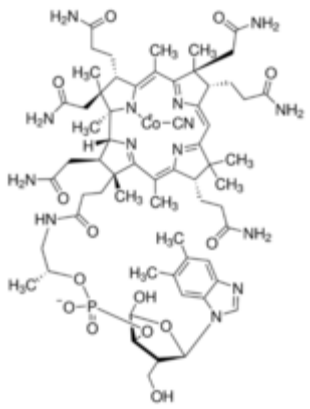
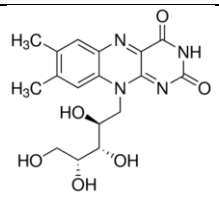
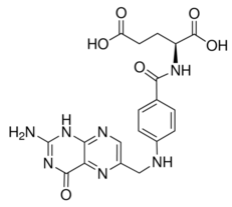
1. Thaw BI medium into room temperature. (It may be better to thaw by placing the frozen

BI medium in 4°C for overnight.)

2. Add 15 mL heat-inactivated ABS (Adult Bovine Serum) and 2 mL vitamin solution, then mix well. Never use vitamin solution that has been exposed light and been stored for more than 3 months.
3. Write “BIS”, the date of preparation, and name on the bottle label.
4. BIS medium is ready for use. Store BIS medium at 4°C**. DO NOT USE BIS medium more than 10 days.** (Try to minimize exposing the medium to air during storage).

Standard Operating Procedure **Preparation of Vitamin Solution for *Entamoeba histolytica* Cell Culture**	**No:**	**Eh-2**
	Page:	**1/2**

- **Composition of Vitamin Solution**

Reagent	Description	Amount (for 200 mL)	Storage	Brand
Niacinamide		45 mg	4°C	Wako 141-01202
Pyridoxal hydrochloride		4 mg	-20°C	Wako 160-23651
Calcium pantothenate		23 mg	4°C	Sigma-Aldrich C8731-100G
Thiamine hydrochloride (Vit B_1)		5 mg	4°C	Wako 203-00851
Vitamin B_{12}		1.2 mg	4°C	Wako 244-00344
Riboflavin (vit B_2)		7 mg	4°C	Wako 180-00171
Folic acid		5.5 mg	4°C	Wako 062-01801

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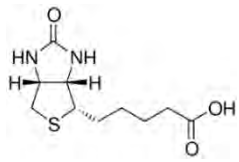
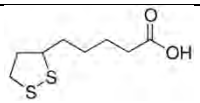
D-biotin (vit B_7)		2 mg	4°C	Wako 029-08713
DL-6,8-thioctic acid (DL-α-lipoic acid)		1 mg	4°C	Sigma T1395-1G
Tween-80		500 mg	RT	Merck

Store vitamin solution at -30°C (stock) or 4°C (being use)

- **Preparation of vitamin solution**

1. Weigh reagents below, then dissolve them in 25 mL DW. Regard this solution as Solution #1. Keep the weighing within 10% deviation.
   - Niacinamide 45 mg
   - Pyridoxal hydrochloride 4 mg
   - Calcium panthothenate 23 mg
   - Thiamine hydrochloride 5 mg
   - Vitamin B_{12} 1.2 mg
2. Weigh 7 mg of riboflavin, then dissolve in 10 mL DW. Drop 1 N (ca. 3 drops) of NaOH until completely dissolved, then add DW up to 45 mL. Regard this solution as Solution #2.
3. Weigh 5.5 mg of folic acid, the dissolve in 10 mL DW. Drop 1 N (ca. 2 drops) of NaOH until completely dissolved, then add DW up to 45 mL. Regard this solution as Solution #3.
4. Weigh 2 mg of D-biotin, then dissolve in 45 mL DW. Regard this solution as Solution #4.
5. Mix Solution #1~#4 above (total 160 mL). Regards this solution as Solution #A.
6. Weigh 1 mg of DL-6,8-thioctic acid, dissolve in 5 mL of 95% ethanol, then add 500 mg Tween-80. Add DW up to 30 mL. Regard this solution as Solution #B.
7. Mix Solution #A and #B, then add DW up to 200 mL.
8. Sterilize by filtration (0.2 μm), then aliquot 10 mL in 15 mL tube. Keep the tube from light by covering with aluminum foil. Write “Vitamin Sol.”, date of preparation, and name of prepared person. Store at -30°C in closed refrigerator.
9. Thaw the solution into room temperature prior to use. Write the date of thawing. Store the thawed solution at 4°C in closed refrigerator. **DO NOT USE the thawed solution after 3 months.**

Prepared by	Verified by	Date of use
Danang W		Feb 5th, 2017

Standard Operating Procedure **Sub-culturing *Entamoeba histolytica* for cell maintenance**	**No:**	**Eh-3 (Rev.1)**
	Page:	**1/1**

- **Sub-culturing *Entamoeba histolytica* for cell maintenance**

1. Sterilize a Pyrex glass tube (dia. 13 mm, height 100 mm, volume 7 mL) with screw cap by autoclave (121°C, 20 min), then dry up in an oven (50°C). Loose the cap during sterilization.
2. Warm BIS medium at 37°C. Fill the tube with 6.5 mL of BIS medium aseptically.
3. Observe *E.histolytica* culture under inverted microscope. Use only exponentially growth phase cell (typically, 50-70% confluence culture). If dead or floating cells are not observed, continue to no.6.

   Note: In case dead or floating cells are observed, mix medium of the culture by inverting the tube 2-3 times (to gently resuspend precipitation of dead cells), then continue to no.4.
4. Discard the medium by decantation gently.
5. Add 6.5 mL of fresh BIS medium into the tube. Invert the tube 2-3 times.
6. Put the tube in ice for 10 min. Flick the tube 2-3 times and mix the medium by inverting the tube to release the attached cells. Observe under inverted microscope to make sure that the cells had been detached from the inner surface of the tube.
7. Mix the medium well, then transfer (typically) 100-1000 μL of the culture into new tube containing fresh BIS medium, then adjust the total medium volume to 6.5 mL with BIS medium.
8. Incubate the new culture at 35.5°C in tilt position. Observe the cell growth every day. The culture should be in exponential growth phase within 3-4 days.

   Note: Use only cells in exponential growth phase for passage.

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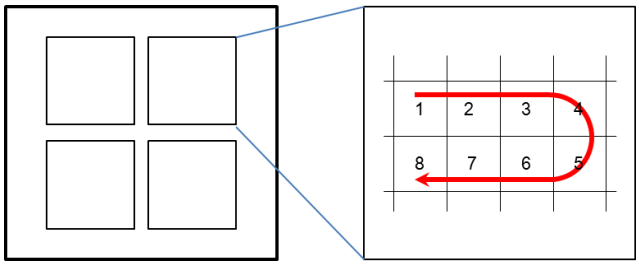
- **General caution**
  - **Caution on attaching tip to multichannel pipette**
  - **Picture of how the tip will put into the well for removing medium**
  - **Describe the way of removing / adding the medium/reagent/extract: and time to do for each plate.**
- **Preparation of *Entamoeba histolytica* cell**

1. Prepare *E.histolytica* culture (incubated at 35.5°C) in exponential growth phase. Typically, 1 plate needs 1-2 tubes.
2. Change medium with the new one and detach cell.
   - Resuspend the precipitation by inverting the tube 2-3 times, then remove the medium by decantation.
   - Add 6.5 mL of fresh pre-warmed BIS medium, then detach the cell by put the tube on ice for 10 min.
3. Transfer the culture into 50 mL tube. Count the cell density using hemocytometer.
   - During counting, put the cell on ice
   - Prepare a hemocytometer (Eosinophil counter, 0.200 mm deep, 1/16 mm^2) with its cover
   - Add 20 µl of 10x diluted culture into 2 grids of hemocytometer (duplicate)
   - Observe under microscope and count the cell on all 8 areas in both 2 grids. Consider the rule of counting the cell (do not count cell overlaid on right and bottom line of the area)
   - Calculate the concentration of cell as follow.

$$Concentration\left(\frac{cells}{\mu l}\right) = \frac{\frac{(\text{number of total cell in 2 grids})}{2}}{2.5} \times (\text{dilution rate})$$



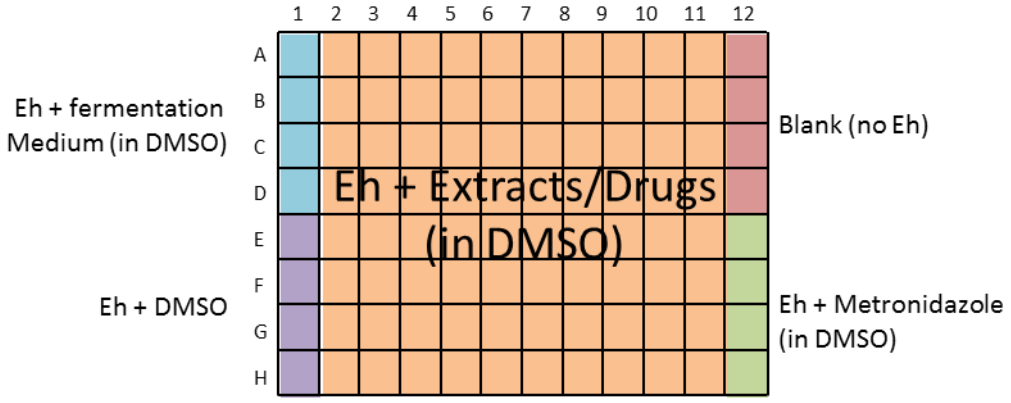
4. Adjust cell density to 5000 cells/200 µL (2.5 x 10^4 cells/mL) with BIS medium. Make this culture at least 22 mL for each 96-well plate.

   Note: Adjust number of cells used for assay if the growth is not good, or reading of sample with cell only (negative control) is not sufficient (Signal-to-background ratio is

less than 3).

5. Transfer the cell into tray, then put 200 µl of the culture into each well of sterile 96-well culture plate (with lid). Be sure to resuspend the cell well by occasionally mix the culture well by shaking the tray gently before transferring the cell.
6. Place the plate in a small anaerobic box (accommodate 4-5 plates), add 4 mesh of anaerocult (pre-immersed with 17.5 mL DW), close the lid, then incubate at 35.5°C for 1 h.

Typical plate layout (Observe the influence of fermentation medium to the cell growth before determining the controls).



- Blank (no Eh) = for checking the contamination of Eh medium
- Eh + Metronidazole (100 µM) (or no cell) = for control positive of inhibition
- Eh + fermentation medium = for control negative of inhibition
- Eh + DMSO = for checking effect of DMSO to Eh

- **Preparation of extract/sample (avoid cross contamination during mixing)**

1. Add dried extract with 40 µL of 100% DMSO, then mix using mixer/vortex and sonicator until the extract is completely dissolve.
2. Transfer the extracts into v-bottom plate as necessary.
3. Store extract/sample in -30°C.

- **Adding extracts/drugs (describe how to remove the medium using pipette more detail)**

1. Prepare extract. If the extract is stored in freezer, thaw in room temperature, mix using mixer/vortex for 3~5 min at 1000~1200 rpm before use.
2. Take out 96-well plate (containing 5000 cells/well preincubated at 35.5°C for 1 h) from

anaerobic box.

3. Transfer 0.4 μL extract solution into cell
4. Place the plate in a small anaerobic box, add 4 mesh of anaerocult (pre-immersed with 17.5 mL DW), close the lid, then incubate at 35.5°C for 2 d.

- **Quantitation**

1. Prepare 1x Opti-MEM medium in 50 mL tube (9 ml for each plate). Warm the medium at 37°C.
2. Remove medium from the plate using multichannel pipette
3. Add 1 mL of 10x WST-1 into 1x Opti-MEM medium, then dispense 90 μL into each well immediately
4. Incubate the plate at 35.5°C for 20 min.
5. Read A450 using plate reader (end-point measurement, without mixing)

- **Calculation** (review the formula based on controls that are used, describe the criteria of acceptable result based on Z-factor and S/B ratio).

1. Calculate the inhibition rate as follow

$$\text{Inhibition rate (\%)} = 100 \times \frac{(A450_{Eh+DMSO} - A450_{Blank}) - (A450_{Eh+extract} - A450_{Blank})}{(A450_{Eh+DMSO} - A450_{Blank})}$$

2. Calculate Z-factor as follow

Z-factor for screening

$$Z_{screening} = 1 - \frac{3(\sigma_{pos\ control} + \sigma_{neg\ control})}{|\mu_{Eh+Metronidazole pos\ control} - \mu_{neg\ control}|}$$

3. Calculate Signal-to-background (S/B) ratio

$$S/B\ \% = \frac{\mu_{neg\ control}}{\mu_{pos\ control}} \times 100\%$$

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Standard Operating Procedure Preparation of *Entamoeba histolytica* Freeze Stock and Recovery the Cell from Frozen Stock	No:	Eh-5 (Rev1)
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- **Preparation of cryoprotective solution**

Component	Amount (mL)	
	Axenic culture (clone 6)	Xenic culture (mixed culture)
DMSO	1.2	0.9
50% Sucrose	0.9	0.7
Vitamin mix solution	2.0	-
ABS	2.0	2.0
BI medium	3.9	6.4
Total	**10**	**10**

Prepare the solution aseptically

- **Preparation of *Entamoeba histolytica* freeze stock (no need to change medium before detaching the cell if the cell is in good condition: no pellet, no floating cell)**

1. Prepare *E.histolytica* culture (incubated at 35.5°C) with <80% confluence, no pellet. **Never used cells in stationary phase.**
2. Discard medium by decantation, then add 5 mL newly prepared BIS medium.
3. Detach the cell by putting on ice 10 min.
4. Transfer all culture into 50 mL tube.
5. Centrifuge the tube at 1000 rpm for 3 min.
6. Discard supernatant, then add 1 mL of cryoprotectant solution. Homogenize the cell by pipetting.
7. Transfer the mixture into 2 cryotubes each 500 μL.
8. Store the tubes in “Bicell” container or Styrofoam box that is prechilled in -30°C, than store the box in -80°C for at least 24 h.
9. Revive 1 tube using reviving method below to check the viability of the cell.
10. If the cell is revived normally, transfer the tubes into liquid nitrogen tank. If not, discard the stock and make new series of freeze stock

- **Reviving *Entamoeba histolytica* frozen stock**

1. Prepare and pre-warm 2 tubes (6 mL) containing recovery medium (for clone6: 6 mL freshly prepared BIS with 10% additional serum) at 35.5°C.
2. Take 1 vial from liquid nitrogen tank/freezer, thaw the tube immediately by warming gently or on 37°C water bath for 2 min.
3. Add 500 μL recovery medium into the vial **SLOWLY, DROP BY DROP**.

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4. Transfer 500 μL from the vial into one of 6 mL tube (containing recovery medium) **SLOWLY, DROP BY DROP** by attaching the pipet tip to inner wall of the tube. Repeat this until all culture in the vial is transferred into 6 mL tube.
   Note: Consider to use sterile blue tip with wide edge (cut edge) when transferring the culture
5. Incubate the tube at 35.5°C for 2 hours on **stand position** (to attach the cell on the bottom of the tube).
6. Observe the cell under microscope, the cell should attach at the bottom of the tube.
7. Discard medium, change with new pre-warmed 6.5 mL recovery medium.
8. Incubate the tube at 35.5°C for 24 hours on stand position.
9. Check the cell growth and any possible contamination every day until 1 week.

Standard Operation Procedure
Cell Culture

Reagents

- Culture medium : DMEM
- Fetal Bovine Serum (FBS)
- Penicillin-Streptomycin Solution (antibiotics)
- Phosphate buffered saline (-) (PBS without Ca and Mg)
- 0.25% Trypsin-EDTA
- Labbanker (cell stock solution)

Procedure

Before experiment

1. Wear a laboratory coat
2. Wear gloves and mask
3. Disinfect the gloves by 70% ethanol
4. Open an incubator (don't speak!) and check the volume of water in the tray
5. Take out a flask from the incubator and observe cell by microscopy
6. Passage when cells are 70-100% confluent → Picture of the cell will be attached (all cells)
7. Turn on UV light in a safety cabinet and keep for 15 minutes
8. Check the volume of fluid waste in the aspirator (if it is over the maximum line, discard it, wash the tank, and add hypochlorous acid)
9. Turn on blower, light, and aspirator of safety cabinet
10. Open a gas cock
11. Disinfect inside of the safety cabinet by 70% ethanol
12. Put pipetmans, media, PBS, and trypsin into safety cabinet

Preparation of culture medium

1. Take out bottles of FBS and Pen/Strep from -30°C and keep at 4°C to melt them
2. Next day, inactive FBS in 56°C water bath for 30 minutes
3. Divide FBS into 10 tubes (50 mL for each) and Pen/Strep into 20 tubes (5 mL for each)

4. Take out 55 mL medium culture (DMEM) from a medium bottle (445 mL remaining)
5. Add 50 mL FBS and 5 mL of Pen/Strep (500 mL total contain 10% FBS, 1% P/S)
6. Keep the medium bottle at 4°C
7. Store other tubes of FBS and Pen/Strep at -30°C

Passage (in the case of T75 flask) (use half volume for T25 flask)

1. Warm up medium and PBS (-) at 37°C water bath for 15 minutes (don't warm up Trypsin/EDTA!)
2. Aspirate media in a flask
3. Add 5 mL of PBS (-) into the flask and rinse cells to wash and remove any FBS in the residual culture media
4. Aspirate the PBS
5. Add 1 mL of Trypsin/EDTA to cover the cell on the bottom of the flask
6. Roll the flask gently to ensure trypsin contact with all cells and pace it in 37°C incubator for 1-2 minutes
7. As soon as cells have detached, add 4 mL culture media into the flask (FBS in media inactive the trypsin) and collect this cell suspension into a 15 mL tube
8. Centrifuge the tube at 1000 x g for 5 minutes
9. Aspirate the supernatant and add 5 mL fresh media into the tube
10. Break cell pellet by pipetting well
11. Place a part of the cell suspension into a new flask and also add fresh media. Total culture 10 mL (0.5 mL cell suspension + 9.5 mL fresh medium)
12. Incubate the culture in incubator 37°C, 5% CO2
13. Depend on cell lines, the culture will be confluence within 3-4 days incubation.

Preparation of frozen stocks

1. Use remaining cell suspension from passage
2. Take 10 µL of cell suspension into hemocytometer (C-chip)
3. Count the cell number of suspension (count for 3 big square)
    - Calculate average of cell number from 3 different squares.
    - Calculate the concentration of cell suspension using formula below

      Concentration = (average of cell number from 3 different squares x 10^4 x dilution rate) cells/mL

- Do not forget to multiple by the dilution rate

4. Put 1 x 10^6 cells into 15 mL tubes
5. Centrifuge the tube at 1000 x g for 5 minutes
6. During this centrifuge, write cell name, cell number, date and own name on cryo-tube
7. Aspirate the supernatant and add 500 µL of cell stock reagent into the tube
8. Transfer the solution to a cryo-tube
9. Put the tube into a styrofoam box and store it at -80°C overnight
10. Next day, move the tube into liquid nitrogen

Waking up frozen cell stocks

1. Warm up water bath at 37°C
2. Put 4.5 mL medium (DMEM) into a 15 mL tube
3. Take out a cryo-tube containing cell from liquid nitrogen
4. As soon as possible, soak the cryo-tube in 37°C water bath
5. Wait for the cells to melt with shaking
6. As soon as possible, add the cells into 15 mL tube containing 4.5 mL medium
7. Centrifuge at 1000 x g for 5 minutes
8. Aspirate the supernatant and add 5 mL fresh media into the tube
9. Transfer all of the cell suspension into T25 flask
10. Incubate 37°C, 5% CO_2
11. Next day, observe the cells by microscope whether the cell is attached to the surface of the flask or not. If the number of floating cells is significant, change the medium.

Standard Operation Procedure

Cytotoxicity Assay

Reagents

- Culture medium : DMEM
- Phosphate buffered saline (-) (PBS without Ca and Mg)
- Cell counting kit 8
- 96 well plate

Procedure

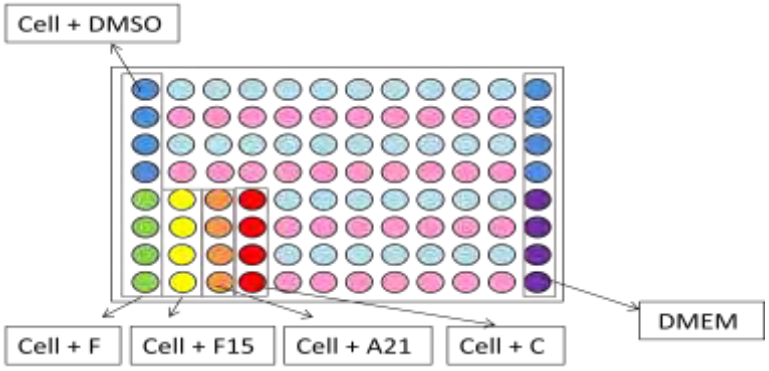
1. Count the cell number of remaining cell suspension from passage
   - Calculate average of cell number from 3 different squares.
   - Calculate the concentration of cell suspension using formula below

     Concentration = (average of cell number from 3 different squares x 10^4 x dilution rate) cells/mL
   - Do not forget to multiple by the dilution rate
2. Prepare cell suspension with the following concentration using fresh medium

   Cell number for DLD1 : 1.25 x 10^5cells/mL (2.5x 10^4cells/200µL)

   Cell number for Panc1, T47D, HepG2: 0.5 x 10^5cells/mL (1x 10^4cells/200µL)
3. Put 100 µL of the cell suspension to each well of 96 well plate
4. Place the plate in 37°C incubator for 24 hour (over night)
5. Add 0.4 µL of each extracts (dissolved in 100% DMSO) for each well
   - Prepare well without cells as blank
   - Do the assay in duplo (n=2) or triplicate (n=3)

   Typical 96-well plate layout for cytotoxicity assay



6. Place the plate in incubator (37°C, 5% CO_2) incubator for 48 hours
7. Removed medium from the plate by aspirator (using 1 mL serology pipette with yellow tip). Change the tip for each sample.
8. Wash the cell by 100 µL of PBS for each well
9. Aspirate the PBS
10. Add 1 mL of CCK-8 into tray, then add 10 mL of DMEM medium on it. Mix well.
11. Add 100 µL of DMEM containing CCK-8 into each well
12. Place the plate in 37°C incubator for 3 hours
13. Measure the absorbance of each well at 450 nm by a plate reader
14. Calculate the survival rate as follow for each extracts and their medium

Survival rate (%) =[(As – Ab) / (Ac – Ab)] x 100

As : Abs of sample well

Ac : Abs of control well (DMSO)

Ab : Abs of Positive control well (Staurosporine)

Note:

1. Cut off: survival rate < 50% is regarded as toxic.
2. First test should be done against DLD1. Secondary test will be done by testing the extracts that passed the 1st test by the other cells. Non-toxic extracts will be regarded as extracts those are passed all the tests.

Cytotoxicity assay under hypoxia and nutrient-free condition

Reagents

- DMEM or RPMI-1640 + 10% FBS + 1% Penicillin/Streptomycin (normal medium)
- DMEM, no glucose, no glutamine + 1% Penicillin/Streptomycin (nutrient-free medium)
- Phosphate buffered saline (-) (PBS)
- Cell counting kit 8
- 96 well plate

Procedure

1. Count the cell number of remaining cell suspension from passage
2. Add fresh normal medium to the cell suspension
   Cell number for DLD-1: 2.5x 10^5cells/mL
3. Put 100 µL of the cell suspension to each well of 96 well plate
4. Place the plate in 37°C incubator (5% CO_2) for 24 hour (over night)
5. Remove the medium and wash the cells by 100 µL of PBS(-)
6. Remove PBS(-) and add 100 µL nutrient-free medium to each well
7. Add 0.4 µL of each extracts (dissolved in 100% DMSO) for each well
8. Place the plate in 37°C incubator (1% O_2, 5% CO_2) for 48 hours
9. Remove medium from the plate and wash the cell by 100 µL of PBS for each
10. Aspirate the PBS
11. Add 100 µL of fresh normal medium and 10 µL of CCK-8 solution
12. Place the plate in 37°C incubator (5% CO_2) for 3 hour
13. Measure the absorbance of each well at 450 nm by a plate reader

Survival rate (%) = (As – Ab) / (Ac – Ab) x 100

As : Abs of sample well

Ac : Abs of control well (medium + DMSO)

Ab : Abs of blank well (only medium)

GENERAL CONSIDERATION FOR MICROBIAL ISOLATION FROM SOIL SAMPLES

Sampling and sample handling

1. Soil samples should be taken from rhizosphere (5-20 cm from surface).
2. Sample should be taken from inhabited location under a plant, preferably from a plant that is commonly used by local people for treating specific diseases.
3. After return to lab, soil samples should be stored in refrigerator (typically 4-8°C).
4. Sample identity (environment, local name, GPS location, etc.) should be recorded and managed.

Microbial isolation

5. The media and solution for microbial isolation should be freshly prepared before the isolation process.
6. The pH of the soil sample should be checked first before the isolation process performed.
7. The identity of the samples should be ensured and record in the form for isolation, including: no. of the sample, sampling site and its geographical condition
8. The isolator should write data below on isolation plate
   - date of isolation
   - name of the isolator
   - code of the isolation method
   - code of the sampling area
   - sample number
   - dilution factor
9. Observed and checked the plate periodically for possible contamination.
10. Isolate code will be generated after all isolation process has finished with the following format.

[type of microbe].[isolation method]-[sampling location].[sample number].[isolate number]

Example: a.WM.PP.54-6

- Code of the isolate : **f** for fungi and **a** for actinomycetes
- Code of the isolation method : **see appendix,** ex. **WM** for sucrose gradient and centrifugation method

- Code of the sampling area : **see from the expedition form,** ex. **PP** for Puspiptek Area
- Number of the sample : ex. **54**
- Number of the isolates : ex. Isolates number **6** from the sample number 54

11. The final result of the isolation should be recorded in the **Isolation Result Form**

Appendix:

Isolation Method		**Code**
Actinomycetes:		
	Dry method : - HV agar - Water Proline Agar	 DM WP
	Sonication Method	SD
	Wet Heating Method	WH
	Dry Heating Method	DH
	High Heating Method	HH
	Sucrose Gradient and Centrifugation Method	WM
	Isolation Using Zhang's Starch Soil Extract Agar	ZM
	Isolation Using Soil Extract Agar	SE
Fungi:		
	Isolation using LCA medium	LCA
	Isolation using MARB medium	MARB
	Isolation using MARB+0.4% LiCl	LiCl
	Isolation using OGA medium	OGA
	Isolation using MEA medium	ME
	Isolation using SEA medium	SE

PROCEDURE FOR ACTINOMYCETES ISOLATION

SUMMARY

No.	Method	Pretreatment	Isolation Media	Isolation Code
1.	Isolation Using HV Agar	No treatment	HV Agar	DM
2.	Isolation Using Water Proline Agar	No treatment	Water Proline Agar	WP
3.	Isolation Using Zhang's Starch Soil Extract Agar	No treatment	Zhang's Starch Soil Extract Agar	ZM
4.	Isolation Using Soil Extract Agar	No treatment	Soil Extract Agar	SE
5.	Sonication Method	Air dried soil is suspended and diluted using Winogradsky Solution (WS), weak sonication for 2 minutes, at RT	HV agar	SD
6.	Wet Heating Method	Air dried soil suspension are sonicated for 2 minutes and heated at 60 ^{o}C in waterbath, for 30 minutes	HV agar	WH
7.	Dry Heating Method	Air dried soil heated at 120 ^{o}C, 1 hour	HV agar	DH
8.	High Heating Method	Air dried soil heated at 140 ^{o}C, 30 minutes	HV agar	HH
9.	Sucrose Gradient and Centrifugation Method	Sucrose Gradient and Centrifugation, 3000 rpm, 30 minutes	HV agar	WM
10.	Acid Treatment Method	Air dried soil suspension was adjusted at pH 3	HV Agar	AT
		Note: All media are supplemented with cycloheximide (50 mg/L), nalidixic acid (20 mg/L) and nystatin (50 mg/L)		

SOIL SAMPLE PREPARATION

1. Air-dry the soil samples for 7 days at room temperature
2. Grind the dry soil with mortar and pass through a sieve

MEDIA AND SOLUTION PREPARATION

(refer to Procedure for media and solution preparation)

ISOLATION PROCEDURES

A. Isolation of actinomycetes using different selective media

1. Weigh 1 gram of soil sample and dissolve in 9 ml sterile distilled water (DW) (10^{-1} dilution)
2. Stir vigorously and stand for 5 min
3. Make a serial dilution from first soil suspension up to 10^{-4}
4. Apply each 100 µl of 10^{-3} and 10^{-4} dilution into duplicate HV Agar (HV), Water Proline Agar (WP), Zhang's Starch Soil Extract Agar (ZSSE) and Soil Extract Agar media
5. Incubate all HV and WP Agar plates at 28 ^{o}C and ZSSE and SE Agar at 30 ^{o}C for 2-3 weeks
6. Pick the suspect colonies of actinomycetes from the isolation media and transfer to ISP2 agar plate using a sterile toothpick
7. Incubate all plates at 28 ^{o}C for 1-2 weeks
8. Select all different colors of actinomycetes isolates and transfer to ISP2 and YS agar slant using a sterile loopful
9. Incubate all inoculated slants at 28 ^{o}C for 1-2 weeks
10. Select all different colors of actinomycetes isolates
11. Select all different colors of actinomycetes isolates and give them the corresponding code
12. Record the isolation results in the **Isolation Result Form**
13. Keep the slant cultures for further identification and preservation not more than 3 months

B. Isolation of Actinomycetes Using Sonication Method

1. Weigh 1 gram of soil sample and dissolve in 9 ml sterile Winogradsky Solution (WS) (10^{-1} dilution)
2. Weak sonicate at room temperature for 2 minutes
3. Stand for 5 min
4. Make a serial dilution from first soil suspension up to 10^{-4} using 9 mL WS solution (each **duplicate**)

5. Apply each 100 µl of a couple 10^{-3} and 10^{-4} dilution into duplicate HV Agar
6. Incubate all plates at 28 ^{o}C for 2-3 weeks
7. Pick the suspect colonies of actinomycetes from the isolation media and transfer to ISP2 agar plate using a sterile toothpick
8. Incubate all plates at 28 ^{o}C, 1-2 weeks
9. Select all different colors of actinomycetes isolates and transfer to ISP2 and YS agar slant using a sterile loopful
10. Incubate all inoculated slants at 28 ^{o}C, 1-2 weeks
11. Select all different colors of actinomycetes isolates and give them the corresponding code
12. Record the isolation results in the **Isolation Result Form**
13. Keep the slant cultures for further identification and preservation not more than 3 months

C. Isolation Using Wet Heating Method

1. Incubate a couple of dilution 10^{-3} and 10^{-4} from the method in point **B** above in the waterbath at 60 ^{o}C for 30 minutes
2. After cooled at room temperature apply each 100 µl from a 10^{-3} and 10^{-4} dilution into duplicate HV Agar
3. Incubate all plates at 30 ^{o}C for 2-3 weeks
4. Pick the suspect colonies of actinomycetes from the isolation media and transfer to ISP2 agar plate using a sterile toothpick
5. Incubate all plates at 28 ^{o}C for 1-2 weeks
6. Select all different colors of actinomycetes isolates and transfer to ISP2 and YS agar slant using a sterile loopful
7. Incubate all inoculated slants at 28 ^{o}C for 1-2 weeks
8. Select all different colors of actinomycetes isolates and give them the corresponding code
9. Record the isolation results in the **Isolation Result Form**
10. Keep the slant cultures for further identification and preservation not more than 3 months

D. Isolation Using Dry-Heating Method

1. Weigh 5 gram of air-dried soil and dry-heat at 120 ^{o}C for 1 hour

2. After cooled at room temperature, spread the soil sample over a sterile tissue paper on to duplicate HV Agar
3. Incubate all plates at 28 °C for 2-3 weeks
4. Pick the suspect colonies of actinomycetes from HV agar and transfer to ISP2 agar plate using a sterile toothpick
5. Incubate all plates at 28 °C for 1-2 weeks
6. Select all different colors of actinomycetes isolates and transfer to ISP2 and YS agar slant using a sterile loopful
7. Incubate all inoculated slants at 28 °C for 1-2 weeks
8. Select all different colors of actinomycetes isolates and give them the corresponding code
9. Record the isolation results in the **Isolation Result Form**
10. Keep the slant cultures for further identification and preservation not more than 3 months

E. Isolation Using High-Heating Method

1. Weigh 5 gram of air-dried soil and dry-heat at 140 °C for 30 minutes
2. After cooled at room temperature, spread the soil sample over a sterile tissue paper on to duplicate HV Agar
3. Incubate all plates at 28 °C for 2-3 weeks
4. Pick the suspect colonies of actinomycetes from HV agar and transfer to ISP2 agar plate using a sterile toothpick
5. Incubate all plates at 28 °C for 1-2 weeks
6. Select all different colors of actinomycetes isolates and transfer to ISP2 and YS agar slant using a sterile loopful
7. Incubate all inoculated slants at 28 °C for 1-2 weeks
8. Select all different colors of actinomycetes isolates and give them the corresponding code
9. Record the isolation results in the **Isolation Result Form**
10. Keep the slant cultures for further identification and preservation not more than 3 months

F. Isolation Using Dry Heating-Phenol Method

1. Prepare the phenol solution as below:

   1.1 Make 5 mM phosphate buffer (pH 7.0)

   a. Na_2HPO_4 (358.14 g/mol) 0.895 g in 500 ml ddH_2O (can also be used K_2HPO_4)

   b. KH_2PO_4 (136.09 g/mol) 0.34 g in 500 ml ddH_2O

   Mix **a** and **b** to make buffer solution at pH 7.0 and sterilize at 121 ^oC, 15b minutes

   1.2 Make a phenol stock solution

   Dissolve phenol (1.7 g) into autoclaved phosphate buffer above 98.2 ml

2. Weigh 5 gram of air-dried soil and dry-heat at 120 ^oC for 1 hour
3. Prepare water bath at 30 ^oC
4. Mix 1.0 g of soil sample with 10 mL sterilized water and stir vigorously for 2 min
5. After allowing the tube to stand for 1 min, transfer 1 ml of supernatan to 9 ml phenol solution to give a final concentration of 1.5 % (w/v) phenol (10^{-2})
6. The mixture was maintained at 30 ^oC with occassional stirring
7. After incubation for 30 min, a 10^{-3} and 10^{-4} dilution of the mixture was prepared in sterile water
8. Aliquots of the diluted mixture are spread onto HV agar supplemented with nalidixic acid
9. All plates are incubated at 28 ^oC for 2 to 3 weeks
10. Pick the suspect colonies of actinomycetes from HV agar and transfer to ISP2 agar plate using a sterile toothpick
11. Incubate all plates at 28 ^oC for 1-2 weeks
12. Select all different colors of actinomycetes isolates and transfer to ISP2 and YSA agar slant using a sterile loopful
13. Incubate all inoculated slants at 28 ^oC for 1-2 weeks
14. Select all different colors of actinomycetes isolates and give them the corresponding code
15. Record the isolation results in the **Isolation Result Form**
16. Keep them for further identification and preservation not more than 3 months

G. Isolation Using Sucrose-Gradient and Centrifugation Method

1. Prepare the sucrose solution as below:
   1.1 Weigh 200 g of sucrose and add into 200 mL sterile DW (100% of *conc*.) in beaker glass. Mix thoroughly
   1.2 Make sucrose solution at concentration of 10, 20, 30, 40 and 50%, each 100 mL, from solution above
   1.3 Sterilize all sucrose solution at 121 ^{o}C, 20 minutes
   1.4 Keep in the refrigerator (4 ^{o}C) until use
2. Prepare the sucrose gradient by layering 1 ml of each 10, 20, 30, 40 and 50% (v/v) sucrose solution carefully in a 10 ml sterile tube
3. Mark the boundaries between the sucrose concentration layer
4. Weigh 1 gram of air-dried soil and dissolved with 10 mL sterile tap water
5. Apply 1 mL of soil suspension to discontinous sucrose gradient and centrifuge at room temperature at 3000 rpm for 30 minutes
6. Remove the 10% concentration layer and transfer all layers to the sterile microtube
7. Aliquot 0.2 ml of sucrose section spread into duplicate HV agar[*)]
8. Incubate all plates at 30 ^{o}C for 2-3 weeks
9. Pick the suspect colonies of actinomycetes from HV agar and transfer to ISP2 agar plate using a sterile toothpick
10. Incubate all plates at 28 ^{o}C for 1-2 weeks
11. Select all different colors of actinomycetes isolates and transfer to ISP2 and YSA agar slant using a sterile loopful
12. Incubate all inoculated slants at 28 ^{o}C for 1-2 weeks
13. Select all different colors of actinomycetes isolates and give them the corresponding code
14. Record the isolation results in the **Isolation Result Form**
15. Keep them for further identification and preservation not more than 3 months

H. Isolation of actinomycetes using different selective media

1. Prepare the 3 M HCl stock solution as below:
   1.1 Volume 24.9 mL of HCl and add to 100 mL of DW
   1.2 Stir vigorously
   1.3 Be carefull because this solution very irritant!

2. Weigh 1 gram of soil sample and dissolve in 9 ml sterile distilled water (DW) (10^{-1} dilution)
3. Stir vigorously for 2 minutes
4. Adjust the pH of suspension by drop-wise addition of 3M HCl stock solution and stand the tube for 30 minutes
5. Make a serial dilution from first soil suspension up to 10^{-4}
6. Apply each 100 µl of 10^{-3} and 10^{-4} dilution into duplicate HV Agar (HV)
7. Incubate all plates at 28 ^{o}C for 2-3 weeks
8. Pick the suspect colonies of actinomycetes from the isolation media and transfer to ISP2 agar plate using a sterile toothpick
9. Incubate all plates at 28 ^{o}C for 1-2 weeks
10. Select all different colors of actinomycetes isolates and transfer to ISP2 and YS agar slant using a sterile loopful
11. Incubate all inoculated slants at 28 ^{o}C for 1-2 weeks
12. Select all different colors of actinomycetes isolates
13. Select all different colors of actinomycetes isolates and give them the corresponding code
14. Record the isolation results in the **Isolation Result Form**
15. Keep the slant cultures for further identification and preservation not more than 3 months

PROCEDURE FOR DETERMINATION CULTURAL CHARACTERISTICS AND MORPHOLOGY OF ACTINOMYCETES ISOLATES

1. Prepare the actinomycetes culture plates to be determined. The culture should be a pure culture and grown at least for 14 days (2 weeks) at 28 ^{o}C in ISP2 medium agar
2. All of the observasion results **should be recorded** in the Identification Result Form **and documented**
3. **Aerial mass colour**. The colour of the mature of sporulating aerial mycelium is observed from the surface of the colony and recorded as white, grey, red, green, blue and violet. When the aerial mass colour fell between two colourss series, both the colourss are recorded. If the aerial mass colour of a strain to be studied shows intermediate tints, then both the colour series are also noted
4. **Melanoid pigments**. The grouping is made on the production of melanoid pigments (i.e., greenish brown, brownish black, or distinct brown, pigment modified by other colours) on the medium. The strains are grouped as melanoid pigment produced (+) and not produced (–). In a few cases, the productions of melanoid pigments are delayed or weak, and therefore, it is not distinguishable.
5. **Reverse side pigments**. The strains are divided into two groups, according to their ability to produce characteristic pigments on the reverse side of the colony (white, yellow, orange, green, red, blue, purple, brown, black and other colourss), namely, **distinctive** (+) and **not distinctive** or none (–). In case, a colour with low chroma such as pale yellow, olive, or yellowish brown occurs, it is included in the latter group (–)
6. **Soluble pigments.** The strains are divided into two groups by their ability to produce soluble pigments other than melanin: namely, produced (+) and not produced (–). The colour is recorded (red, orange, green, yellow, blue, and violet)
7. **Spore chain morphology.** The basic morphology of the spore-bearing hypae and spore chains are observed by light microscope directly from culture surface. Adequate magnification (400x) could be used to establish the presence or absence of spore chains and to observe the nature of sporophores. The results are grouped in the genus *Streptomyces* and non-streptomyces (the spores forms can be seen in the book of Bergey's Manual, Determinative Bacteriology *Ninth Edition*)

8. All of the identified actinomycetes **should be registered** in Biotech Microbial Culture Collection Database

ISOLATION OF FUNGI USING USING DIFFERENT MEDIA ISOLATION

Isolation procedure

1. Mix 1 g of soil sample with 9 ml sterilized distilled water (DW) and stir vigorously for 2 min
2. Stand the soil suspension for 1 min
3. Make a 10^{-3} and 10^{-4} dilution from the first soil suspension
4. Aliquots of the diluted mixture (100 µl) are spread onto LC Agar, MARB agar, MARB agar supplemented with 0.4 % LiCl, Onion Garlic Agar (OGA), Malt Extract Agar (MEA) and Soil Extract Agar (SEA) ***)**
5. Incubate all plates at 28 ^{o}C for 1-3 weeks
6. Periodically observe the growth colonies in the isolation medium and transfer the mycelium into PDA medium using a sterile toothpick
7. Incubate at 28 ^{o}C for 1-2 weeks
8. Select all different colors of fungal isolates and transfer to YPSs and LCA: Miura's agar slant using a sterile loopful ***)**
9. Incubate the slant cultures at 28 ^{o}C for 1-2 weeks
10. Select all different colors of fungal isolates in slant culture and give them the corresponding code in the beginning of the code as follows :

    f.LCA : for isolates from LCA medium

    f.MARB : for isolates from MARB medium

    f.LiCl : for isolates from MARB + 0.4% LiCl

    f.OGA: for isolates from OGA medium

    f.ME : for isolates from MEA medium

    f.SE : for isolates from SEA medium

11. Keep them on refrigerator for further identification and preservation not more than 3 months

Note: *) refers to the Procedure for media and solution preparation

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

IDENTIFICATION OF FUNGI BY MORPHOLOGICAL CHARACTERISTICS

Reference :

Reviewed and approved by :

Date last modification

IDENTIFICATION OF FUNGI BY MORPHOLOGICAL CHARACTERITICS

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to identified of fungi using morphological characteristics such as colony appearance, colony pigmentation and shapes of spores/conidia.

B. METHOD

- **All handling in this SOP should be performed under ASEPTIC conditions!!!**

Fungi culturing

Make a cutted square PDA on plate and put on preparate glass. And then pick a surface of colony fungi isolation result with sterile loop and transfer to cutted square agar and cover with steril cover glass. Incubation at 25 ^{o}C. Observe growth of colony from 3 to 7 days. Recorde a morphological characteristics such as colony appearance, colony pigmentation and shapes of spores/conidia on identification form. Use The books for identification of fungi to compare a spora and a micellium that we get with identification book. The identification books that we are used : Identification Key of Fungi imperfecti by Katsuhiko Ando, Ilustrated genera of imperfect fungi by Barnett H.L and Barry B. Hunter (1972) and Pictorial atlas of soil and seed fungi : morphologies of cultured fungi and key to species by Tsuneo Watanabe.

Required solutions :

- PDA medium
- Sterile H2O

Required equipment:

- Sterile petri dish glass
- sterile cover glass
- sterile tisues
- Incubator
- Microscope
- Cover glass
- Object glass
- Micropippte 1000µL
- Sterile pippet tip

Procedure to make a cover slide method:

1. Prepare a petri dish glass

2. Put on two folds tissue and
3. Put object glass above the tisues
4. Put a cover glass on another side petri disk and then cover with a cover glass petri dish.
5. Sterile by autoclave at 121 ^{o}C for 25 minutes.
6. A cuttled square PDA medium put on object glass in sterile glass petri dish.
7. Pick up a surface colony culture that will be identified with sterile loop
8. Transfer to cuttled square PDA medium.
9. Cover it with sterile cover glass.
10. Wet a fold tisue with 1 ml of sterile water using micropippete
11. Incubation at 25 ^{o}C on incubator.
12. Observe a growth of fungi every day under microscope.
13. Compare the spore/conidia with the book of identification of fungi.

Appendix :

Preparation of PDA medium (composition 1000 mL) :
Potatoes Dextrose Agar 39 g,
Distilled water 1000 mL
Weight all ingredients and put on 1500 mL Erlenmeyer flask. Sterilize the medium by autoclave at 121 ^{o}C for 15 minutes. After cooling down the temperature to 50-60 ^{o}C, pour the medium into plastic disposable petri dish (about 20 mL for each plate). Keep the plate on the table for overnight, then store at 4^{o}C until use.

Sterile water :
Distilled water 100 mL
Put a distilled water on a bottle and sterilize using autoclaved at 121 ^{o}C, for 15 minutes and store at 4^{o}C.

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

PRESERVATION OF ACTINOMYCETES USING CRYOPRESERVATION

Reference :

Reviewed and approved by :

Date last modification

PRESERVATION OF ACTINOMYCETES

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for long term storage of actinomycetes microbe. The microbes should be stored at low temperature -80 °C. Cryopreservation is technique storage microbes in cryovial contains 20% glycerol solution and cutted agar culture disk of microbes. The cryovials tube are stored at -80°C deep freezer.

B. METHOD

- **All handling in this SOP should be performed under ASEPTIC conditions!!!**

This procedure is use for preservation of actinomycetes isolates from the isolation results and reviving cultures. The actinomycetes isolates should be preserved in form of **a well sporulating culture and have been registered in Biotech Microbial Culture Collection Database.**

Required solutions (see Appendix):

- Sterile 20% (v/v) glycerol stock solution in distilled water

Required equipment:

- 2 ml sterile cryogenic vial
- 4 °C refrigerator
- -80 °C deep freezer

Procedure:

1. Prepare an actinomycete isolate with well sporulated growth on an agar plate (refer to SOP of isolation of actinomycetes or SOP of reviving actinomycetes).
2. Create an access code label for each isolate to be preserved and attach the labels around to the cryogenic vial (duplicate)
3. Distribute 1 mL of 20% glycerol into a sterile cryogenic vial tube
4. Cut the culture agar as much as 10-12 culture disks using a sterile straw (Ø 7 mm)
5. Transfer 4-5 of cutting off culture disks into a sterile cryogenic vial using a sterile toothpick. Make sure the isolates are inserted into the right tube.
6. Shake the cryogenic vial slowly so all agar disks are submerged in the glycerol solution
7. Store the vial in the 4 °C refrigerator for 2-24 h (typically overnight), then move into the -80 °C deep freezer.

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Appendix :

Preparation of glycerol solution (20% (v/v)) :

Glycerol	50 mL
Distilled water	200 mL
Total	250 mL

Sterilize by autoclave at 121 °C, 15 minutes and store at 4°C.

Preparation of ISP Medium 2 (composition in 1000 mL):

Yeast Extract	4.0 g
Malt Extract	10.0 g
Dextrose	4.0 g
Agar	20.0 g

Distilled water up to 1000 mL
pH 7,2

Weight all ingredients and put on 1500 mL Erlenmeyer flask and adjust pH to 7.2 using NaOH 5M or HCl 2N. Sterilize the medium by autoclave at 121 °C for 15 minutes. After cooling down the temperature to 50-60 °C, pour the medium into plastic disposable petri dish (about 20 mL for each plate). Keep the plate on the table for overnight, then store at 4°C until use.

Fungi with well

15% Glycerol solution

Distribute 1 mL of glycerol sol.

Steril cryovial with label

Cutting off a culture agar with steril straw

Transfer a disk into steril cryovial

Store cryovial in the -80 °C deepfreezer

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

PRESERVATION OF FUNGI ISOLATES USING CRYOPRESERVATION

Reference :

Reviewed and approved by :

Date last modification

PRESERVATION OF FUNGI ISOLATES USING CRYOPRESERVATION SYSTEM

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for long time storage of fungi microbe. Microbes should be stored at low temperatures -80 ^{o}C. Cryopreservation is technique storage microbes in cryovial contains 15% glycerol solution and cutted agar culture disk of microbes. The cryovials tube are stored at -80°C deep freezer.

B. METHOD

- **All handling in this SOP should be performed under ASEPTIC conditions!!!**

This procedure is use for preservation of fungi isolates from the isolation results and reviving cultures. The fungi isolates should be preserved in form of a well sporulating culture and have been registered in Biotech Microbial Culture Collection Database.

Required solutions (see Appendix):

- Glycerol stock solution (15% v/v)

Required equipment:

- 2 ml sterile cryogenic vial
- 4 ^{o}C refrigerator
- -80 ^{o}C deep freezer

Procedure:

1. Prepare a fungi isolate with well sporulated growth on an agar plate (refer to SOP of isolation of fungi or SOP of reviving fungi).
2. Create an access code label for each isolate to be preserved and attach the labels around to the cryogenic vial (duplicate)
3. Distribute 1 mL of 15% glycerol into a sterile cryogenic vial tube
4. Cut the culture agar as much as 10-12 culture disks using a sterile straw (Ø 7 mm)
5. Transfer 4-5 of cutting off culture disks into a sterile cryogenic vial using a sterile toothpick. Make sure the isolates are inserted into the right tube.
6. Shake the cryogenic vial slowly so all agar disks are submerged in the glycerol solution
7. Store the vial in the 4 ^{o}C refrigerator for 2-24 h (typically overnight), then move into the -80 ^{o}C deep freezer.

Appendix :

Preparation of Glycerol solution (15%) :

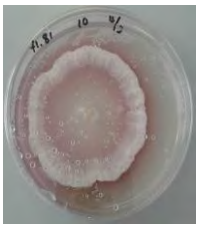
Glycerol	22,5 mL
Trehalose	11,25 g
Distilled water up to	250 mL
Total	250 ml

Sterilize by autoclave at 121 °C, 15 minutes and store at 4°C.

Preparation of PDA medium for plate :

Potatoes Dextrose Agar 39.0 g,
Distilled water up to 1000 mL

Weight all ingredients and put on 1500 mL Erlenmeyer flask. Sterilize the medium by autoclave at 121 °C for 15 minutes. After cooling down the temperature to 50-60 °C, pour the medium into plastic disposable petri dish (about 20 mL for each plate). Keep the plate on the table for overnight, then store at 4°C until use.



Fungi with well

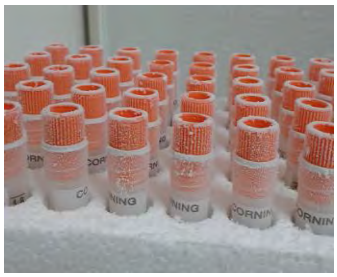
15% Glycerol solution

Distribute 1 mL of glycerol sol.

Steril cryovial with label

Cutting off a culture agar with steril straw

Transfer a disk into steril cryovial



Store cryovial in the -80 °C deepfreezer

Balai Bioteknologi BPPT

SOP

STANDARD OPERATION PROCEDURE

REVIVING OF ACTINOMYCETES ISOLATES FROM FROZEN STOCK

Reference :

Reviewed and approved by :

Date last modification

REVIVING OF ACTINOMYCETES ISOLATES FROM FROZEN STOCK

A. PURPOSE & PRINCIPLE

The purpose of this SOP is to describe the procedure for reviving of microbe from frozen/ Glycerol stock. Take a frozen/ glycerol stock from -80 ^{o}C deep freezer and transfer it to refrigerator 4 ^{o}C. Use a sterile loop and take out one piece of agar in a cryovial and then put on ISP2 medium plate crush the agar then streak them on agar plate. Incubate at 28 ^{o}C for 14-21 days.

B. METHOD

- **All handling in this SOP should be performed under ASEPTIC conditions!!!**

Required equipment:

- Laminar air flow
- Refrigerator 4 ^{o}C
- Sterile sterile loop
- Frozen/ Glycerol stock
- ISP2 medium

Procedure

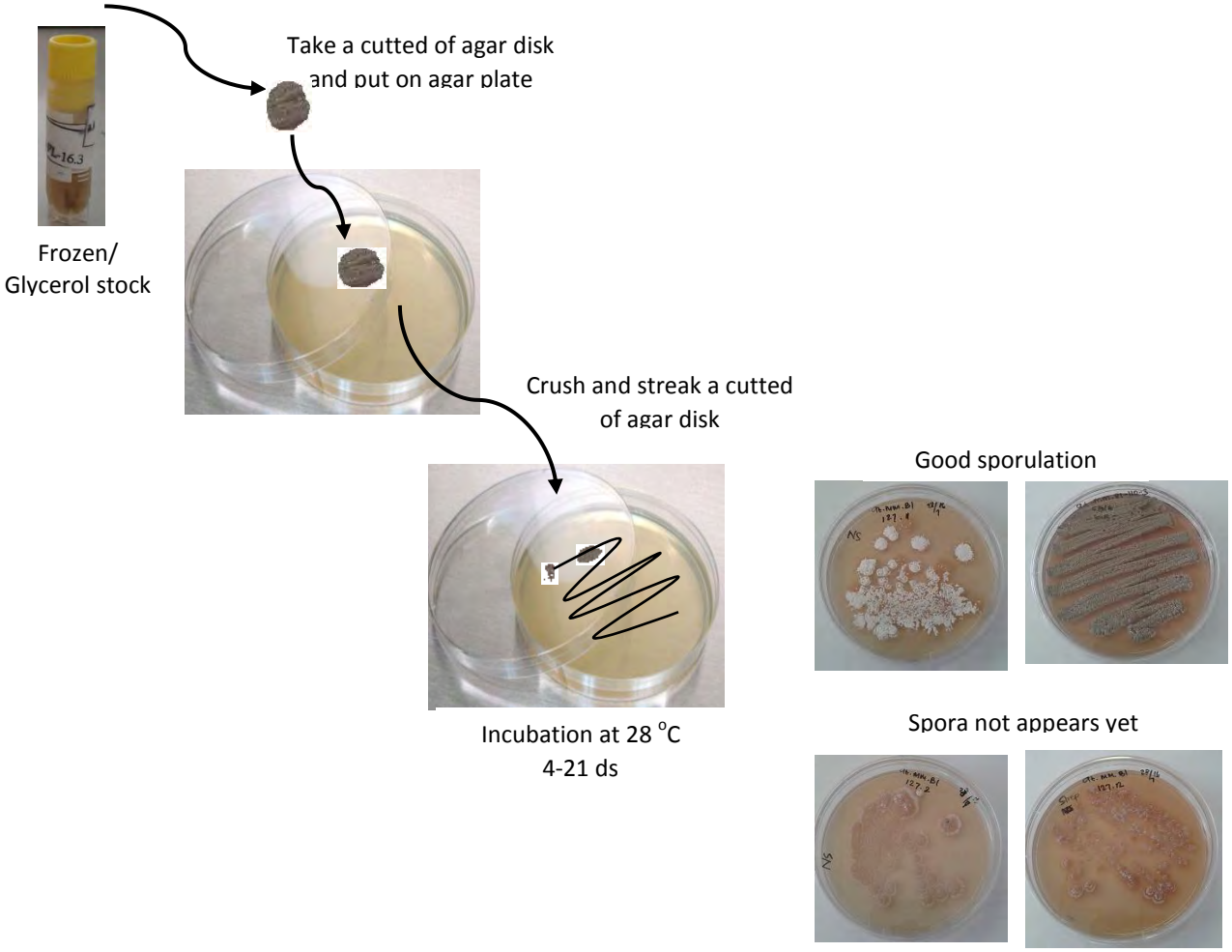
1. Transfer a frozen (glycerol) stock from -80 ^{o}C deep freezer into 4 ^{o}C refrigerator, then keep for 2 hours.
2. Take one piece of cutted agar disk from the frozen tube using a steril loop, then put it on ISP2 agar medium.
3. Crush the cutted agar disk using loop, then streak on agar medium.
4. Seal the plate with plastic wrap.
5. Incubate at 28 ^{o}C.
6. Observe the formation of spore after 7 days incubation. Continue the incubation and observe the plate every 7 days until the spores could be observed from the whole streaked line.
7. Record the length of incubation time from the revival date until the day of using of the isolate (inoculation day) in “Regeneration form”.
8. Cut the agar using sterile straw, then put into fermentation medium (30 mL medium in 250 mL Erlenmeyer flask).
9. Preserve the isolate in form of frozen stock in -80 ^{o}C according to SOP of Preservation of Actinomycetes.

Appendix :

Preparation of ISP2 agar medium

Yeast extract 4.0 g,
Malt extract 10.0 g,
Dextrose 4.0 g,
Agar 20.0 g
Distilled water 1000 mL

Weight all ingredients and put on 1500 mL Erlenmeyer flask after pH adjust to 7.2 and then add agar. Sterilize the medium by autoclave at 121 ^{o}C for 15 minutes. After cooling down the temperature to 50-60 ^{o}C, pour the medium into plastic disposable petri dish (about 20 mL for each plate). Keep the plate on the table for overnight, then store at 4^{o}C until use.



- **Preparation of 10% giemsa solution (typically 3 mL for 1 slide glass)**

Giemsa	0.3 mL
PBS 1x	2.7 mL
10% Giemsa solution	3.0 mL

1. Add 2.7 mL of PBS 1x (prepared by dissolve 1 table of PBS in 1000 mL milli-Q water then store at r.t.) and 0.3 mL giemsa solution into a 100 mL Erlenmeyer flask.
2. Mix well.
3. The solution is ready to use.

Note:

- Prepare fresh 10% giemsa solution for each staining
- Prevent giemsa solution from light (use amber bottle or wrap the bottle with aluminum foil)

- **Giemsa staining of thick blood films from parasite cultures**

1. Pipette 200 µL of parasite culture into microtube
2. Spin down using mini centrifuge for 5 sec at r.t.
3. Discard half of the supernatant and resuspend the pellet using the remaining supernatant
4. Put it onto an object glass and make a thin film as shown in the picture below.

How to make thick blood films

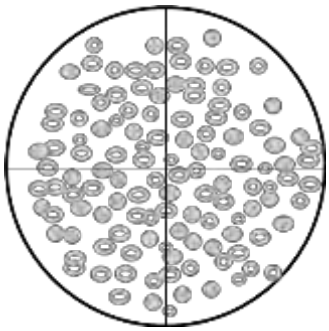
5. Dry the thin film by cool wind (using cool dryer)
6. Put the slide in staining rack
7. Fix the film with methanol (2-3 mL) while prepare a fresh 10 % Giemsa solution in PBS 1x (typically 3 mL for each sample)
8. Discard the methanol and pour the Giemsa solution on the slide.
9. Leave it to stain for 15 min.
10. Rinse it carefully and thoroughly under running tap water.
11. Dry the slide by cool wind (using cool dryer)
12. Observe the film with immersion oil under microscope with 1000x magnification

Note:

- Clean microscope lens using a lens paper immersed with hexane.

- **Estimation of the percentage of erythrocytes infected with *P. falciparum* in a thin blood film**

1. Use microscope with 1000× magnification to view cells under oil immersion.
2. Choose an area of Giemsa-stained thin blood film where the erythrocytes are evenly distributed.
3. Count all erythrocytes observed under viewfinder window.



4. Move the slide to randomly adjacent fields and continue counting all infected erythrocytes in the ocular lens viewfinder window.
5. Repeat the counting at least 10 fields
   Note: If one erythrocyte contains ≧2 parasites it is still counted as one infected erythrocytes
6. Calculate the parasitemia using formula below.

$$\text{Parasitemia } (\%) = 100 \times \frac{number\ of\ infected\ erythrocytes}{number\ of\ erythrocytes\ \times number\ of\ observed\ field}$$

Prepared by	Verified by	Date of use
Dian Japany Puspitasari	DanangWaluyo	May 22nd,2019

- **Preparation of Mediums and Reagents**

A. Basal medium (RPMI (-)) 1000 mL

- Composition
  - RPMI 1640 (w/o $NaHCO_3$, with 25 mM Hepes, with L-glutamine)
  - $NaHCO_3$ 23.8 mM
  - Hypoxanthine 50 mg/L
  - Gentamycin 25 mg/L

  (pH 7.2)
- Preparation protocol
  1. Prepare sterile 1000 mL glass bottle
  2. Dissolve RPMI 1640 powder (Gibco Cat.No.23400-013) in 900 mL MilliQ water
  3. Add 2.0 g of $NaHCO_3$, 50 mg hypoxanthine, and 1 mL gentamycin solution (25 mg/mL, stored at -30°C)
  4. Adjust pH to 7.2 using 5 M NaOH (stored at 4°C)
  5. Add MilliQ water up to 1 L
  6. Filter sterilization (0.22 μm) using bottle top filter
  7. Store 4°C for 2 months

B. Complete medium (RPMI (+)) 1000 mL

- Composition
  - Basal medium RPMI (-) containing 0.5% Albumax II
- Preparation protocol
  1. Prepare sterile 500 mL glass bottle
  2. Add 50 mL of 5% albumax II into 450 mL RPMI (-) medium
  3. Store 4°C (~ 1 month)

C. Gentamycin solution 25 mg/mL (10 mL)

- Composition
  - 25 mg/mL gentamycin in water
- Preparation protocol
  1. Dissolve 250 mg gentamycin in 10 mL MilliQ water
  2. Filter sterilization (0.22 μm) using syringe filter
  3. Aliquot into 1.5 mL tube, @ 1 mL
  4. Store at -30°C

D. Albumax II 5% (w/v) 1000 mL

- Composition
  - 5% Albumax II in PBS buffer
- Preparation protocol
  1. Prepare sterile 1000 mL glass bottle
  2. Dissolve 50 g Albumax II (Gibco, Cat.No.11021-029) and 1 tablet of PBS (w/o CaCl2, w/o MgCl2, Takara) in 800 mL MilliQ water
  3. Mix slowly until completely dissolved (it takes about 1 hour to completely dissolve)
  4. Filter sterilization (0.22 μm) using disposable bottle top filter
  5. Aliquot into 50 mL tube, @ 50 mL
  6. Store at -30°C

E. RBC (red blood cell) 50% hematocrit

- Composition
  - 50% (v/v) red blood cell in RPMI (-) medium
- Preparation protocol
  1. Prepare 1 bag of blood (approx.150-250 mL)
  2. Transfer the blood from the bag into 50 mL @30 mL using sterile needle and 50 mL syringe (the blood is taken by piercing the needle to the bag from its outlet tube).

  3. Centrifuge at 1700 x g, r.t., 10 min (slow deceleration)
  4. Remove supernatant by aspiration
  5. Distribute RBC evenly between tubes using serological pipette
  6. Add 1 volume of RPMI (-) into each tube
  7. Mix by inverting gently
  8. Centrifuge at 1700 x g, r.t., 10 min (slow deceleration)
  9. Wash RBC with RPMI (-) 3 times

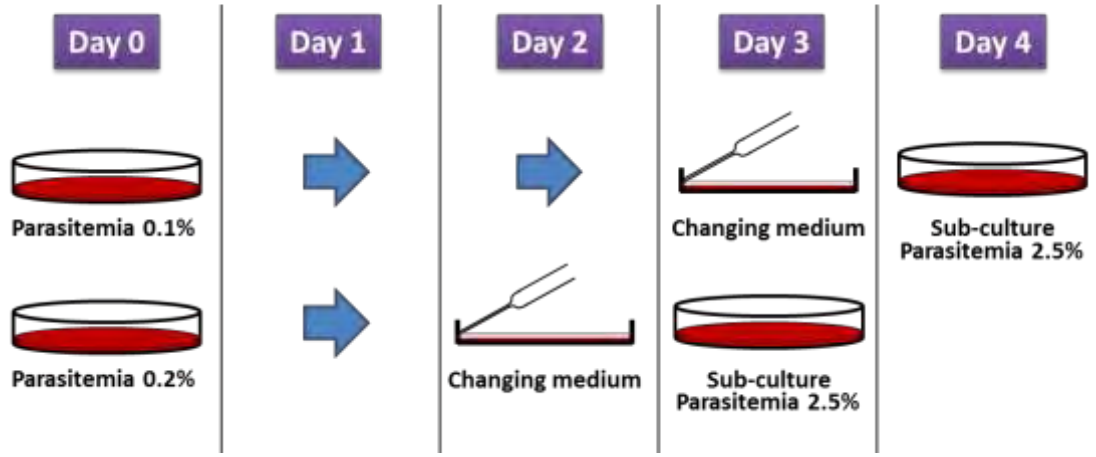
10. Add 1 volume of RPMI (-) medium, mix well
11. Store at 4°C for 3 weeks

- Note
  1. Typically, red blood is obtained from local Red Cross
  2. Use any kind of blood type, O is preferred
  3. "Waste blood" (blood that does not prefer for transfusion) can be used.
  4. Do not use blood that has been stored for 4 weeks
  5. Discard blood properly according to local authorities regulation
  6. After receive the blood, check its sterility by doing the protocol below. **Discard the blood if contaminant is observed.**
     a. Spread 200 μL blood on NA agar, incubate at 37°C for 2 days, and observe the growth of any contaminant.
     b. Add 1 drop of blood into FTM and phenol red medium, incubate 37°C for 2 days, and observe the growth of any contaminant.

- **Sub-culturing *Plasmodium falciparum* for cell maintenance**

1. Count the parasitemia of the culture
2. Prepare culture medium composing from RPMI(+) with 3% of hematocrit (typically, 10 mL medium, made by adding 9.4 mL RPMI (+) and 0.6 mL 50% erythrocytes)
3. Add infected RBC to new medium. Typical initial parasitemia of new culture is 0.1 (for 4 days culture until next passage cycle) or 0.2 (for 3 days culture until next passage cycle).
   Note:
   - The parasites will grow 5x within 2 days.
   - Never set initial parasitemia exceed 5%
4. Incubate at 37°C, 5% CO_2, 5% O_2.
5. Change medium if the parasitemia>1%

Typical culture schedule



- **Medium change**

1. Remove medium by aspiration
2. Add RPMI (+) medium (typically 10 mL), resuspend RBC
3. Estimate parasitemia to monitor the growth of parasites if necessary

- **Preparation Reagents**

A. Thawing solution A 100 mL
   - Composition
     - 12% (w/v) NaCl
   - Preparation protocol
     1. Dissolve 12 g NaCl in 100 mL deionized water
     2. Sterilize by filtration (0.22 mm) or by autoclave
     3. Store 4°C

B. Thawing solution B 250 mL
   - Composition
     - 1.6% (w/v) NaCl
   - Preparation protocol
     1. Dissolve 4 g NaCl in 250 mL deionized water
     2. Sterilize by filtration (0.22 μm) or by autoclave
     3. Store 4°C

C. Thawing solution C 250 mL
   - Composition
     - 0.9% (w/v) NaCl
     - 0.2% Glucose
   - Preparation protocol
     1. Dissolve 2.25 g NaCl and 0.5 g glucose in 250 mL deionized water
     2. Sterilize by filtration (0.22 μm) or by autoclave
     3. Store 4°C

D. Freezing solution (use SF-60 or prepare freezing solution as follow)
   - Composition
     - 35% glycerol
     - 30% D-sorbitol
     - 0.65% NaCl
   - Preparation protocol
     1. Dissolve 13.9 mL glycerol 100%, 1.5 g D-sorbitol, and 0.325 g NaCl in 50 mL MilliQ water.
     2. Sterilize by filtration (0.22 μm) or by autoclave
     3. Store at 4°C

- **Preparation of frozen stock of *Plasmodium falciparum***

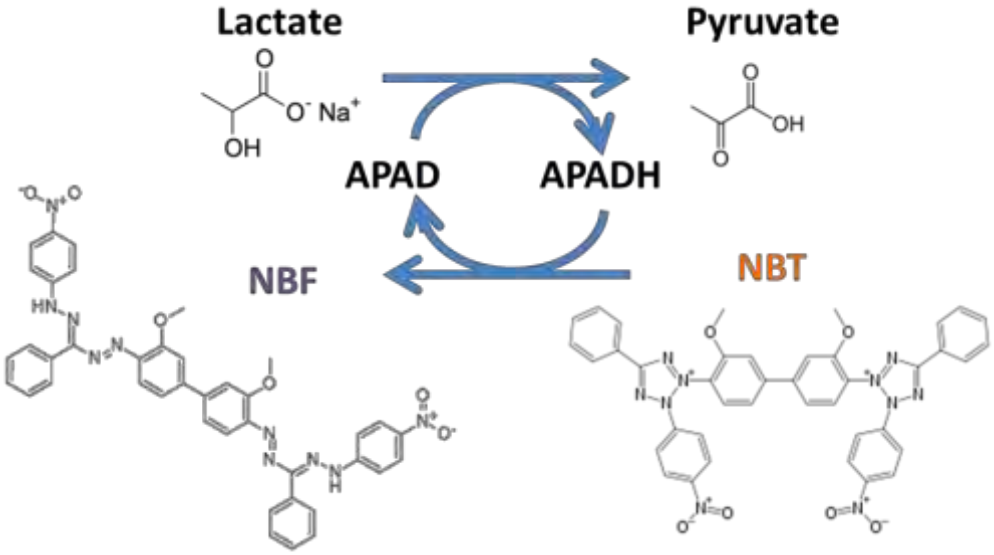
1. Transfer 10 mL culture into a 50 mL tube.
2. Count percentage of ring form: (the number of ring form / total erythrocytes) x 100%
    - Percentage of ring form should be adjusted to 3 ~ 5%
3. Centrifuge at 800×g, for 5 min at r.t.
4. Discard supernatant by aspiration
5. Take 4 mL freezing solution using syringe+needle
6. Change needle with 0.22 μM syringe filter
7. Add 5 drops of freezing solution dropwise (pass through syringe filter), incubate 5 min at r.t.
8. Add additional 2 mL of freezing solution (pass through syringe filter) dropwise, 1 drop/sec with shaking the tube gently.
9. Aliquot into 2 frozen tubes @1 mL.
10. Pun in styrofoam box (or in a bicell vessel) with stand position, store at -80°C.
11. Confirm the parasitemia of the frozen stock by thawing one tube, incubate for overnight, then count the parasitemia.
12. Transfer the tube into liquid nitrogen tank in the following day.

- ***Plasmodium falciparum* cell recovery from frozen stock**

1. Take a frozen serum tube and thaw it at 37°C for 1 to 2 min (immediate thawing)
2. Transfer the culture to 50 mL tube.
3. Add 0.2 mL of thawing solution A slowly, dropwise, while shaking the tube gently.
4. Let the tube stand for 5 min.
5. Add 10 mL of thawing solution B slowly, dropwise, while shaking the tube.
6. Centrifuge the tube at 800 x g for 5 min at RT.
7. Aspirate the supernatant and add 10 mL of thawing solution C slowly, dropwise, while shaking the tube.
8. Centrifuge the tube at 800 x g for 5 min at RT, then aspirate the supernatant.
9. Resuspend pelleted blood cells in 5 mL pre-warmed RPMI(+) with 3% RBC (**use fresh RBC**) and transfer to a small culture plate (or T25 flask).
10. Incubate the culture at 37°C, 5% CO_2, 5% O_2.
11. Observe the cell after 2 days incubation. If the cell does not grow well or contaminated and due to bad frozen stock, discard all frozen vial those are prepared in the same batch.

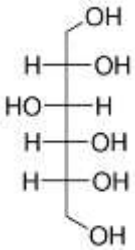
- **Principle of LDH assay**

APAD and Lactate (present in substrate buffer) are converted by pfLDH to APADH and Pyruvate. APADH reduces the chromogenic substrate NBT using the enzyme Diaphorase. This results in the formation of Nitro Blue Formazan (NBF), a deep purple soluble stain that can be measured at a wavelength of 650 nm.



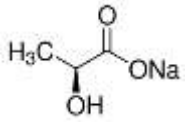
- **Preparation of Reagents**

A. 5% (w/v) Sorbitol (500 mL)



- Composition
  - 5% (w/v) Sorbitol in milli-Q water
- Preparation protocol
  1. Dissolve 25 g sorbitol powder in 500 mL of milli-Q water
  2. Sterilize by filtration (0.22 μm) or by autoclave
  3. Store 4°C

B. LDH assay buffer



- Composition
  - 100 mMTris-HCl (pH 8.0)
  - 50 mM Sodium-L-lactate
  - 0.25% (v/v) Triton X-100
- Preparation protocol
  1. Mix 50 mL of 1 M Tris-HCl (pH 8.0, stored at r.t.) into 450 mL milli-Q water

Prepared by	Verified by	Date of use
Dian Japany Puspitasari	Danang Waluyo	May 22nd, 2019

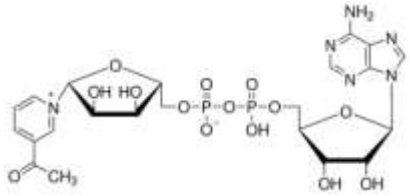
2. Add 2.8 g sodium L-lactate and 1.25 mL triton X-100, mix with stirrer gently at room temperature for at least 30 minutes.
3. Aliquot the solution into 15 mL tube each 10 mL
4. Store -30°C

C. Diaphorase 50 U/mL (from *Clostridium kluyveri*, Sigma) (30 mL)

- Composition
  - Diaphorase 50 U/mL
- Preparation protocol
  1. Dissolve 1500 U Diaphorase in 30 mL milli-Q water. Add water directly into new sealed reagent bottle.
  2. Aliquot the solution into 1.5 mL tube each 200 μL
  3. Store -30°C

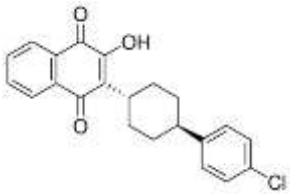
  Note: This protocol is made for Diaphorase only from Sigma. For Diaphorase from Oriental Yeast, prepare a solution with concentration of 333 U/mL by dissolving 10,000 U of Diaphorase in 30 mL milli-Q water.

D. APAD (3-acetylpyridine adenine dinucleotide) 10 mg/mL (10 mL)



- Composition
  - 10 mg/mL APAD
- Preparation protocol
  1. Dissolve 100 mg of APAD in 10 mL milli-Q water. Add water directly into new sealed reagent bottle.
  2. Aliquot into 1.5 mL tube each 50 μL.
  3. Store -30°C

E. 250 μg/mL Atovaquone



- Composition
  - 250 μg/mL Atovaquone (MW=366.84 g/mol)
- Preparation protocol
  1. Dissolve 1 mg of atovaquone 10.9 mL DMSO. Add DMSO directly into new sealed reagent bottle.
  2. Aliquot into 1.5 mL tube each 50 μL.
  3. Store -30°C

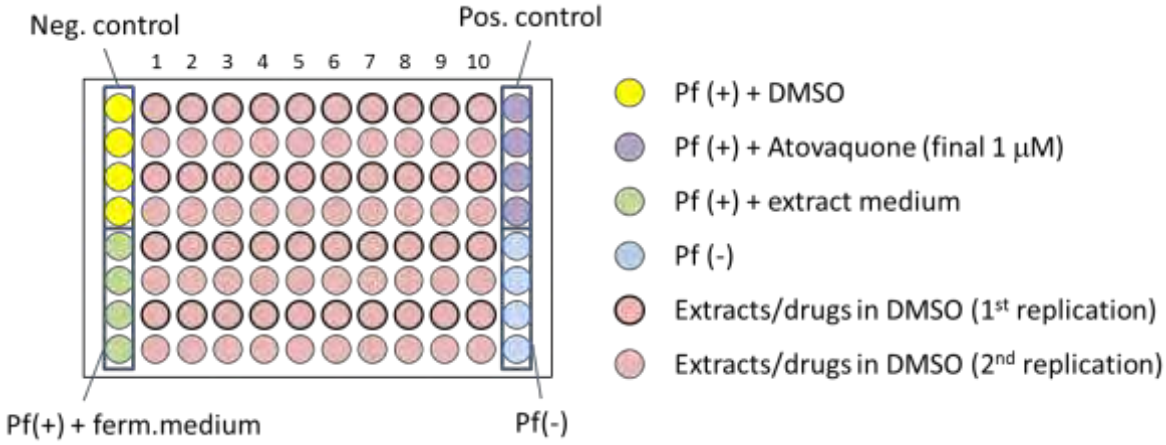
Prepared by	Verified by	Date of use
Dian Japany Puspitasari	Danang Waluyo	May 22nd, 2019

- **Sorbitol-synchronization of *Plasmodium falciparum***

1. Transfer 10 mL of culture into 50 mL centrifuge tube
2. Spin down the parasite culture at 800×g for 5 min at room temperature (RT), slow brake. Removed supernatant by aspirator.
3. Add 6 ml of 5% sorbitol solution and incubate for 15 min at RT. Shake 2 or 3 times.
4. Centrifuge the culture at 800×g for 5 min at RT, slow brake, then remove supernatant by aspirator.
5. Resuspend the cell with 6 mL RPMI(-), centrifuge the culture at 800×g for 5 min at RT, slow brake, then remove supernatant by aspirator.
6. Resuspend the cell with 10 ml of RPMI(+)
7. Count parasitemia (all forms).

- **Cell-based assay**

1. Use sterile 96-well plate flat-bottom culture plates with individual lid.
2. Label all plates with the parasite-line used, date, initials and plate-number. Each experiment should be done in duplicate on the same assay plate.
    - Typical assay plate layout



3. Determine % parasitemia of malarial culture treated with 5 %(w/v) D(-) sorbitol
4. Dilute the culture into 0.3% parasitemia using RPMI (+) medium with 3% hematocrit.
5. Pipette 0.4 µL extracts into red well (mix the deep well or dilution plate using plate mixer for 1 min at 650 rpm prior use)
6. Pipette 0.4 µL DMSO into yellow well (negative control).
7. Pipette 0.4 µL atovaquone 250 µM (stored -30°C)into purple well (positive control).
8. Pipette 0.4 µL extract medium into green well
9. Pipette 100 µL non-infected medium into blue well.

10. Pipette 100 µL parasite culture (initial parasitemia 0.3 %) into the rest well.
11. Incubate the plates at 37°C for 72 hours under 5%O_2 , 5%CO_2.

- **Harvest of assay plate**

1. Harvest the plate at 72 hours.
2. Fill each well with 200 µL cold PBS. Seal the plate with plastic seal.
3. Spin plate for 5 minutes at 1300×g at r.t with low brake.
4. Remove 240µL of supernatant without disturbing the pellet. Seal the plate again, then wrap the plate with plastic wrap.
5. Freeze the plates at −30°C to lyse erythrocytes. Plate can be frozen up to 1 month.

- **LDH assay**

1. Thaw LDH-buffer and warm up room temperature (10 mL LDH-buffer / plate).
2. Dissolve 2 mg of NBT* (nitroblue tetrazolium, stored at 4°C) in 10 mL of LDH buffer. Mix gently and keep the substrate in dark.
3. Add 50 µL APAD stock (10 mg/ml) to every 10 mL substrate.
4. Add 200 µL Diaphorase** stock (50 units/mL) to every 10 mL substrate.
5. Thaw the plates
6. Add 90 µL substrate per well of the harvested plates. One plate every minute.
7. Cover with aluminium-foil and place on a flatbed shaker at 650 rpm, room temperature.
8. Incubate for 30 minutes.
9. Measure A_{650} using plate reader. One plate every minute.
10. Calculate Z-factor using formula below

$$\mathrm{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

δ_p = standard deviation of A_{650} of Pf(+)+atovaquone samples

δ_n = standard deviation of A_{650} of Pf(+)+DMSO

μ_p = mean of A_{650} of Pf(+)+atovaquone samples

μ_n = mean of A_{650} of Pf(+)+DMSO

11. Calculate Δ value of signal

$$\Delta \text{ signal} = A_{650} negative\ control - A_{650} positive\ control$$

A_{650} negative control = average of A_{650} of Pf(+) + DMSO

A_{650} positive control = average of A_{650} of Pf(+) + atovaquone

12. Calculate % inhibition using formula below

$$\%\ \text{inhibition} = (1 - \frac{A_{650}\text{extracts or drug} - A_{650}\ \text{positive control}}{A_{650}\ \text{negative control} - A_{650}\ \text{positive control}}) \times 100\ \%$$

Positive control = Pf(+) + atovaquone

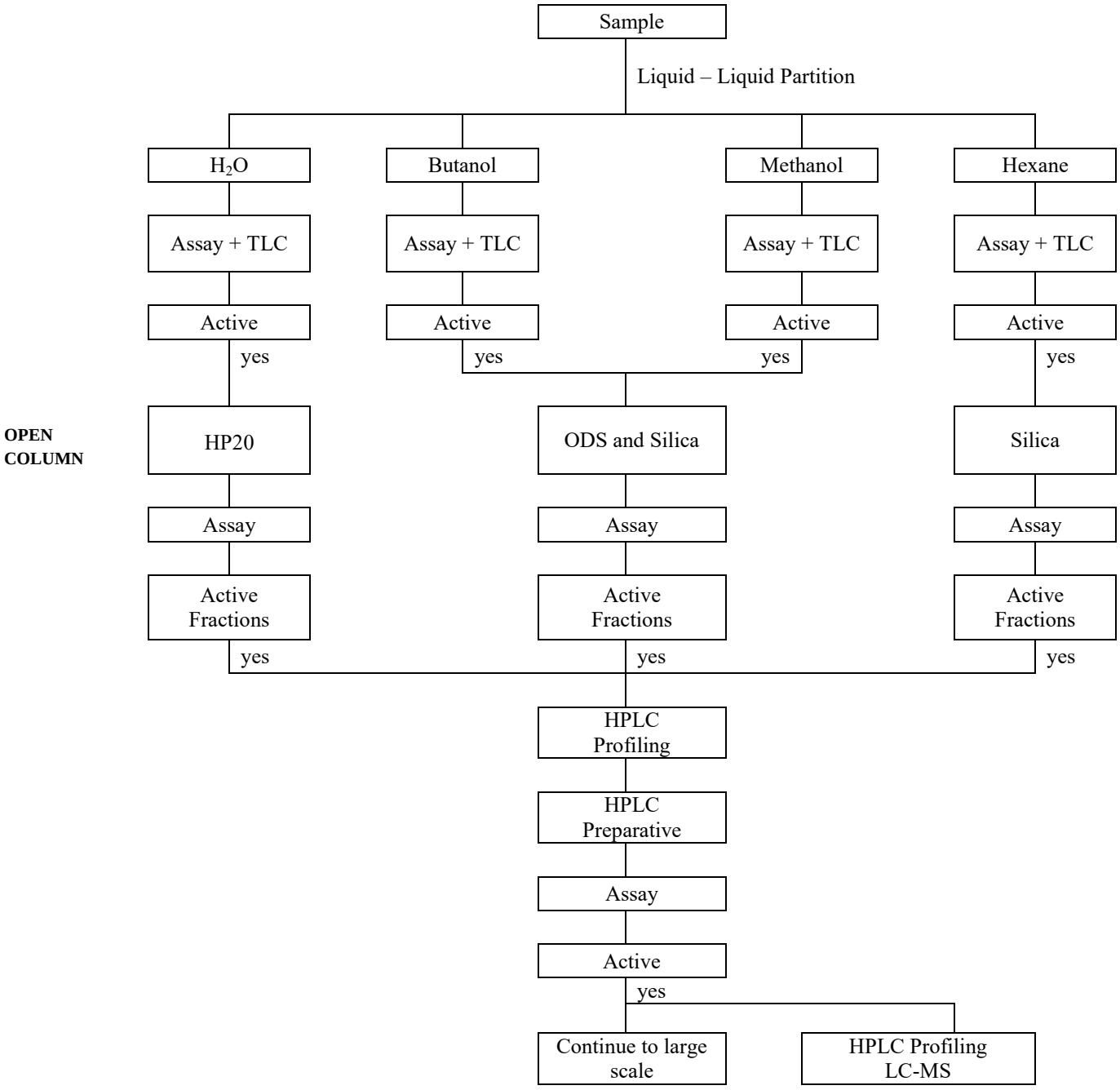
Negative control = Pf(+) + DMSO

NOTE:

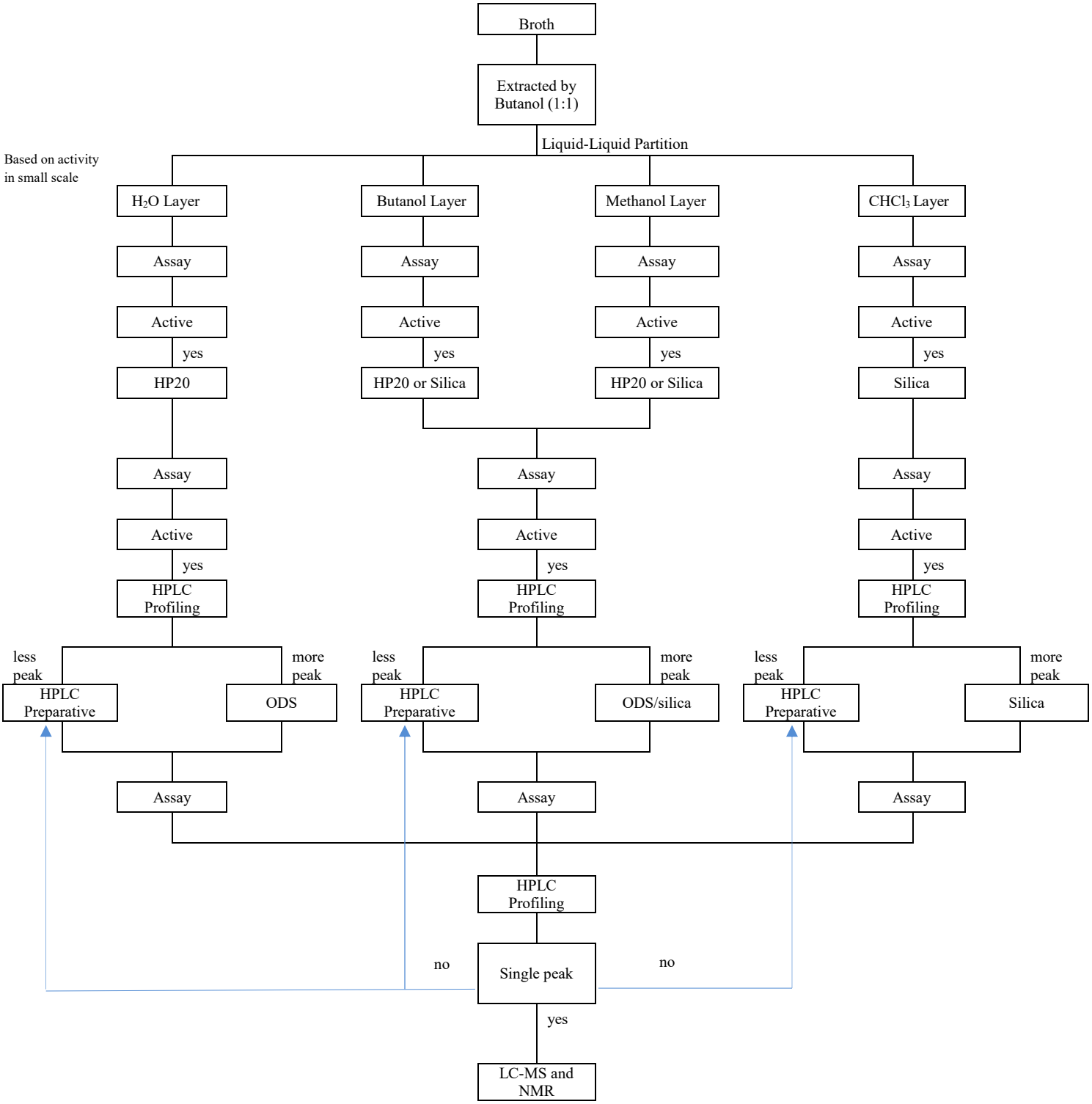
* NBT is light sensitive, so avoid direct light and keep solutions and plates with substrate in the dark (covered with aluminium-foil).

** Prepare diaphorase (from Sigma) solution stock in 50 units/mL. The unit may different for diaphorase produced by other company (Oriental yeast's diaphorase should be prepared by adding 10,000 units diaphorase with 30 mL MilliQ water).

Small Scale (20 mL)



Large Scale (5 L)



Liquid-Liquid Partition (for 20 mL dried butanol extract)

1. Resuspend dried crude extract in 20 ml of chloroform:water mixture (1:1).
2. Transfer the mixture into 100 mL separating funnel and shake for 1 minute. Do not forget to release gas from the funnel by opening the cock 3 times.
3. Wait until the layers are separated by placing the funnel on funnel stand. Transfer the chloroform layer (lower layer) into 100 mL Erlenmeyer flask.
4. Add 10 mL chloroform into separating funnel, shake for 1 minute, wait until the layers are separated, then recover the chloroform layer into the same flask as no.3.
5. Repeat this chloroform extraction 3 times in total (3 volumes of chloroform layer).
6. Dry up this chloroform layer (approx. 30 mL) using rotary evaporator. **Continue extraction of chloroform layer to produce hexane and methanol layers as described in no.7,** and **extraction of water layer to produce water and butanol layers as described in no.14.**

Hexane and Methanol Layer

7. Resuspend dried chloroform layer in 20 mL of hexane:methanol:water mixture (10:9:1)
8. Transfer the mixture into 100 mL separating funnel and shake for 1 minute. Do not forget to release gas from the funnel by opening the cock 3 times.
9. Wait until the layers are separated by separate placing the funnel on funnel stand. Transfer the hexane layer (lower layer) into 100 mL Erlenmeyer flask.
10. Add 10 mL hexane into separating funnel, shake for 1 minute, wait until the layers are separated, then recover the hexane layer into the same flask as no.9.
11. Repeat this hexane extraction 3 times in total (3 volumes of hexane layer).
12. Take 4 μL, 20 μL and 100 μL in triplicate of each layer into 96-well plate, then dry up using vacuum concentrator for further activity check.
13. Dry up this hexane layer (approx. 30 mL) and methanol layer (approx. 10 mL) using rotary evaporator.

Water and Butanol Layer

14. Add 10 mL of butanol into water layer (from step no.5, total volume ± 20 ml), shake for 1 minute, wait until the layers are separated, then recover the butanol layer (lower layer into 100 mL Erlenmeyer flask.
15. Repeat this butanol extraction 3 times in total (3 volumes of butanol layer).
16. Take 4 μL, 20 μL and 100 μL in triplicate of each layer into 96-well plate, then dry up using vacuum concentrator for further activity check.
17. Dry up this butanol layer (approx. 30 mL) and water layer (approx. 10 mL) using rotary evaporator.

Liquid-Liquid Partition (for 5 L dried butanol extract)

1. Resuspend dried crude extract (5 – 10 g) in 800 ml of chloroform:water mixture (1:1).
2. Transfer the mixture into 2 L separating funnel and shake for 1 minute. Do not forget to release gas from the funnel by opening the cock 3 times.
3. Wait until the layers are separated by placing the funnel on funnel stand. Transfer the chloroform layer (lower layer) into 2000 mL Erlenmeyer flask.
4. Add 400 ml chloroform into separating funnel, shake for 1 minute, wait until the layers are separated, then recover the chloroform layer into the same flask as no.3.
5. Repeat this chloroform extraction 3 times in total (3 volumes of chloroform layer).
6. Dry up this chloroform layer (approx. 1200 mL) using rotary evaporator. Continue extraction of chloroform layer to produce hexane and methanol layers as described in no.7, and extraction of water layer to produce water and butanol layers as described in no.14.

Hexane and Methanol Layer

7. Resuspend dried chloroform layer in 800 mL of hexane:methanol:water (10:9:1)
8. Transfer the mixture into 2 L separating funnel and shake for 1 minute. Do not forget to release gas from the funnel by opening the cock 3 times.
9. Wait until the layers are separated by separate placing the funnel on funnel stand. Transfer the hexane layer (lower layer) into 2000 mL Erlenmeyer flask.
10. Add 400 ml hexane into separating funnel, shake for 1 minute, wait until the layers are separated, then recover the hexane layer into the same flask as no.9.
11. Repeat this hexane extraction 3 times in total (3 volumes of hexane layer).
12. Take 4 μL, 20 μL and 100 μL in triplicate of each layer into 96-well plate, then dry up using vacuum concentrator for further activity check.
13. Dry up this hexane layer (approx. 1200 mL) and methanol layer (approx. 400 mL) using rotary evaporator.

Water and Butanol Layer

14. Add 400 mL of butanol into water layer (from step no.5, total volume ± 400 ml), shake for 1 minute, wait until the layers are separated, then recover the butanol layer (lower layer into 2000 mL Erlenmeyer flask.
15. Repeat this butanol extraction 3 times in total (3 volumes of butanol layer).
16. Take 4 μL, 20 μL and 100 μL in triplicate of each layer into 96-well plate, then dry up using vacuum concentrator for further activity check.
17. Dry up this butanol layer (approx. 1200 mL) and water layer (approx. 400 mL) using rotary evaporator.

OPEN COLUMN: SILICA GEL CHROMATOGRAPHY

Preparation of silica gel

1. Prepare silica gel (60-150 nm particle size, cat. Number XXXMerck) 30 x of sample weight in a beaker glass or 50 mL tube (example: for 1 g dried sample, prepare 30 mL bed volume of silica gel in chloroform).
2. Transfer the gel into column (glass or plastic) gently using Komagome-pipette (for 1 g sample the size column is ID: length: xxx).
3. Wash with 3 volumes of chloroform.

Preparation of sample

1. Weigh sample, then add chloroform until all dried sample are resuspended.
2. Transfer 1/20 bed volume of silica gel into sample suspension. Mix well, then apply onto surface of column using glass Pasteur pipette.

Fractionation

(The steps and composition of eluents may need to be modified)

1. Prepare 3 x of column bed volume of eluent with composition ratio as follow.

Eluent	Chloroform	Methanol	Water
A	100	-	-
B	95	5	-
C	90	10	-
D	80	20	1
E	70	30	5
F	60	40	10
G	50	50	15

2. Elute sample with eluent A – G sequentially. Collect eluted fraction from each eluent into 2 flasks/tubes.
3. Weigh empty glass tube. Transfer 5 mL sample from each fraction into glass tube, dry up using vacuum concentrator, and weigh the dried sample. Resuspend the sample with 1 mL MeOH.
   Note: For small scale purification, omit this step.
4. Transfer 2 μL, 10 μL and 50 μL of sample in triplicate into 96-well plate, dry up for further assay.
5. Dry active fraction using rotary evaporator for further process.
6. Discard used silica gel

OPEN COLUMN: Octadecylsilyl (ODS) GEL CHROMATOGRAPHY

Preparation of ODS gel

1. Prepare ODS gel (40 nm, 100 A, YMC) 30 x of sample weight in a beaker glass or 50 mL tube (example: for 0.1 g dried sample, prepare 3 mL bed volume of ODS gel in chloroform).
2. Transfer the gel into column (column size) (glass or plastic) gently (specific instruction) using Komagome-pipette.
3. Wash with 3 volumes of water.

Preparation of sample

1. Weigh sample, then add water until all dried sample are resuspended.
2. Transfer 1/20 bed volume of ODS gel into sample suspension. Mix well, then apply onto surface of column.
   .

Fractionation

1. Prepare 3 x of column bed volume of eluent with composition ratio as follow.

Eluent	Water	Methanol
A	100	-
B	80	20
C	60	40
D	40	60
E	20	80
F	0	100

2. Elute sample with eluent A – F sequentially. Collect eluted fraction from each eluent into 2 flasks/tubes.
3. Weigh empty glass tube. Transfer 5 mL sample from each fraction into glass tube, dry up using vacuum concentrator, and weigh the dried sample. Resuspend the sample with 1 mL MeOH.
   Note: For small scale purification, omit this step.
4. Transfer 2 μL, 10 μL and 50 μL of sample in triplicate into 96-well plate, dry up for further assay.
5. Dry active fraction using rotary evaporator for further process.
6. Wash used ODS with methanol 5x bed volumes.

OPEN COLUMN: Diaion-HP-20 GEL CHROMATOGRAPHY

Preparation of HP-20 gel

1. Prepare HP-20 gel (Diaion, Mitsubishi Chemicals) 30 x of sample weight in a beaker glass or 50 mL tube (example: for 1 g sample, prepare 30 mL bed volume of HP-20 gel in chloroform).
2. Transfer the gel into column (glass or plastic) gently (column size, specific instruction).
3. Wash with 3 volumes of water.

Preparation of sample

1. Weigh sample, then add water until all dried sample are resuspended.
2. Transfer1/20 bed volume of HP-20 gel into sample suspension. Mix well, then apply onto surface of column.

Fractionation

1. Prepare 3 x of column bed volume of eluent with composition ratio as follow.

Eluent	Water	Methanol
A	100	-
B	80	20
C	60	40
D	40	60
E	20	80
F	0	100

2. Elute sample with eluent A – F sequentially. Collect eluted fraction from each eluent into 2 flasks/tubes.
3. Weigh empty glass tube. Transfer 5 mL sample from each fraction into glass tube, dry up using vacuum concentrator, and weigh the dried sample. Resuspend the sample with 1 mL MeOH.
   Note: For small scale purification, omit this step.
4. Transfer 2 μL, 10 μL and 50 μL of sample in triplicate into 96-well plate, dry up for further assay.
5. Dry active fraction using rotary evaporator for further process.
6. Discard used HP-20 gel.

Thin Layer Chromotography (TLC) with Silica Gel Plate

1. Prepare each 5 ml solvent with composition as follow:
    - chloroform:methanol (9:1)
    - chloroform:methanol:water (7:3:0,5)
    - chloroform:methanol:water (5:5:1,5)
2. Add solvent into the chamber (alternatively glass bottle with flat bottom and wide neck) and cover it. Wait at least 10 minutes before applying plate into the chamber for saturating the headspace of chamber (Make sure, the solvent surface 7 mm from the bottom).
3. Prepare TLC plate (Silica Gel 60 F254 20 cm x 20 cm, Merck) by cutting silica gel plates 5 cm in height with various sizes of width depend on number of samples (typically 2 cm for 3 samples, or 4 cm for 7 samples). Mark the starting and end line with pencil at 1 cm from bottom and at 0.5 cm from upper edge. Mark the starting point for each sample with 0.5 cm in space between the points.
4. Prepare sample in 10 mg/mL of concentration.
   Typically, this is made by dry up 5 mL of fraction sample from each step of large scale purification and dissolves in methanol, or dry up all fraction samples from each step of small scale purification and dissolves in methanol.
5. Load samples on the plate with a capillary tube on each sample mark on the plate. Allow the spots to dry for 2 minutes.
6. Place the plate into the chamber carefully, with the top leaning against the chamber wall. Cover the chamber and let the solvent move up the plate by capillary action.
7. When solvent has reached the top mark of the plate, remove plate from chamber using forceps and dry by dryer.
8. After the plate dried, visualize spots under visible light (366 nm) and UV light (254 nm) and mark the spots with a pencil, and take picture of the plate.

ANALYTICAL HPLC

Preparation of sample

Prepare 1 ml sample with concentration 1-5 mg/ml in methanol in 1.5 ml Eppendorf tube. Centrifuge at 13,000 rpm for 10 min. Transfer at least 500 µL sample into a HPLC vial.

Preparation of eluent

Place 1000 mL of water (Eluent A) and 1000 mL of HPLC grade methanol (Eluent B) in 2 separate bottles and degas in water bath sonicator for 30 minutes.

HPLC condition

Injection volume : 10 µl

Flow rate : 1 ml/min

Gradient method :

Time	Eluent A	Eluent B
0.01	95	5
20	0	100
25	0	100
25.5	95	5
30	95	5

1. Prepare HPLC (L-20, Shimadzu):
   - Turn on the instrument
   - Turn on the pump to deliver eluent (A:B=95:5) with flow rate 1 mL/min. Let it stand by for 15 min.
   - Check the stability of pump pressure (Δ=5 kgf). Purging the line or check the eluent if the pressure is not stable.
   - Check the value of pump pressure. Typically, it should between 100 to 140 kgf. Check for any leakage if the pressure under 100 kgf, or check the condition of column if the pressure above 140 kgf.
2. Run the instrument by injecting 10 µL methanol, then observe the base line at typically wavelength 254 nm.
3. Run samples.
4. After finished, wash column with 50% MeOH for 30 min with flow rate 1 mL/min.
5. If column is not used for more than one week, remove from column oven, put column cap and properly store.
6. Turn off the instrument after finished.

Standard Operation Procedure
Assay for EhCS3 inhibitor

A. Preparing stock solution and reagents

1. Preparing 200 mM Tris-HCl pH=7.5 (500 mL)

a. Weigh 15.756 g of Tris-HCl (MW=157.56 g/mol).
b. Dissolve in 300 ml milli-Q water
c. Adjust pH to 7.6 using 10 M KOH
d. Adjust volume to 500 mL, then transfer into 500 mL glass bottle. Autoclve at 121ºC for 15 minutes
e. When needed transfer an aliquot into separate tube aseptically (use as working solution)
f. Store at r.t

2. Preparing 50 mM O-Acetyl L-Serine (OAS) (1 mL)

a. Weigh 9.2 mg of OAS (MW=183.6 g/mol) using 1.5 mL tube
b. Dissolve in 1 ml 10 mM HCl
c. Aliquot in 1.5 mL tube each 200 µL (smaller aliquot)
d. Store at -30ºC for maximum 6 months
e. After used do not return to -30ºC

3. Preparing solution of 500 mM Sodium sulphide (Na_2S) (1 mL)

a. Weigh 120 mg of Na_2S (MW=240.18 g/mol) using 1.5 mL tube
b. Dissolve in 1 ml milli-Q water
c. Aliquot in 1.5 mL tube each 200 µL
d. Wrap tube using aluminium foil
e. Store at -30ºC for maximum 6 months

4. Preparing Acid ninhydrin reagent (100 mL)

a. Transfer 40 mL of concentrated hydrochloric and 60 mL concentrated acetic acid to amber bottle, then mix well by stirring
b. Weight 2.5 g of ninhydrin
c. Add ninhydrin into the bottle containing mix of hydrochloric acid and acetic acid
d. Mix thoroughly using stirrer until all ninhydrin dissolved
e. Store the reagent at r.t

5. Preparing diluted EhCS3 recombinant enzyme

a. Transfer 3499 µL of 1x PBS into 15 mL tube

b. Transfer 1 µL of -30ºC stock enzyme EhCS3 (35 mg/ml) into 15 mL tube containing 3499 µL 1x PBS. Mix well by inverting the tube gently ([EhCS3= 10 ng/µL). (Have to verified to purification team)

c. After used discard diluted remaining enzyme

d. Aliquot diluted enzyme in 1.5 µL tube each 200 µL.

e. Store at -30ºC

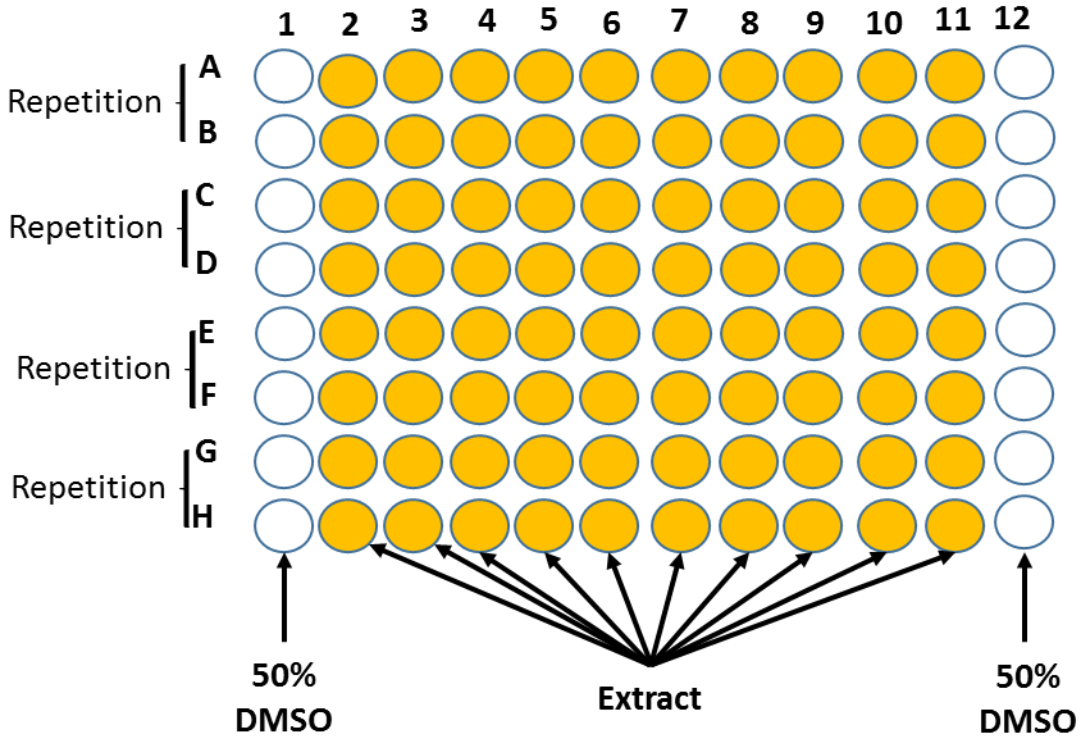
f. Before used, transfer 150 µL of 10 ng/µL EhCS3 into 15 mL tube containing 1350 1x PBS. Mix well by inverting the tube gently ([EhCS3= 1 ng/µL)

B. Preparing microbial extract for screening

1. Add 1 mL of DMSO in milli-Q into dried extract.
2. Resuspend the sample by water-bath sonicator 20 min, and mix by multi-well plate mixer (1000 rpm, 10 min). If the extract has not been resuspended, continue the mixing.
3. Transfer 10 µL of extract into 96-well assay plate (flat bottom) using electric multichannel pipette.

* Assay should be done in at least duplicate

* Refer to typical plate layout below to put the extract in the assay plate



C. Assay of screening enzyme inhibitor

1. Prepare assay mix in 15 mL tube (for 1 (one) 96-well assay plate), mix 1875 µL of 200 mM Tris-HCl pH = 7.5, 300 µL of 50 mM OAS (final concentration 2 mM), 30 µL of 500 mM Na_2S (final concentration 2 m), and 2295 µL milli-Q
2. Transfer all of assay mic into reagent reservoir
3. Transfer 30 µL assay mix into each well (pre-added with extract) using electric multi-channel pipette.
4. Mix the plate using plate mixer at 1000 rpm for 30 sec
5. Add 10 µL off 1 ng/µL EhCS3 into each well (except wells in row 12)
6. Incubate the plate at 37°C (water bath/PCR) for 10 minutes
7. Add 50 µL of concentrated acetic acid and 50 µL of nihydrin reagent into each well.
8. Mix the plate using plat mixer at 700 rpm for 30 sec
9. Heat the plate at 96ºC for 5 minutes
10. Transfer the plate into ice to cool it
11. Read the absorbance in end point mode using SpectraMax micro plate reader at 560 nm

D. Calculation

1. Inhibition

Inhibition of extract against enzyme was calculated as equation below:

$$100 - \left[\left(\frac{sample - negative\ control}{positive\ control}\right) \times 100\ \%\right]$$

Sample	= absorbance of sample
Positive control	= absorbance average of row no 12
Negative control	= absorbance average of row no 1

2. Z factor

Z factor is calculated as equation below:

$$Z\ factor = 1 - \left(\frac{3x\ \sigma\ positive\ control + 3x\ \sigma\ negative\ control}{\mu\ positive\ control - \mu\ negative\ control}\right)$$

Positive control	= absorbance decrease of row no 12
Negative control	= absorbance decrease of row no 1

A. Preparing stock solution and reagents

1. Preparing 1 M HEPES pH=8.0 (1000 mL)

a. Weigh 238.32 g of HEPES (MW=238.32 g/mol).
b. Dissolve in 500 ml milli-Q water
c. Adjust pH to 8.0 using 10 M KOH
d. Adjust volume to 1000 mL, then transfer into 1000 mL glass bottle.
e. Store at 4°C

2. Preparing 5 M NaCl (1000 mL)

a. Weigh 292.2 g NaCL (MW=58.44 g/mol)
b. Dissolve in 800 mL milli-Q water
c. Adjust volume to 1000 mL, then transfer into 1000 mL glass bottle.
d. Store at 4°C

3. Preparing 20% (v/v) Triton-X 100 (50 mL)

a. Transfer 10 mL triton-X 100 into 50 mL measuring cylinder.
b. Adjust volume to 50 mL by adding millli-Q water.
c. Mix well using stirrer gently to avoid bubbling.
d. Transfer to 50 mL tube.
e. Store at 4°C

4. Preparing assay buffer (composition: 100 mM HEPES pH=8, 50 mMNaCl, 10% (v/v) glycerol; 0.05 % triton-X 100) (1000 mL)

a. Mix 100 mL HEPES 1 M pH=8.0, 10 mL NaCl 5 M, 100 mL glycerol 100%, and 2.5 mL Triton-X 100 20% in 1000 mL measuring cylinder
b. Add milliQ water up to 1000 mL
c. Mix well using stirrer gently to avoid bubbling
d. Transfer to 1000 mL glass bottle
e. Store at 4°C

5. **Preparing solution of 200 mMdecyl ubiquinone (d-UQ) (MW=322.44 g/mol)**

   *** Keep d-UQ solution from light**

   a. Take a new 100 mg d-UQ stock.
   b. Add 1550 μL ethanol absolute into the reagent bottle directly.
   c. Dissolve by vortexing or water-bath sonicator
   d. Aliquot the solution into 1.5 mL tube, each 50 μL.
   e. Wrap the tube using aluminium foil to avoid from light.
   f. Seal the cap using parafilm to avoid evaporation.
   g. Store at -30°C.

6. **Preparing solution of 50 mM L-dihydroOrotate (L-DHO) (10 mL)**
   a. Weigh 79.055 mg L-DHO powder (stored at r.t., MW=158.11 g/mol)
   b. Dissolve in 10 mL milli-Q water using water-bath sonicator, and mix well by vortex
   c. Aliquot the solution into 15 mL tube each 1 mL.
   d. Store at -30°C
   e. Before use, thaw a tube on ice, add 9 mL milli-Q water to make a 10x dilution
   f. Mix well by vortexing

7. **Preparing solution 12 mMdichloro phenol indol phenol (DCIP) (5 mL)**

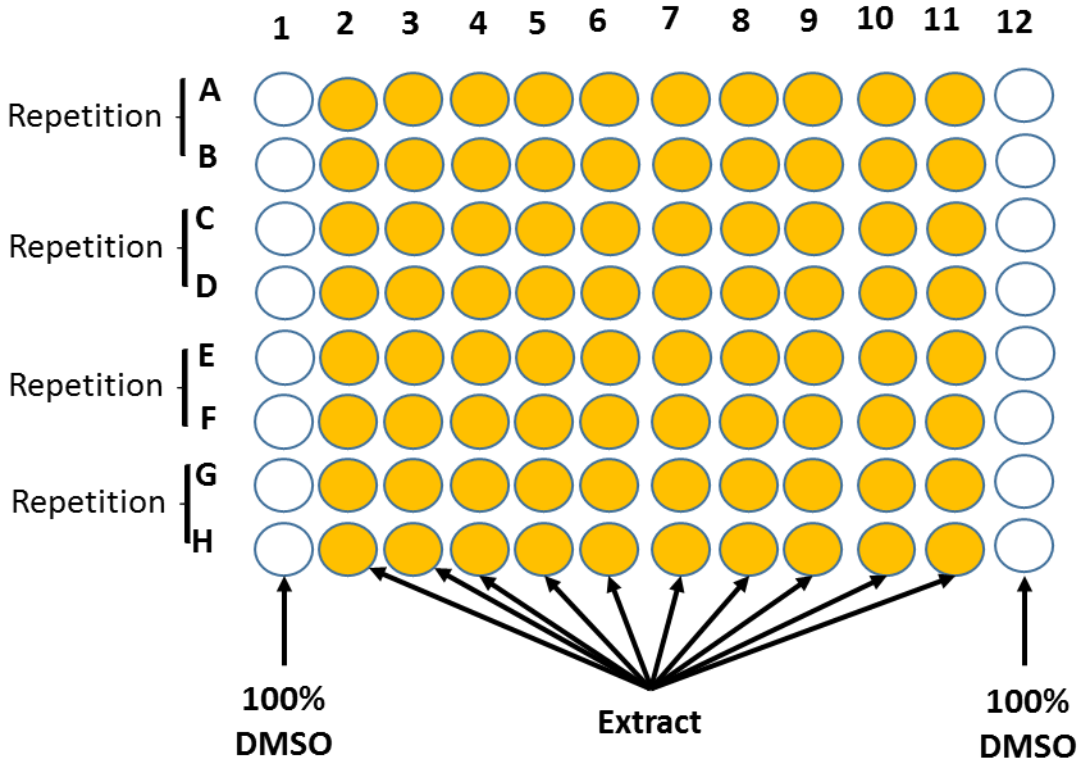
   *** Always prepare fresh DCIP solution**

   a. Weigh 17.4 mg DCIP powder (stored at r.t., MW=290.08 g/mol) in a small (10 mL) amber bottle.
   b. Dissolve in 5 mL milli-Q using water-bath sonicator, and mix well by vortex
   c. Use only for the same day, not to be stored.
   d. Keep from light.

B. Preparing extract for assay

* Assay should be done in at least duplicate

* Refer to typical plate layout below to put the extract in the assay plate



1. Microbial extract

a. Add 40 μL DMSO into dried extract.

b. Resuspend the extract by water-bath sonicator 20 min, and mix by multi-well plate mixer (1000 rpm, 10 min). If the extract has not been resuspended, continue the mixing.

c. Transfer 2 μL of extract into 96-well assay plate (flat clear bottom, nunc cat no 430341bottom) using electric multichannel pipette.

2. Plant extract

a. Prepare plant extract in DMSO at concentration 10 mg/ml

b. Transfer 2 μL of extract into 96-well assay plate (flat clear bottom, nunc cat no 430341) using electric multichannel pipette.

C. Assay of screening enzyme inhibitor

1. Prepare 20 mL assay mix in 50 mL tube (for 1 96-well assay plate), by mix 20 ml assay buffer, 200 μL of 12 mM DCIP (final concentration 120 μM), 1.8 μL of 200mMdecyl ubiquinone (final concentration 18 μM), 1.6 μL of recombinant enzyme PfDHODH (final concentration 20 nM).
2. Transfer all of assay mix into reagent reservoir.
3. Transfer 190 μL assay mix into each well (pre-added with extract) using electric multi-channel pipette.
4. Mix the plate using plate mixer at 500 rpm for 10 sec, then increase to 600 rpm for 10 sec, then increase again to 700 rpm for 10 sec.
5. Read the absorbance in kinetic mode using SpectraMax micro plate reader at 600 nm, 25°C for 1 minute. Save the data as background (refer to SOP for SpectraMaxMicroplate Reader)
6. Transfer the plate into plate mixer, add 8 μL of 5 mM L-DHO into each well (except wells in row 12).
7. Mix the plate as step no.3 above.
8. Read the absorbance in kinetic mode using SpectraMax micro plate reader at 600 nm, 25°C for 20 minutes. Save the data as activity (refer to SOP for SpectraMaxMicroplate Reader).

D. Calculated the inhibition of extract

1. Inhibition

Inhibition of extract against enzyme is calculated as equation below:

$$\%\ inhibition = 100 - \left[\left(\frac{sample - positive\ control}{negative\ control}\right) \times 100\ \%\right]$$

Sample = absorbance decreaseof sample after 20 minutes reading

Positive control = average of absorbance decrease of row no 12

Negative control = average of absorbance decrease of row no 1

2. Z factor

Z factor is calculated as equation below:

$$Z\ factor = 1 - \left(\frac{3x\ \sigma\ negative\ control + 3x\ \sigma\ positive\ control}{\mu\ negative\ control - \mu\ positive\ control}\right)$$

Positive control = absorbance decrease of row no 12

Negative control = absorbance decrease of row no 1

Note:

1. During absorbance reading in kinetic mode observe the graph of absorbance decrease
2. Absorbance decrease in the end of kinetic reaction should be around -1
3. If absorbance decrease is above -1, it is needed to add amount of enzyme in reaction mix
4. If absorbance decrease is under -1, it is needed to reduce amount of enzyme in reaction mix

A. Preparing stock solution and reagents

1. Preparing 1 M HEPES pH=8.0 (1000 mL)

a. Weigh 238.32 g of HEPES (MW=238.32 g/mol).

b. Dissolve in 500 ml milli-Q water

Put HEPES crystal little by little into milli-Q water while stirring. HEPES will be solidified if put first in the jar then added by water.

c. Adjust pH to 8.0 using 10 M KOH

d. Adjust volume to 1000 mL, then transfer into 1000 mL glass bottle.

e. Store at 4°C

2. Preparing solution of 60 mM decyl ubiquinone (d-UQ) from 200 mM d-UQ stock (200 µL)

*** Keep d-UQ solution from light**

a. Take 60 µL of 200 mM d-UQ stock.

b. Add140 µL of absolute ethanol

c. Mix well by vortexing

d. Aliquot the solution into 1.5 mL tube, each 50 µL.

e. Wrap the tube using aluminium foil to avoid from light.

3. Preparing solution of 1 M L-malate (20 ml)

a. Weight 2.64 g of L-malate (MW=132.071 g/mol)

b. Dissolve in 20 mL milli-Q water using water-bath sonicator, and mix well by vortex

c. Aliquot the solution in 15 mL tube each 4 mL

d. Store in -30ºC

e. Before use, thaw a tube on ice, add 6 mL milli-Q water to make 400 mM solution

f. Mix well by vortexing

4. Preparing 1 M Potassium cyanide (KCN) (5 mL)

a. Weight 325 mg of potassium (MW=65.117 g/mol)

b. Dissolve in 20 mL milli-Q water using water-bath sonicator, and mix well by vortex
c. Aliquot the solution in 1.5 mL tube each 1 mL
d. Store in -30ºC

5. Preparing solution 12 mM dichloro phenol indol phenol (DCIP) (5 mL)

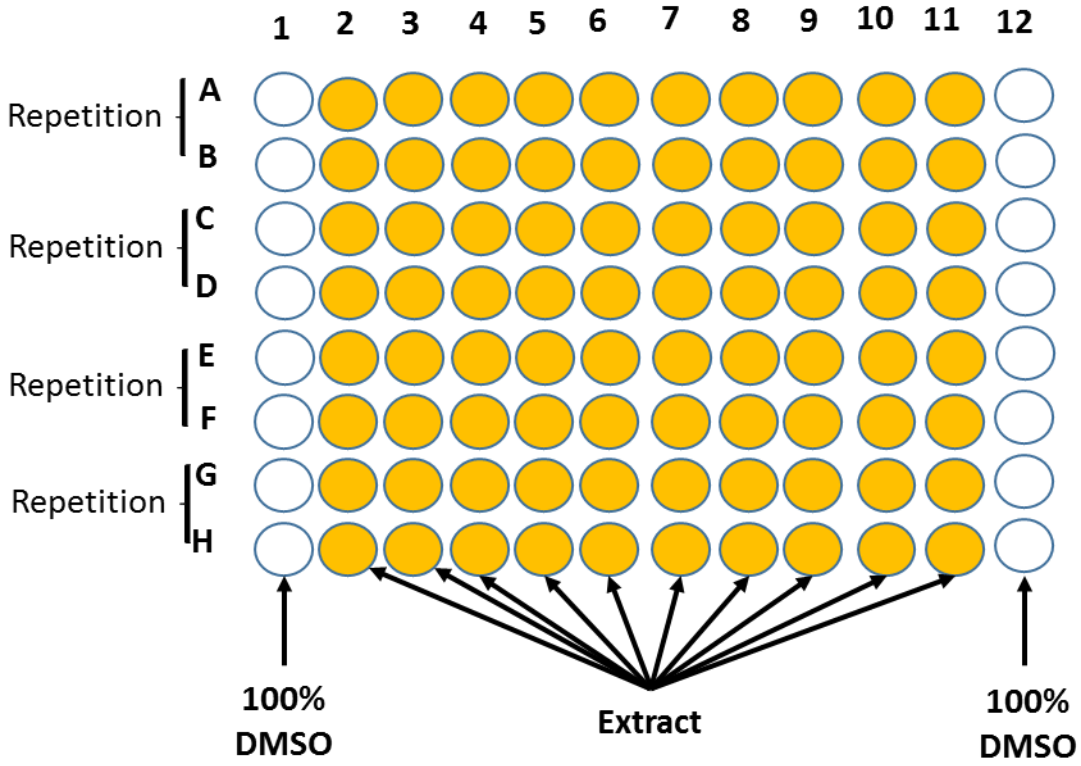
*** Always prepare fresh DCIP solution**

a. Weigh 17.4 mg DCIP powder (stored at r.t., MW=290.08 g/mol) in a small (10 mL) amber bottle.
b. Dissolve in 5 mL milli-Q using water-bath sonicator, and mix well by vortex
c. Use only for the same day, not to be stored.
d. Keep from light.

B. Preparing extract for screening

* Assay should be done in at least duplicate

* Refer to typical plate layout below to put the extract in the assay plate



1. Microbial extract

a. Add 40 µL DMSO into dried extract.

b. Resuspend the extract by water-bath sonicator 20 min, and mix by multi-well plate mixer (1000 rpm, 10 min). If the extract has not been resuspended, continue the mixing.

c. Dilute resuspended extract with DMSO using 96 well plate (V bottom).
Microbial extract produced using F1 and C medium : 25 fold
Microbial extract produced using F15 and A21 medium : 10 fold

d. Transfer 2 µL of diluted extract into 96-well assay plate (flat clear bottom, nunc cat no 430341bottom) using electric multichannel pipette

2. Plant extract

a. Prepare plant extract in DMSO at concentration 10 mg/ml

b. Transfer 2 μL of extract into 96-well assay plate (flat clear bottom, nunc cat no 430341bottom) using electric multichannel pipette.

C. Assay of screening enzyme inhibitor

1. Prepare 20 ml assay mix in 50 ml tube (for one 96-well assay plate)by mix20 ml 50 mM HEPES pH = 7.5, 200 uL of 12 mM DCIP (final concentration = 120 uM), 8.3uL of 60 mM decyl ubiquinone (final concentration = 25uM), 3.1uL of recombinant enzyme PfMQO (final concentration 2.5 μg/ml)
2. Transfer all of assay mix into reagent reservoir.
3. Transfer 193 μL of assay mix into each well micro plate using electric multi-channel pipette.
4. Mix the plate using plate mixer at 700 rpm for 10 sec, then increase to 900 rpm for 10 sec, then increase again to 1350 rpm for 10 sec
5. Read the absorbance in kinetic mode using SpectraMax micro plate reader at 600 nm, 37°C for 1 minute. Save the data as background (refer to SOP for SpectraMax Microplate Reader)
6. Transfer the plate into plate mixer, add 5μL of 400 mM L-malate into each well (except wells in row 12).
7. Mix the plate using plate mixer at 700 rpm for 10 sec, then increase to 900 rpm for 10 sec, then increase again to 1350 rpm for 10 sec
8. Read the absorbance in kinetic mode using SpectraMax micro plate reader at 600 nm, 37°C for 8 minute. Save the data as activity (refer to SOP for SpectraMax Microplate Reader)

D. Calculation

1. Inhibition

Inhibition of extract against enzyme was calculated as equation below:

$$\%\ inhibition = 100 - \left[\left(\frac{sample - positive\ control}{negative\ control}\right) \times 100\ \%\right]$$

Sample = absorbance decrease of sample after 20 minutes reading

Positive control = average of absorbance decrease of row no 12

Negative control = average of absorbance decrease of row no 1

2. Z factor

Z factor is calculated as equation below:

$$Z\ factor = 1 - \left(\frac{3x\ \sigma\ negative\ control + 3x\ \sigma\ positive\ control}{\mu\ negative\ control - \mu\ positive\ control}\right)$$

Positive control = absorbance decrease of row no 12

Negative control = absorbance decrease of row no 1

Note:

1. During absorbance reading in kinetic mode observe the graph of absorbance decrease
2. Absorbance decrease in the end of kinetic reaction should be around -1
3. If absorbance decrease is above -1, it is needed to add amount of enzyme in reaction mix
4. If absorbance decrease is under -1, it is needed to reduce amount of enzyme in reaction mix

Standard Operation Procedure
Assay for EhSAT1 inhibitor

A. Preparing stock solution and reagents

1. Preparing 200 mM Tris-HCl pH=7.5 (500 mL)

a. Weigh 15.756 g of Tris-HCl (MW=157.56 g/mol).
b. Dissolve in 300 ml milli-Q water
c. Adjust pH to 7.6 using 10 M KOH
d. Adjust volume to 500 mL, then transfer into 500 mL glass bottle.
e. Store at 4°C

2. Preparing solution of 500 mM Sodium sulphide (Na_2S) (1 mL)

a. Weigh 120 mg of Na_2S (MW=240.18 g/mol) using 1.5 mL tube
b. Dissolve in 1 ml milli-Q water
c. Wrap tube using aluminium foil
d. Store at -30ºC

3. Preparing 50 mM L-serine (1 mL)

a. Weight 5.25 mg of L-serine (MW=105.09 mg/mol) using 1.5 mL tube
b. Dissolve in 1 mL milli-Q water
c. Store at -30ºC

4. Preparing 25 mM Acetyl Co-A (1 mL)

a. Weight 20.239 mg of Acetyl Co-A (MW=809.57 g/mol) using 1.5 mL tube
b. Dissolve in 1 mL milli-Q water
c. Aliquot in 1.5 mL tube each 200 µL
d. Store at -30°C

5. Preparing Acid ninhydrin reagent.

a. Transfer 40 mL of concentrated hydrochloric and 60 mL concentrated acetic acid to amber bottle, then mix well by stirring
b. Weight 2.5 g of ninhydrin
c. Add ninhydrin into the bottle containing mix of hydrochloric acid and acetic acid
d. Mix thoroughly using stirrer until all ninhydrin dissolved
e. Store the reagent at r.t

6. Preparing diluted EhSAT1 recombinant enzyme

a. Transfer 1450 µL of 1x PBS into 15 mL tube
b. Transfer 50 µL of frozen stock enzyme EhSAT1 (3 mg/ml) into 15 mL tube containing 3490 µL 1x PBS. Mix well by inverting the tube gently ([EhSAT1 3= 100 ng/µL)

c. Directly use for 1 (one) of 96-well assay mix

7. Preparing diluted EhCS3 recombinant enzyme

a. Transfer 3499 µL of 1x PBS into 15 mL tube

b. Transfer 1 µL of frozen stock enzyme EhCS3 (35 mg/ml) into 15 mL tube containing 3499 µL 1x PBS. Mix well by inverting the tube gently ([EhCS3= 10 ng/µL)

c. Aliquot diluted enzyme in 1.5 µL tube each 200 µL.

d. Store at -30ºC

B. Preparing microbial extract for screening

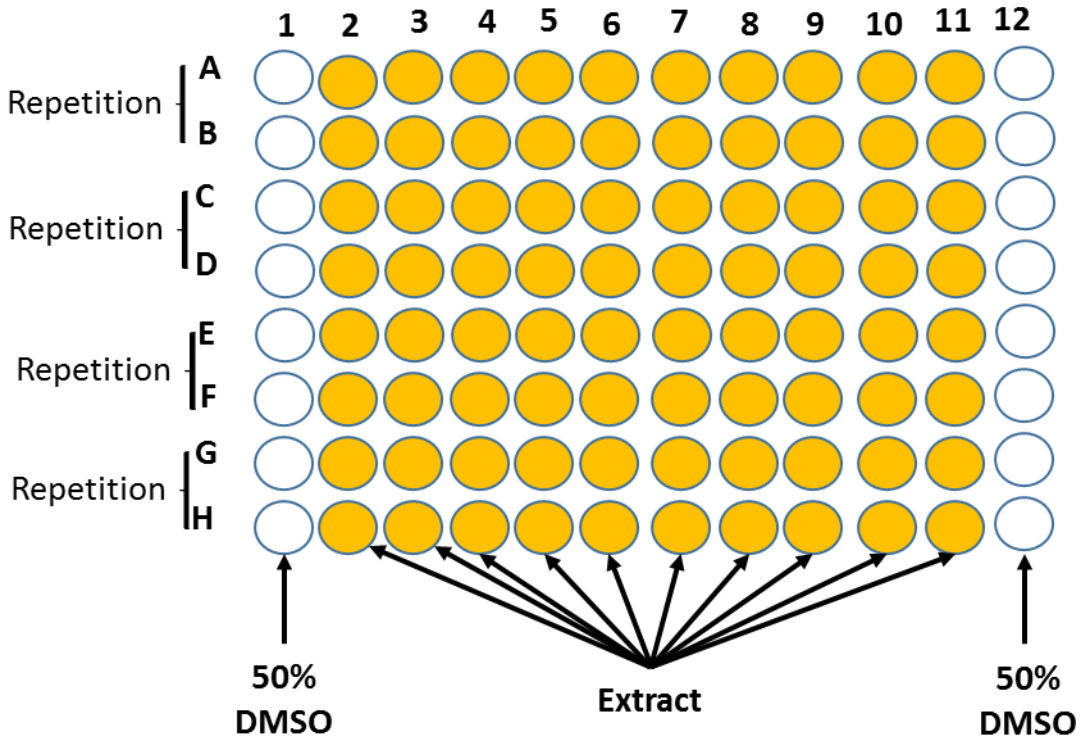
a. Add 1 mL of DMSO in milli-Q into dried extract.

b. Resuspend the extract by water-bath sonicator 20 min, and mix by multi-well plate mixer (1000 rpm, 10 min). If the extract has not been resuspended, continue the mixing.

c. Transfer 10 µL of extract into 96-well assay plate (flat bottom) using electric multichannel pipette.

* Assay should be done in at least duplicate

* Refer to typical plate layout below to put the extract in the assay plate



C. Assay of screening enzyme inhibitor

1. Prepare assay mix in 15 mL tube (for 1 (one) 96-well assay plate), mix : 1875 µl of 200 mM Tris-HCl pH = 7.5, 300 µL of 50 mM OAS, 30 µL of 500 mM Na_2S, 25 µL of

diluted EhCS3 (10 ng/µL), 90 µL of 50 mM L-serine, 180 µL of 25 mM Acetyl Co-A, 2000 µL milli-Q

2. Transfer all of assay mic into reagent reservoir
3. Transfer 30 µL assay mix into each well (pre-added with extract) using electric multi-channel pipette.
4. Mix the plate using plate mixer at 1000 rpm for 30 sec
5. Add 10 µL off 100 ng/µL EhSAT1 into each well (except wells in row 12)
6. Incubate the plate at 37°C for 10 minutes
7. Add 50 µL of concentrated acetic acid and 50 µL of nihydrin reagent into each well.
8. Mix the plate using plat mixer at 700 rpm for 30 sec
9. Heat the plate at 96ºC for 5 minutes
10. Transfer the plate into ice to cool it
11. Read the absorbance in end point mode using SpectraMax micro plate reader at 560 nm

D. Calculatition

1. Inhibition

Inhibition of extract against enzyme was calculated as equation below:

$$100 - \left[\left(\frac{sample - negative\ control}{positive\ control} \right) \times 100\ \% \right]$$

Sample = absorbance of sample

Positive control = absorbance average of row no 12

Negative control = absorbance average of row no 1

2. Z factor

Z factor is calculated as equation below:

$$Z\ factor = 1 - \left(\frac{3x\ \sigma\ positive\ control + 3x\ \sigma\ negative\ control}{\mu\ positive\ control - \mu\ negative\ control} \right)$$

Positive control = absorbance decrease of row no 12

Negative control = absorbance decrease of row no 1

Standard Operation Procedure

Analysis of protein concentration (Bradford method)

1. Prepare reaction mix of protein standard and sample as following table:

	Control buffer (µL)	Milli-Q water (µL)	2 mg/ml BSA (µL)	Protein sample (µL)	Bio-rad protein assay dye (µL)
Standard 0	2	798	0	-	200
Standard 1	2	797	1	-	200
Standard 2	2	796	2	-	200
Standard 3	2	795	3	-	200
Standard 4	2	794	4	-	200
Standard 5	2	793	5	-	200
Sample	-	798	-	2	200

Note:

- Control buffer = buffer and glycerol in same amount, which is used for enzyme storage
- Volume of control buffer must be same with sample volume
- Total reaction is 1 mL. If volume sample and control buffer change, adjust volume of milli-Q water.
- BSA = bovine serum albumin

2. Mix all reaction mix using vortex
3. Incubate at 37ºC for 30 minutes
4. Read A_{595} using spectrophotometer
5. Plot concentration of BSA standard vs A_{595} as a linier graph by Microsoft excel and calculate the equation of the graph.
6. Calculate concentration of protein based on the equation of protein standard.
   Note: Don't forget to take sample dilution into account.

Standard Operating Procedure **Production of PfDHODH (For 500 mL main culture, typically producing for 40 mg protein)**	No:	PfDHODH-1
	Page:	1/5

Microbial revival

1. Take a frozen stock of *Eschericia coli*BL21 star (DE3) pET Sumo PfDHODH out from -80°C, then transfer into ice box.
2. Scratch the frozen stock using a loop then streak on an LB agar plate supplemented with 50 mg/mL kanamycin.
3. Incubate the plate at 37°C for overnight.
4. Discard the frozen stock that has been used (do not return into deep freezer).

Pre-culture

1. Inoculate a colony of *E.coli* BL21 star (DE3) pET Sumo PfDHODH that is freshly revived from frozen stock on LB-Kanamycin (Brand) agar medium into 50 mL LB medium (supplemented with 50 μg/mL kanamycin) in 500 mL Erlenmeyer flask. Add 25 μL of 100 mg/mL kanamycin stock into 50 mL medium.
2. Incubate at 37^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Main culture

1. Transfer 50 mL pre-culture broth of *E.coli* BL21 star (DE3) pET Sumo PfDHODH into 500 mL TB medium(supplemented using 50 μg/ml kanamycin) in 2000 mL Erlenmeyer flask. (Add 250μL of 100 mg/mL kanamycin stock into 500 mL medium)
2. Incubated the culture at 37^{o}C under vigorous orbital shaking (200 rpm)
3. Check OD_{600} of the culture. If OD_{600} culture reach 0.6 (typically in 1-2 hours), add IPTG so that final concentration is 250 μM(add 125 μL of 1 M IPTG to 500 ml medium). Continue the incubation at 20^{o}C under vigorous orbital shaking (200 rpm)for overnight.

Harvest *E. coli* cell

1. Transfer overnight culture into 500 ml centrifuge bottle.
2. Centrifuge at 4000 x g, 4°C, for 15 minutes. (Kubota 7780, rotor AG 5006)

Prepared by	Verified by	Date of use
Erwahyuni EP	Danang Waluyo	May 24th,2019

3. Discard supernatant by carefully decantation, and resuspend the cells using 20 ml lysis buffer for each 500 ml *E.coli* culture. (Lysis buffer: 50 mM HEPES-KOH pH=7.6; 50 mMNaCl; 5 mM Imidazole; 20% (v/v) Glycerol). Cell can be washed by resuspending pellet with 25 ml 1xPBS and centrifuge as no 2 then discard the supernatant by pipetting.
4. Transfer cell suspension in lysis buffer into a metal cup (100 mL) for cell lysis. The suspension can be temporary stored at -80°C in 50 mL tube. (We will add the length of storage cell suspension in lysis buffer)

Cell lysis

1. Break the cell using sonicator (Branson digital sonifier, model 102C, second biggest probe)5 sec on, 20 sec off 10 set at 4°C (Do it on ice).
2. Check whether the cells are broken completely. Extend the time for sonication if the cells are not completely broken. The colour of cell suspension will turn from milky-white into clearer suspension. Alternatively examine the suspension in the microscope, compare between sonicated cell and unsonicated cell (save unsonicated sample as control)
3. Centrifuged at 18000 x g, 4°C for 60 minutes.
4. Transfer supernatant into 50 mL tube, keep on ice until purification.

Enzyme purification.

1. Mix 50% Ni-NTA resin (in 20% ethanol, stored at 4°C) well, then immediately transfer 1.5 mL into 15 mL tube.
2. Centrifuge at 800 x g for 5 min at r.t.
3. Discard supernatant, resuspend with 10 mL milli-Q water.
4. Centrifuge at 800 x g for 5 min at r.t.
5. Discard supernatant, resuspend with 10 mL milli-Q water.
6. Centrifuge at 800 x g for 5 min at r.t. Discard supernatant.

7. Transfer a part lysed cell suspension (typically 1 mL) into resin, then transfer back into lysed cell suspension tube. Repeat this step until all resins are transferred.
8. Mix the lysed cell suspension and resin by mild shaking at 4°C for overnight.
9. Transfer the suspension into disposable 15 mL plastic column. Collect the flowthrough with 50 mL tube.
10. Wash the resin using 20 mL washing buffer (50 mM HEPES-KOH pH 7.6; 300 mMNaCl; 10% (v/v) glycerol; 0.2 mMorotate; 0.05% C12E9; 20 mM Imidazole). Collect the wash wafer with 50 mL tube.
11. Elute using 4 mL elution buffer (50 mM HEPES-KOH pH 7.6; 300 mMNaCl; 10% (v/v) glycerol; 0.2 mMorotate; 0.05% C12E9; 300 mM Imidazole). Collect the elutedprotein with 15 mL tube.
12. Analysis protein concentration and calculated total protein in eluted protein
13. Add Sumo protease with ratio protein PfDHODH:Sumo protease = 50:1, to cleave pET Sumo
14. Fill up to 40 ml with cleavage buffer (50 mMTrisHCl pH=8.0; 0.05% C9H12; 0.2 mMOrotate).
15. Shakeenzyme gently at 4°C for overnight
16. Prepare resin Ni-NTA as described before (1-6).
17. Transfer resin into cleavage enzyme, continue shaking for 2.5 hours
18. Transfer all of cleavage enzyme with resin into 15 mL plastic column
19. Collect eluted PfDHODH protein using 50 mL tube
20. Transfer all the protein into centrifugal filter unit (Centricon, 15 mL, MWCO 30kDa), then centrifuge at 4500xg, 4°C until the remained volume is about 500 μL.
21. Transfer the retained protein into 15 mL tube, then add 100% glycerol so the final concentration of glycerol is 50%. (Do not forget to calculate the amount of glycerol in elution buffer).

    Example for calculating glycerol adding:

    (500 μL x 10%) + (V μL x 100%) = (500 + V) μL x 50%

    $$5000 + 100V = 25000 + 50V$$

$$(100-50)\ V = 25000-5000$$

$$50V = 20000$$

$$V = 400\ \mu L$$

22. Aliquot into 1.5 mL tube each 200 μL.
23. Put label : enzyme name, preparation date, concentration, name of person, no of tube
24. Store at -30°C until used (length of storage will be added later)
25. Analyse protein concentration and purity by following each SOP
26. Do not take out enzyme from freezer, work by the freezer.

Appendix

A. LB medium plate (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl Brand (Merck)	: 10 g
Agar (Oxoid)	: 20 g

1. Put 800 mL milli-Q water into a beaker glass.
2. Weigh all ingredients except agar, then dissolve in milli-Q water using a stirrer.
3. Add agar after other all ingredients already dissolve, mix well using stirrer
4. Add milli-Q water up to 1000 mL using a measuring cylinder.
5. Sterilize the medium using autoclave at 121°C for 15 minutes.
6. Pour warm medium into sterile disposable petri dish, wait until cool then cover it
7. Store medium in 4ºC until use
8. Transfer medium into incubator 37ºC prior to use. Put 20 µL of 50 mg/ml kanamycin then spread kanamycin in the surface of plate using sterile glass spreader

B. LB medium broth (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl (Merck)	: 10 g

1. Put 800 ml milli-Q into beaker glass

2. Weight all ingredient, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Use the medium after cooled down at room temperature

C. TB medium broth (1000 mL)

Trypton (Oxoid)	: 12.0 g
Yeast extract (Oxoid)	: 24.0 g
Potassium phosphate dibasic (Wako)	: 9.4 g
Potassium phosphate monobasic (Wako)	: 2.2 g
Glycerol 80%	: 5.0 ml

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, except glycerol, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Sterilize 80% glycerol separately using autoclaved 121ºC for 15 minutes
6. Use the medium after cooled down at room temperature
7. Add sterilized 80% glycerol aseptically just before using the medium

D. 50 mg/ml Kanamycin stock (Sigma-Aldrich, K4000-25g) (10 mL)
1. Weight 500 mg of kanamycin powder
2. Dissolve in 8 mLmilli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using sterile membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

E. 1M Isopropyl-β-D-(-)-thiogalactopyranoside (IPTG) (Wako, 099-02534, MW=238.30 g/mol) (10 mL)
1. Weight 2.389 g of IPTG powder
2. Dissolve in 8 mL milli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using streil membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

Standard operation procedure

Enzyme EhCS3 production

(For 500 mL main culture, typically producing for 14 mg protein)

Microbial revival

1. Take a frozen stock of *Eschericia coli* BL21 star (DE3) pET 15b EhCS3 out from -80°C, then transfer into ice box.
2. Scratch the frozen stock using a loop then streak on an LB agar plate supplemented with 100 mg/mL amphicilin or carbenicilin.
3. Incubate the plate at 37°C for overnight.
4. Discard the frozen stock that has been used (do not return into deep freezer).

Pre-culture

1. Inoculate a colony of *E.coli* BL21 star (DE3) pET 15b EhCS3 that is freshly revived from frozen stock on LB-amphicilin or LB-carbenecillin agar medium into 50 mL LB medium (supplemented with 100 μg/mL amphicilin or carbenicillin) in 500 mL Erlenmeyer flask. Add 50 μL of 100 mg/mL amphicilin or carbenicilin stock into 50 mL medium
2. Incubate at 37^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Main culture

1. Transfer 50 mL pre-culture broth of *E.coli* BL21 star (DE3 pET 15b EhCS3 into 500 mL 2YT medium (supplemented using 100 μg/mL amphicilin or carbenicillin) in 2000 mL Erlenmeyer flask. Add 500 μL of 100 mg/mL amphicilin or carbenicilin stock into 500 mL medium
2. Incubated the culture at 37^{o}C under vigorous orbital shaking (200 rpm)
3. Check OD600 of the culture. If OD_{600} culture reach 0.6 (typically in 1-2 hours), add IPTG so that final concentration is 5 mM (add 500 μL of 1 M IPTG into 500 mL medium). Continue the incubation at 20^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Harvest *E. coli* cell

1. Transfer overnight culture into 500 ml centrifuge bottle.
2. Centrifuged at 4000 x g, 4°C, for 15 minutes. (Kubota 7780, rotor AG 5006).
3. Discard supernatant, and resuspend the cells using 20 ml lysis buffer for each 500 ml *E.coli* culture. (Lysis buffer: 50 mM Tris HCl pH=8.0; 300 mM NaCl; 10% glycerol).

4. Transfer cell suspension into a metal cup (100 mL) for cell lysis. The suspension can be temporary stored at -80°C in 50 mL tube.

Cell lysis

1. Break the cell using sonicator (Branson digital sonifier, model 102C, second biggest probe) 5 sec on, 20 sec off 10 set at 4°C (Do it on ice).
2. Check whether the cells are broken completely. Extend the time for sonication if the cells are not completely broken. The colour of cell suspension will turn from milky-white into clearer suspension. Alternatively examine the suspension in the microscope, compare between sonicated cell and unsonicated cell (save unsonicated sample as control)
3. Centrifuged at 18000 x g, 4°C for 60 minutes.
4. Transfer supernatant into 50 mL tube, keep on ice until purification

Enzyme purification.

1. Mix 50% Ni-NTA resin (in 20% ethanol, stored at 4°C) well, then immediately transfer 1.5 mL into 15 mL tube.
2. Centrifuge at 800 x g for 5 min at r.t.
3. Discard supernatant, resuspend with 1 mL milli-Q water.
4. Centrifuge at 800 x g for 5 min at r.t.
5. Discard supernatant, resuspend with 1 mL milli-Q water.
6. Centrifuge at 800 x g for 5 min at r.t. Discard supernatant.
7. Transfer a part lysed cell suspension (typically 1 mL) into resin, then transfer back into lysed cell suspension tube. Repeat this step until all resins are transferred.
8. Mix the lysed cell suspension and resin by mild shaking at 4°C for overnight.
9. Transfer the suspension into disposable 15 mL plastic column. Collect the flow through with 50 mL tube.
10. Wash the resin using 20 mL washing buffer (50 mM Tris HCl pH=8.0; 300 mM NaCl; 10% glycerol; 20 mM Imidazole). Collect the flow through with 50 mL tube.
11. Elute using 4 mL elution buffer (50 mM Tris HCl pH=8.0; 300 mM NaCl; 10% glycerol; 250 mM Imidazole). Collect the flow through with 15 mL tube.
12. Transfer all the elution liquid into centrifugal filter unit (Centricon, MWCO 30kDa), then centrifuge at 4500 x g, 4°C until the remained volume is about 500 μL.
13. Transfer the retained liquid into 15 mL tube, then add 100% glycerol so the final concentration of glycerol is 50%. (Do not forget to calculate the amount of glycerol in elution buffer).

Example for calculating glycerol adding:

(500 μL x 10%) + (V μL x 100%) = (500 + V) μL x 50%

$$5000 + 100V = 25000 + 50V$$

$$(100-50)\,V = 25000-5000$$

$$50V = 20000$$

$$V = 400\ \mu L$$

14. Aliquot into 1.5 mL tube each 200 μL.
15. Store at -30°C until used
16. Analyse protein concentration and purity by following each SOP
17. Do not take out enzyme from freezer, work by the freezer.

Appendix

A. LB medium plate (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl (Merck)	: 10 g
Agar (Oxoid)	: 20 g

1. Put 800 mL milli-Q water into a beaker glass.
2. Weigh all ingredients except agar, then dissolve in milli-Q water using a stirrer.
3. Add agar after other all ingredients already dissolve, mix well using stirrer
4. Add milli-Q water up to 1000 mL using a measuring cylinder.
5. Sterilize the medium using autoclave at 121°C for 15 minutes.
6. Pour warm medium into sterile disposable petri dish, wait until cool then cover it
7. Store medium in 4ºC until use
8. Transfer medium into incubator 37ºC prior to use. Put 20 µL of 50 mg/ml kanamycin then spread kanamycin in the surface of plate using sterile glass spreader

B. LB medium broth (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl (Merck)	: 10 g

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Use the medium after cooled down at room temperature

C. 2 YT medium broth (1000 mL)

Trypton (Oxoid)	: 16 g
Yeast extract (Oxoid)	: 10 g
NaCl (Merck)	: 5 g

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Use the medium after cooled down at room temperature

D. 100 mg/mL Carbenicilin stock (Wako, Cat No. 035-23694), 10 mL
1. Weight 1 g of Carbenicilin powder
2. Dissolve in 8 mL milli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using sterile membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

E. 1M Isopropyl-β-D-(-)-thiogalactopyranoside (IPTG) (Wako, 099-02534, MW=238.30 g/mol) (10 mL)
1. Weight 2.389 g of IPTG powder
2. Dissolve in 8 mL milli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using streil membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

Standard Operating Procedure **Production of PfMQO (For 500 mL main culture, typically producing for 93 mg protein)**	No:	PfMQO-1
	Page:	1/4

Microbial revival

1. Take a frozen stock of *Eschericia coli*BL21 star (DE3) pET Sumo PfMQO out from -80°C, then transfer into ice box.
2. Scratch the frozen stock using a loop then streak on an LB agar plate supplemented with 50 mg/mL kanamycin.Add 25 μL of 100 mg/mL kanamycin stock into 50 mL medium.
3. Incubate the plate at 37°C for overnight.
4. Discard the frozen stock that has been used (do not return into deep freezer).

Pre-culture

1. Inoculate a colony of *E.coli*BL21 star (DE3) pET Sumo PfMQO that is freshly revived from frozen stock on LB-Kanamycin agar medium into 50 mL LB medium (supplemented with 50 μg/mL kanamycin) in 500 mL Erlenmeyer flask. Add 250 μL of 100 mg/mL kanamycin stock into 500 mL medium.
2. Incubate at 37^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Main culture

1. Transfer 50 mL pre-culture broth of *E.coli*BL21 star (DE3) pET Sumo PfMQO into 500 mL TB medium(supplemented using 50 μg/ml kanamycin) in 2000 mL Erlenmeyer flask.
2. Incubated the culture at 37^{o}C under vigorous orbital shaking (200 rpm)
3. Check OD600 of the culture. If OD_{600} culture reach 0.6 (typically in 1-2 hours), add IPTG so that final concentration is 20 μM (Add 10 μL of 1M IPTG stock into 500 mL medium). Continue the incubation at 20^{o}C under vigorous orbital shaking (200 rpm)for overnight.

Harvest *E. coli* cell

1. Transfer overnight culture into 500 ml centrifuge bottle.
2. Centrifuged at 4000 x g, 4°C, for 15 minutes.

Prepared by	Verified by	Date of use
Erwahyuni EP	Danang Waluyo	May 24th,2019

3. Discard supernatant, and resuspend the cells using 20 ml lysis buffer for each 500 ml *E.coli* culture. (Lysis buffer: 50 mM HEPES-KOH pH 7.6; 0.25 mM PMSF).
4. Transfer cell suspension into a metal cup (100 mL) for cell lysis. The suspension can be temporary stored at -80°C in 50 mL tube.

Cell lysis

1. Break the cell using sonicator (Branson digital sonifier, model 102C, second biggest probe) 5 sec on, 20 sec off 10 set at 4°C (Do it on ice).
2. Check whether the cells are broken completely. Extend the time for sonication if the cells are not completely broken. The colour of cell suspension will turn from milky-white into clearer suspension. Alternatively examine the suspension in the microscope, compare between sonicated cell and unsonicated cell (save unsonicated sample as control)
3. Centrifuged at 18000 x g, 4°C for 60 minutes.
   Transfer supernatant into 50 mL tube, keep on ice until purification

Enzyme isolation

1. Fill centrifuge tube until full using lysis buffer and weight **precisely** in a pair.
2. Centrifuge supernatant using ultracentrifuge at 104.000xg for 2 hours.
3. Discard supernatant
4. Remove pellet gently from ultracentrifuge tube using spatula (Pellet was membrane fraction of PfMQO).
5. Transfer gently membrane fractioninto glass homogenizer.
6. Add resuspend buffer until all membrane fraction submerged. (Resuspend buffer: 50 mM HEPES-KOH pH 7.6; 150 mMKCl; 5 mM Imidazole; 0.02 mM FAD)
7. Homogenize membrane fraction using glass homogenizer.
8. Transfer homogenized PfMQO membrane fraction into 50 mL tube.
9. Add glycerol so final concentration of glycerol is 50%. (1 volume of enzyme : 1 volume of glycerol)

10. Aliquot to 1.5 mL tubes, eachtain 200-500 µL
11. Store at -30°C until used (length of storage will be added later)
12. Analyse protein concentration and purity by following each SOP
13. **Do not take out enzyme from freezer, work by the freezer**.

Appendix

A. LB medium plate (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl (Merck)	: 10 g
Agar (Oxoid)	: 20 g

1. Put 800 mL milli-Q water into a beaker glass.
2. Weigh all ingredients except agar, then dissolve in milli-Q water using a stirrer.
3. Add agar after other all ingredients already dissolve, mix well using stirrer
4. Add milli-Q water up to 1000 mL using a measuring cylinder.
5. Sterilize the medium using autoclave at 121°C for 15 minutes.
6. Pour warm medium into sterile disposable petri dish, wait until cool then cover it
7. Store medium in 4ºC until use
8. Transfer medium into incubator 37ºC prior to use. Put 20 µL of 50 mg/ml kanamycin then spread kanamycin in the surface of plate using sterile glass spreader

B. LB medium broth (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl (Merck)	: 10 g

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Use the medium after cooled down at room temperature

C. TB medium broth (1000 mL)

Trypton (Oxoid)	: 12.0 g
Yeast extract (Oxoid)	: 24.0 g

Potassium phosphate dibasic (Wako) : 9.4 g
Potassium phosphate monobasic (Wako) : 2.2 g

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, except glycerol, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Sterilize 80% glycerol separately using autoclaved 121ºC for 15 minutes
6. Use the medium after cooled down at room temperature
7. Add sterilized 80% glycerol aseptically just before using the medium

D. 50 mg/ml Kanamycin stock (Sigma-Aldrich, K4000-25g) (10 mL)
1. Weight 500 mg of kanamycin powder
2. Dissolve in 8 mL milli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using sterile membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

E. 1M Isopropyl-β-D-(-)-thiogalactopyranoside (IPTG) (Wako, 099-02534, MW=238.30 g/mol) (10 mL)
1. Weight 2.389 g of IPTG powder
2. Dissolve in 8 mL milli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using streil membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

Standard operation procedure

Enzyme EhSAT1 production

Microbial revival

1. Take a frozen stock of *Eschericia coli* BL21 star (DE3) pET 15b EhSAT1 out from -80°C, then transfer into ice box.
2. Scratch the frozen stock using a loop then streak on an LB agar plate supplemented with 100 mg/mL amphicilin or carbenicilin.
3. Incubate the plate at 37°C for overnight.
4. Discard the frozen stock that has been used (do not return into deep freezer).

Pre-culture

1. Inoculate a colony of *E.coli* BL21 star (DE3) pET 15b EhSAT1 that is freshly revived from frozen stock on LB-Amphicilin or LB-carbenecillin agar medium into 50 mL LB medium (supplemented with 100 μg/mL amphicilin or carbenicillin) in 500 mL Erlenmeyer flask.
2. Incubate at 37^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Main culture

1. Transfer 50 mL pre-culture broth of *E.coli* BL21 star (DE3 pET 15b EhSAT1 into 500 mL 2YT medium (supplemented using 100 μg/mL amphicilin or carbenicillin) in 2000 mL Erlenmeyer flask.
2. Incubated the culture at 37^{o}C under vigorous orbital shaking (200 rpm)
3. Check OD600 of the culture. If OD_{600} culture reach 0.6 (typically in 1-2 hours), add IPTG so that final concentration is 5 mM. Continue the incubation at 20^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Harvest *E. coli* cell

1. Transfer overnight culture into 500 ml centrifuge bottle.
2. Centrifuged at 4000 x g, 4°C, for 15 minutes.
3. Discard supernatant, and resuspend the cells using 20 ml lysis buffer for each 500 ml *E.coli* culture. (Lysis buffer: 50 mM Tris HCl pH=8.0; 300 mM NaCl; 20% glycerol).
4. Transfer cell suspension into a metal cup (100 mL) for cell lysis. The suspension can be temporary stored at -80°C in 50 mL tube.

Cell lysis

1. Break the cell using sonicator for 21 minutes (10 sec on, 20 sec off) at 4°C (on ice). Use big probe.
2. Check whether the cells are broken completely. Extend the time for sonication if the cells are not completely broken. The colour of cell suspension will turn from milky-white into clearer suspension.
3. Centrifuged at 18000 x g, 4°C for 60 minutes.
4. Transfer supernatant into 50 mL tube, keep on ice until purification.

Enzyme purification.

1. Mix 50% Ni-NTA resin (in 20% ethanol, stored at 4°C) well, then immediately transfer 1.5 mL into 15 mL tube.
2. Centrifuge at 800 x g for 5 min at r.t.
3. Discard supernatant, resuspend with 1 mL milli-Q water.
4. Centrifuge at 800 x g for 5 min at r.t.
5. Discard supernatant, resuspend with 1 mL milli-Q water.
6. Centrifuge at 800 x g for 5 min at r.t. Discard supernatant.
7. Transfer a part lysed cell suspension (typically 1 mL) into resin, then transfer back into lysed cell suspension tube. Repeat this step until all resins are transferred.
8. Mix the lysed cell suspension and resin by mild shaking at 4°C for overnight.
9. Transfer the suspension into disposable 15 mL plastic column. Collect the flow through with 50 mL tube.
10. Wash the resin using 20 mL washing buffer (50 mM Tris HCl pH=8.0; 300 mM NaCl; 20% glycerol; 20 mM Imidazole). Collect the flow through with 50 mL tube.
11. Elute using 4 mL elution buffer (50 mM Tris HCl pH=8.0; 300 mM NaCl; 20% glycerol; 250 mM Imidazole). Collect the flow through with 15 mL tube.
12. Add 100% glycerol to the flow through so the final concentration of glycerol is 50%. (Do not forget to calculate the amount of glycerol in elution buffer).
13. Aliquot into 1.5 mL tube each 200 μL.
14. Store at -30°C until used

Appendix

A. LB medium plate (1000 mL)

Tryptone	: 10 g
Bacto-yeast extract	: 5 g
NaCl	: 10 g
Agar	: 20 g

1. Put 800 mL milli-Q water into a beaker glass.
2. Weigh all ingredients except agar, then dissolve in milli-Q water using a stirrer.
3. Add agar after other all ingredients already dissolve, mix well using stirrer
4. Add milli-Q water up to 1000 mL using a measuring cylinder.
5. Sterilize the medium using autoclave at 121^{o}C for 15 minutes.
6. Pour warm medium into sterile disposable petri dish, wait until cool then cover it
7. Store medium in 4ºC until use
8. Transfer medium into incubator 37ºC prior to use. Put 20 µL of 50 mg/ml kanamycin then spread kanamycin in the surface of plate using sterile glass spreader

B. LB medium broth (1000 mL)

Tryptone	: 10 g
Bacto-yeast extract	: 5 g
NaCl	: 10 g

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Use the medium after cooled down at room temperature

C. 2 YT medium broth (1000 mL)

Trypton	: 16 g
Yeast extract	: 10 g
NaCl	: 5 g

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Use the medium after cooled down at room temperature

Standard operation procedure

Production of Sumo Protease

(For 500 mL main culture, typically producing for 32.5 mg protein)

Microbial revival

1. Take a frozen stock of *Eschericia coli* BL21 (DE3) pET 28a Sumo Protease out from -80°C, then transfer into ice box.
2. Scratch the frozen stock using a loop then streak on an LB agar plate supplemented with 50 mg/mL kanamycin.
3. Incubate the plate at 37°C for overnight.
4. Discard the frozen stock that has been used (do not return into deep freezer).

Pre-culture

1. Inoculate a colony of *E.coli* BL21 (DE3) pET 28a Sumo Protease that is freshly revived from frozen stock on LB-Kanamycin (Brand) agar medium into 50 mL LB medium (supplemented with 50 μg/mL kanamycin) in 500 mL Erlenmeyer flask. Add 25 μL of 100 mg/mL kanamycin stock into 50 mL medium.
2. Incubate at 37^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Main culture

1. Transfer 50 mL pre-culture broth of *E.coli* BL21 (DE3) pET 28a Sumo Protease into 500 mL TB medium (supplemented using 50 μg/ml kanamycin) in 2000 mL Erlenmeyer flask. (Add 250 μL of 100 mg/mL kanamycin stock into 500 mL medium)
2. Incubated the culture at 37^{o}C under vigorous orbital shaking (200 rpm)
3. Check OD_{600} of the culture. If OD_{600} culture reach 0.6 (typically in 1-2 hours), add IPTG so that final concentration is 100 μM (add 50 μL of 1 M IPTG to 500 ml medium). Continue the incubation at 20^{o}C under vigorous orbital shaking (200 rpm) for overnight.

Harvest *E. coli* cell

1. Transfer overnight culture into 500 ml centrifuge bottle.
2. Centrifuge at 4000 x g, 4°C, for 15 minutes. (Kubota 7780, rotor AG 5006)
3. Discard supernatant by carefully decantation, and resuspend the cells using 20 ml lysis buffer for each 500 ml *E.coli* culture. (Lysis buffer: 25 mM Tris-HCl pH=8.0; 300 mM NaCl; 10% (v/v) Glycerol). Cell can be washed by resuspending pellet with 25 ml 1xPBS and centrifuge as no 2 then discard the supernatant by pipetting.

4. Transfer cell suspension in lysis buffer into a metal cup (100 mL) for cell lysis. The suspension can be temporary stored at -80°C in 50 mL tube. (We will add the length of storage cell suspension in lysis buffer)

Cell lysis

1. Break the cell using sonicator (Branson digital sonifier, model 102C, second biggest probe) 5 sec on, 20 sec off 10 set at 4°C (Do it on ice).
2. Check whether the cells are broken completely. Extend the time for sonication if the cells are not completely broken. The colour of cell suspension will turn from milky-white into clearer suspension. Alternatively examine the suspension in the microscope, compare between sonicated cell and unsonicated cell (save unsonicated sample as control)
3. Centrifuged at 18000 x g, 4°C for 60 minutes.
4. Transfer supernatant into 50 mL tube, keep on ice until purification.

Enzyme purification.

1. Mix 50% Ni-NTA resin (in 20% ethanol, stored at 4°C) well, then immediately transfer 1.5 mL into 15 mL tube.
2. Centrifuge at 800 x g for 5 min at r.t.
3. Discard supernatant, resuspend with 10 mL milli-Q water.
4. Centrifuge at 800 x g for 5 min at r.t.
5. Discard supernatant, resuspend with 10 mL milli-Q water.
6. Centrifuge at 800 x g for 5 min at r.t. Discard supernatant.
7. Transfer a part lysed cell suspension (typically 1 mL) into resin, then transfer back into lysed cell suspension tube. Repeat this step until all resins are transferred.
8. Mix the lysed cell suspension and resin by mild shaking at 4°C for overnight.
9. Transfer the suspension into disposable 15 mL plastic column. Collect the flow through with 50 mL tube.
10. Wash the resin using 20 mL washing buffer (25 mM Tris-HCl pH=8.0; 300 mM NaCl; 10% (v/v) glycerol; 20 mM Imidazole). Collect the wash wafer with 50 mL tube.
11. Elute using 4 mL elution buffer (25 mM Tris-HCl pH=8.0; 300 mM NaCl; 10% (v/v) glycerol; 250 mM Imidazole). Collect the eluted protein with 15 mL tube.
12. Transfer the eluted protein into 15 mL tube, then add 100% glycerol so the final concentration of glycerol is 50%. (Do not forget to calculate the amount of glycerol in elution buffer).

Example for calculating glycerol adding:

(500 μL x 10%) + (V μL x 100%) = (500 + V) μL x 50%

$$5000 + 100V = 25000 + 50V$$

$$(100-50)\,V = 25000-5000$$

$$50V = 20000$$

$$V = 400\ \mu L$$

13. Aliquot into 1.5 mL tube each 200 μL.
14. Put label : enzyme name, preparation date, concentration, name of person, no of tube
15. Store at -30°C until used (length of storage will be added later)
16. Analyse protein concentration and purity by following each SOP
17. Do not take out enzyme from freezer, work by the freezer.

Appendix

A. LB medium plate (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl Brand (Merck)	: 10 g
Agar (Oxoid)	: 20 g

1. Put 800 mL milli-Q water into a beaker glass.
2. Weigh all ingredients except agar, then dissolve in milli-Q water using a stirrer.
3. Add agar after other all ingredients already dissolve, mix well using stirrer
4. Add milli-Q water up to 1000 mL using a measuring cylinder.
5. Sterilize the medium using autoclave at 121°C for 15 minutes.
6. Pour warm medium into sterile disposable petri dish, wait until cool then cover it
7. Store medium in 4ºC until use
8. Transfer medium into incubator 37ºC prior to use. Put 20 μL of 50 mg/ml kanamycin then spread kanamycin in the surface of plate using sterile glass spreader

B. LB medium broth (1000 mL)

Tryptone (Oxoid)	: 10 g
Bacto-yeast extract (Oxoid)	: 5 g
NaCl (Merck)	: 10 g

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Use the medium after cooled down at room temperature

C. TB medium broth (1000 mL)

Trypton (Oxoid)	: 12.0 g
Yeast extract (Oxoid)	: 24.0 g
Potassium phosphate dibasic (Wako)	: 9.4 g
Potassium phosphate monobasic (Wako)	: 2.2 g
Glycerol 80%	: 5.0 ml

1. Put 800 ml milli-Q into beaker glass
2. Weight all ingredient, except glycerol, then dissolve in milli-Q water using stirrer
3. Add milli-Q water up to 1000 mL using measuring cylinder
4. Sterilize the medium using autoclaved at 121ºC for 15 minutes
5. Sterilize 80% glycerol separately using autoclaved 121ºC for 15 minutes
6. Use the medium after cooled down at room temperature
7. Add sterilized 80% glycerol aseptically just before using the medium

D. 50 mg/ml Kanamycin stock (Sigma-Aldrich, K4000-25g) (10 mL)

1. Weight 500 mg of kanamycin powder
2. Dissolve in 8 mL milli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using sterile membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

E. 1M Isopropyl-β-D-(-)-thiogalactopyranoside (IPTG) (Wako, 099-02534, MW=238.30 g/mol) (10 mL)

1. Weight 2.389 g of IPTG powder
2. Dissolve in 8 mL milli-Q water using stirrer
3. Add milli-Q water up to 10 ml in measuring cylinder
4. Sterilize using streil membrane filter 0.22 μL
5. Aliquot aseptically into 1.5 mL tube each 1 mL
6. Store at -30ºC (duration of storage will added later)

SATREPS SLeCAMA Project

The Project for Searching Lead Compound for Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Program Book

International Symposium on

Natural Resources-based Drug Development

August 21-22, 2017

BPPT Building II, 3rd Floor, Jl. MH Thamrin 8, Jakarta, Indonesia

Organized by:

Laboratory for Biotechnology, BPPT

Japan International Cooperation Agency

Agency for Medical Research and Development, Japan

Co-organized by:

LIPI Indonesian Institute of Science

Airlangga University

東京大学 The University of Tokyo

長崎大学 Nagasaki University

北里大学 Kitasato University

MicroBiopharm Japan

CONTENTS

PREFACE AND ACKNOWLEDGEMENT
FROM ORGANIZING COMMITTEE

Dr. Agung Eru Wibowo
Chair of Organizing Committee

Dear distiguished participants,

Welcome to the International Symposium on Natural Resources-based Drug Development!

Indonesia has been recognized internationally as a mega-biodiversity country. The diversity and uniqueness of bioresources became a big and important capital for Indonesia to increase wealth andp prosperity of the people, where innovation is the key for transforming this biodivesity into a useful products.

In other side, Indonesia is still suffering from many diseases, and infectious diseases are still a major health problem. Extensive treatment of diseases with certain drug, however, revealed in rapidly growing cases of drug resistance. Thus, drug development to search a new drug for battling infectious diseases become an unneglectable effort to be done.

Under cooperation with Japan International Cooperation Agency (JICA) and Japan Agency for Medical Research and Development (AMED) under SATREPS Project, Agency for the Assessment and Application of Technology (BPPT) is currently developing drug candidates originated from Indonesian bioresources for two major parasitic diseases, malaria and amebiasis. This project aims to build capacity of Indonesian research institution on drug development, especially based on utilization of natural resources.

Since innovation in drug development is a multi-disciplinary field, strong network between researchers involved in related fields is the key for successful development. This symposium is designed to build a research network on drug development in Indonesia. Prominent leading researchers in drug development from Japan and Indonesia will deliver recent update on advanced technology in drug development. Some of key persons from related ministries and industries will also share strategies and policies that are needed for promoting innovation on drug development in Indonesia. The 2015 Nobel Laurette in Physiology/Medicine, Prof. Satoshi Omura (Kitasato University, Japan), will also join to the symposium by delivering his valuable message to the participants.

We wish you have a valuable time during this symposium!

GREETING AND OPENING SPEECH FROM DEPUTY CHAIRPERSON OF BPPT

Dr. Unggul Priyanto, MSc., BPPT Chairman
Ms. Mari Takada, Minister of Economic Affairs, Embassy of Japan in Indonesia
Mr. Naoki Ando, Chief Representative, JICA Indonesia Office
Mr. Masahiro Takahata, First Secretary, Embassy of Japan in Indonesia
Dr. Kaname Kanai, Executive Technical Advisor, JICA Headquarter Tokyo
Invited speakers
Distinguished Guests, Ladies and Gentlemen,

Prof. Dr.Eng. Eniya Listiani Dewi, B.Eng., M.Eng.
BPPT Deputy Chairperson

Good morning,
Ohayou gozaimasu,
Assalamu'alaikum Wr. Wb.

It is my greatest pleasure to welcome all of you in International Symposium on Natural Resources-based Drug Development.
This symposium is one of activities in SATREPS Project for searching lead compounds of antimalarial and antiamebic agents by utilizing diversity of Indonesian bio-resources (SATREPS Slecama). This year is the second years of the project, which was started in 2015. The project is aimed to increase the capacity of BPPT and Indonesian counterparts in utilization of bioresources for drug development, especially antimalarial and antiamebiasis drug.

Ladies and Gentlemen,
During these 2 years, BPPT together JICA and all counterparts in this project has been cooperated in enriching the microbial collection that is managed by BPPT, increasing capacity and quality of bioresource extract production, establishing screening method of extracts for anti-malaria and anti-amebic activity, and purification of active compounds. Some of 2000 newly isolated microbes have been added to the collection. Last year, BPPT also could produce more than 8000 extracts for screening. Screening system for searching anti-malarial and anti-amebic active extract has also been established. The system includes screening for searching inhibitors of parasite specific enzymes, as well as inhibitors of proliferation of parasites. Moreover, the team has also successfully purified and elucidated the structure of some active compounds.

Distinguished Guests, Ladies and Gentlemen,
Capacity building of BPPT and Indonesian counterparts for drug development has also been done in form of training of researchers involved. More than 26 persons have been dispatched to Japan to have training in various fields. On-site training has also been conducted by 21 experts from Japan to make sure that the system in drug discovery learned by researchers has been implemented properly in Indonesia. Moreover, 3 researchers from Indonesia are currently dispatched to Japan for PhD degree program as long-term training. In addition, in order to establish the system for drug discovery

activities from bioresources in Indonesia, JICA also provided technical support in form of delivering laboratory equipment.

Ladies and Gentlemen,

BPPT considers that strong networking and close collaboration between research institutes and researchers is the key for successful innovation in drug development.

This symposium is aimed to promote and strengthen local and international network and collaboration on drug development in Indonesia. Co-organized with JICA and AMED, this symposium will present 17 leading scientists as speakers from both Indonesia and Japan. Some of key persons from related ministries and industry will also share strategies and policies that are needed for promoting innovation on drug development in Indonesia. Initially, this symposium is designed to be attended by 100 researchers. However, due to large enthusiasts from researchers in this field, the committee increased the number of participant to 130. I believe that we can achieve the objective of this symposium together with all of participants.

Ladies and Gentlemen,

Today, BPPT is celebrating its 39th anniversary. Therefore, this symposium is special, because it became part of BPPT anniversary celebration event.

I invite all of participants to involve actively during symposium and share thoughts and experiences in drug development. I do also hope this symposium will soon reflect to acceleration of innovation in drug development in Indonesia.

Wassalamu'alaikum wr.wb.

Jakarta, August 21, 2017

BPPT Deputy Chairperson

Prof. Dr.Eng. Eniya Listiani Dewi, B.Eng., M.Eng.

GREETING AND OPENING SPEECH FROM BPPT CHAIRPERSON

Dr. Unggul Priyanto, MSc.
BPPT Chairperson

Ms. Mari Takada, Minister of Economic Affairs, Embassy of Japan in Indonesia
Mr. Naoki Ando, Chief Representative, JICA Indonesia Office
Mr. Masahiro Takahata, First Secretary, Embassy of Japan in Indonesia
Dr. Kaname Kanai, Executive Technical Advisor, JICA Headquarter Tokyo
Invited speakers
Distinguished Guests, Ladies and Gentlemen,

Good morning,
Ohayou gozaimasu,
Assalamu'alaikum Wr. Wb.

It is my greatest pleasure to welcome all of you in this very important event. It is also an honor for BPPT to host International Symposium on Natural Resources-based Drug Development as part of SATREPS (Science and Technology Research Partnership for Sustainable Development) Project activities, which is co-organized together with Japan International Cooperation Agency (JICA) and Japan Agency for Medical Research and Development (AMED).
In this opportunity, please allow me to express my greatest and sincere appreciation to JICA, AMED, and counterparts from both Indonesia and Japan. I really do appreciate the establishment of this technical assistance and mutual cooperation between BPPT, LIPI, Airlangga University and Japanese partner institutions especially in the area drug development.

Ladies and Gentlemen,
BPPT, the Agency for the Assessment and Application of Technology, is one of the non-ministerial government institutions under coordination of the Ministry of Research, Technology and Higher Education. BPPT's vision is to be the center of leading technology which prioritizes on innovation and technology services to achieve national resilience, increase competitiveness and improvement of public services.
In order to realize the vision, BPPT has missions to generate innovation and technology services through engineering, technology clearing, technology audit, diffusion and commercialization, technology transfer, and intermediation.

Distinguished Guests, Ladies and Gentlemen,
Indonesia is still importing more than 95% of bulk pharmaceuticals needed by local pharma-companies. BPPT consistently supports those industries in term of providing innovation and technology services, based on roadmap prepared by the Ministry of Health for national sovereignty in bulk pharmaceuticals. BPPT also released a Health Technology Outlook, which production technology of bulk pharmaceuticals is part of the proposed technology.

Blessed with highly diverse natural bio-resources, Indonesia has comparative advantages in term of source for drug development. Based on this, BPPT emphasises innovation on production of bulk pharmaceuticals including Active Pharmaceutical Ingredient (API) in order to overcoming national health problems, especially those for infectious diseases including malaria and amebiasis.

People are suffering with these two parasitic diseases in term increasing drug resistance cases. BPPT highly concerns with this situation and promotes innovation of anti-malarial and anti-amebiasis new drugs based on Indonesian bio-resources.

Therefore, I really ask BPPT team to put all efforts as much as possible to make this SATREPS Project of Searching Lead Compounds of Anti-Malarial and Anti-Amoebic Agents by Utilizing Diversity of Indonesia Bio-resources successfully. I also hope that all partners from Indonesian and Japanese institutions together with related stakeholders will also make every endeavor to achieve our mutual benefits.

Distinguished Guests, Ladies and Gentlemen,

I understand and do realize that developing a drug is not an easy task. However, I do believe nothing is impossible as long as we work together and do the best. Strong networks and tight collaboration will be the success key in drug development. So, I do believe that this symposium, that is aimed to strengthen network and collaboration between research institutes in both Indonesia and Japan, will be very beneficial for BPPT to accelerate innovation in drug development.

Have a very fruitful symposium and networking. Thank you very much for your kind attention.

Wassalamu'alaikum wr.wb.

Jakarta, August 21, 2017

BPPT Chairman

Dr. Unggul Priyanto, MSc

BPPT in Brief

Agency for the Assessment and Application of Technology (BPPT) is a non-ministry government institution that is directly responsible to the President of the Republic of Indonesia under the coordination of the Ministry of Research, Technology and Higher Education. BPPT was established in 1978, and Prof. Dr. Ing. Bacharudin Jusuf Habibie was the first Chairperson.

BPPT's vision is "To be a technology center of excellent that prioritizing innovation and technology services for promoting national competitiveness and sovereignity". BPPT has mission to assess and apply technology to create innovation and technology services in field of technology for food, health, electricity, fuel, ICT, transportation, security and defense, material, machinery, disaster reduction, natural resources and marine, environment, and innovation system.

BPPT has governmental duties in the assessment and application of technology in accordance with the provisions of applicable laws. BPPT does not only play a role as an intermediary agency that bridges the interests of customers and technology providers, but also serves to provide approval to the key technologies that will be used in Indonesia. The role of BPPT as a clearing house agency is realized here. Other roles performed by BPPT are technology assessment (engineering) and technology audit including providing technology solutions. Its entire activities are aimed to provide innovation and technology services to support the improvement of the people's welfare. Its technology services comprise recommendation, advocacy, technology transfer, consultancy, testing, operation services, pilot project, pilot plant, prototype, surveys, technical reference, technology audit, and technology-based startups.

These roles of BPPT should be able to provide value proposition to the beneficiaries of BPPT's output in the improvement of competitiveness and sovereignity in technology mastery through technology transfer as well as the acquisition of the latest technology. These roles are implemented by BPPT through principle secretary and 5 deputies: Deputy for Technology Policy Assessment, Deputy for Natural Resource Development Technology, Deputy for Agroindustrial Technology and Biotechnology, Deputy for Information, Energy and Material Technology, and Deputy for Industrial Technology, Design and Engineering.

Currently, BPPT has more than 3000 employees (engineers, researchers, administrative staffs) and advanced laboratories to support its technology innovation and service activities. BPPT also has wide collaboration and networking with numerous local and international research institutes and industries.

Deputy of Agroindustrial Technology and Biotechnology-BPPT in Brief

Deputy of Agroindustrial Technology and Biotechnology (TAB) is one of technical deputy in BPPT that has duties of planning and implementation of policies in field of agroindustrial technology and biotechnology. There are 4 centers and 2 implementing units under TAB: Center for Agroindustrial Technology, Center for Agricultural Production Technology, Center for Bioindustry, Center for Pharmaceutical and Medical Technology, Center for Starch Technology, and Laboratory for Biotechnology.

TAB has been largely contributed to create innovation and services in field of technology for food and health for promoting national competitiveness and sovereignity.

Gelatin from seaweed: alternatif for halal ingedient

Standardized herbal medicine *Neurat* for lowering blood uric acid

Pharmaceutical grade salt (currently manufactured by local pharmaceutical industry)

Black garlic, a health supplementary food with stong antioxidant activity

Rice made from cassava flour for food diversification

Rice and noodle products made from local row material such as sago and corn

Development of salt water-tolerance Tilapia

Ex-vitro propagation technology for pepper seedlings production

Complete feed for grower cattle qualified from oil palm waste

Laboratory for Biotechnology-BPPT in Brief

Laboratory for Biotechnology (previously known as Biotech Center, BC) is one of implementing unit in Deputy of Agrotechnology and Biotechnology, BPPT. It was inaugurated by President Soeharto in December 29, 1995. Located in Puspiptek, South Tangerang, the laboratory has vision "To be center of excellent of Biotechnology that prioritizing biotechnological innovation and services for promoting industrial competitiveness and national sovereignity".

BC enroles two major competencies, industrial biotechnology and agricultural biotechnology, which contributes to BPPT's mission in field of technology for health and food. BC has 106 employees and more than half of them are govermental officials as engineers, researchers, and administrative staffs. Equipped with advanced laboratory facilities, including pilot-scale fermentation and recovery facility and pilot-scale *in-vitro* and *ex-vitro* plant propagation facility, BC has been developed numerous bio-based technology innovation that are useful for industries and communities. Accreditated by National Accreditation Committee as internationally standardized testing laboratory (ISO17025) and strong networking with both local and international research institutes and industries, as well as with local government, BC has diseminated numbers of technologies developed, as well as assessed, in BC to numerous stake holders.

Plant propagation technology

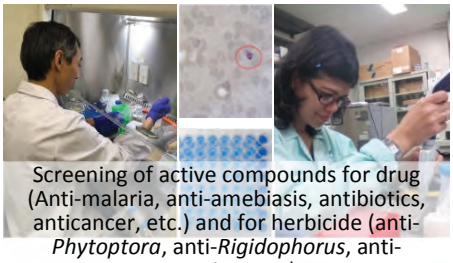
Plant Productivity and Field Improvement

Bitumman (self-growing seedling), a biofertilizer-coated seedling for revegetation of ex-mining field

Technofert, a biofertilizer containing microbial consorsia (*Mycorrhiza arbuscular, Corynebacterium* sp.) for increasing nutrient adsorption from soil

Technology for Drug Development

Microbial collection: field sampling, isolation, identification, preservation (focus on Fungi and Actinomycetes)



Screening of active compounds for drug (Anti-malaria, anti-amebiasis, antibiotics, anticancer, etc.) and for herbicide (anti-*Phytoptora*, anti-*Rigidophorus*, anti-*Fusarium*, etc)

Fermentation, purification, and characterization of active compounds (lab and pilot scale fermenter up to 2500 L, lab and pilot scale down stream process, mass spectrometry, etc.)

Laboratory for Biotechnology
(Biotech Center)
Building 630, Puspiptek Area, Setu,
South Tangerang 15314, Banten, Indonesia
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AGENDA

INTERNATIONAL SYMPOSIUM ON

NATURAL RESOUCES-BASED DRUG DEVELOPMENT

Monday, August 21st,2017	
08.00 – 08.25	Registration
Opening Session	
08.25 – 09.00	Welcome speech by Project Director
	Opening remark by Minister, Embassy of Japan in Indonesia
	Opening remark by Chief Representative, JICA Indonesia Office
	Opening remark by Chairperson of BPPT
	Photo session
Special Speach	
09.00 – 09.15	Overview of JICA's cooperation related to the Science and Technology Research for Sustainable Development **Kaname Kanai, MD., PhD.** (Executive Technical Advisor, Human Development Department, JICA Headquarter Tokyo)
Keynote speech **Chair : Prof. Tomoyoshi Nozaki**	
09.15 – 09.45	Magic bullets for parasitic diseases: Gifts from Nature **Prof. Kiyoshi Kita** (School of Tropical Medicine and Global Health, Nagasaki University)
Plenary 1 – Exploration of Bioresources for Drug Development **Chair : Dr. Achmad Fuad**	
09.45 – 10.15	Session 1: The Role of Microbial Culture Collection in Drug Discovery **Dr. Atit Kanti** (Indonesia Culture Collection, Indonesian Institute of Science)
10.15 – 10.45	Session 2: Analysis of Eponemycin (α'β' epoxyketone) Analog Compound from *Streptomyces hygroscopicus* subsp. *hygroscopicus* and Its Antiplasmodial Activity *in vivo* and *in vitro* through Inhibition of Ubiquitin-proteasome System **Prof. Loeki Enggar Fitri** (Faculty of Medicine, Brawijaya University)
10.45 – 11.15	Coffee break
Plenary 2 – Utilization of Natural Resources for Drug Development **Chair : Drs. Tarwadi, MSi.**	
11.15 – 11.45	Session 3: Role and Potency of Marine Biodiversity on Drug Development **Suciati Iryani, PhD.** (Faculty of Pharmacy, Airlangga University)
11.45 – 12.15	Session 4: Exploration of bioactive compound from marine organisms for drug discovery **Prof. Ekowati Chasanah** (Research Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine Affairs and Fisheries)
12.15 – 13.30	Lunch

Plenary 3 – Recent Update of Technology for Drug Development Chair : Dr. Kurnia Agustini	
13.30 – 14.00	Session 5: Development of animal model for pre-clinical studies of anti-protozoan agents **drh. Fitriya Nur Annisa Dewi, Ph.D** (Primate Study Center, Bogor Agricultural University)
14.00 – 14.30	Session 6: Development of screening system for anti-toxoplasmolysis agents **Dr. Yoshifumi Nishikawa** (National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine)
Plenary 4 – Current Status in Parasitic Diseases Chair : Dr. Daniel Ken Inaoka	
14.30 – 15.00	Session 7: Anti-malaria drug resistant parasite in Indonesia **Prof. Din Syafruddin** (Eijkman Institute for Molecular Biology)
15.00 – 15.30	Coffee break
15.30 – 16.00	Session 8: Genetic diversity of *Plasmodium* sp. in Indonesia and its potential for malaria vaccine development **Dr. Rintis Noviyanti** (Eijkman Institute for Molecular Biology)
16.00 – 16.30	Session 9: Epidemiology study of malarial parasites in Indonesia **Dr. Pretty Multihartina Sasono** (Agency for Health Research and Development, Ministry of Health)
16:30-16:40	Photo session
16:40	End of day 1
Reception Dinner and Networking	
17.30 – 19.00	Reception Dinner and Networking (Sari Pan Pacific Hotel)

Tuesday, August 22nd,2017	
08.00 – 08.30	Registration
Plenary 5 - Current Status of Drug Discovery Chair : Dr.rer.nat. Chaidir	
08.30 – 08.35	A message from 2015 Nobel Laurette in Physiology/Medicine **Prof. Satoshi Omura** (Kitasato University)
08.35 – 09.05	Session 10: Search for new antibiotics from natural resources **Prof. Kazuro Shiomi** (Kitasato Institute for Life Sciences, Kitasato University)
09.05 – 09.20	Session 11: Searching for Lead Compounds of Antimalarian and Antiamebic Agents by Utilizing Indonesian Bioresources **Danang Waluyo, MEng.** (Laboratory for Biotechnology, BPPT)
09.20 – 09.50	Session 12: Development of anti-malarial drug from Indonesian Plants **Dr. Eti Nurwening Sholikhah** (Faculty of Medicine, Gadjah Mada University)
09.50 – 10.20	Coffee break

Plenary 6 – Promotion of Drug Development Research Chair : Dr. Ir. Roy Alexander Sparringa, M.AppSc.	
10.20 – 10.50	Session 13: Strategic planning for natural resources-based drug discovery in Indonesia **Dr. Ira Nurhayati Djarot** (Director, Ministry of Research, Technology, and Higher Education)
10.50 – 11.20	Session 14: LPDP scheme for Research and Development in Indonesia **Dyah Kartiningdyah S.E, M.M, M.Ed** (Indonesia Endowment Fund for Education, Ministry of Finance)
11.20 – 11.50	Session 15: Business scheme for drug discovery/Business opportunities in Drug Development Reseach **Prof. Dr. Wahono Sumaryono** (Commissioner, PT. Kimia Farma)
Closing session	
11.50 – 11.55	Closing remark by Chair of Organizing Committee
11.55 – 12.00	Photo session
12.00 – 13.00	Lunch
Lab Visit	
14.30 – 16.00	Lab Visit (Laboratory for Biotechnology)

Abstract and Curriculum Vitae of Speakers

Special Speech

JICA's cooperation related to Science and Technology Research for Sustainable Development (SATREPS)

Kaname KANAI
Executive Technical Advisor, Human Development Department, JICA Headquarter Tokyo
e-mail: Kanai.Kaname@jica.go.jp

JICA is an agency for providing international cooperation assistance for more the 150 countries and areas. SATREPS is one of the ODA technical cooperation. There are 5 research fields: 1) Infectious disease control, 2) Disaster Prevention and Mitigation, 3) Bioresource, 4) Low Carbon Society / Energy, and 5) Global-scale Environmental.

Indonesia has had 16 SATREPS researches, including 3 researches related to the infectious disease control.

Curriculum Vitae

Name : **Kaname KANAI, MD., PhD.**
Affiliation : **JICA Headquarter Tokyo**
E-mail address : **Kanai.Kaname@jica.go.jp**

After 3 year medical practice Dr. KANAI decided to join the health ministry. He has served for the government for more than 25 years and his experiences are mainly based on international cooperation such as an embassy secretary (ODA) , WHO official, an international cooperation director of the ministry, a chief of quarantine stations, and JICA.

Keynote Speech

Magic bullets for parasitic diseases: Gifts from Nature

Kiyoshi KITA
School of Tropical Medicine and Global Health, Nagasaki University
e-mail: kitak@nagasaki-u.ac.jp

Parasites have developed a variety of physiological functions necessary for their survival within the specialized environment of the host. Using metabolic systems that are very different from those of the host, they can adapt to low oxygen tension present within the host animals. Most parasites do not use the oxygen available within the host to generate ATP even they reside oxygen rich circumstance such as blood, but rather employ systems anaerobic metabolic pathways. In addition, all parasites have a life cycle. In many cases, the parasite employs aerobic metabolism during their free-living stage outside the host. In such systems, parasite mitochondria play diverse roles. In particular, marked changes in the morphology and components of the mitochondria during the life cycle are very interesting elements of biological processes such as developmental control and environmental adaptation. As mitochondrial function is essential for the survival of the parasites, it should be promising target of chemotherapy (Siregar et al., Science, 2016). Recent our results on the inhibitors of parasites respiratory chains discovered from natural resources, such as ascofuranone, will be presented.

Curriculum Vitae

Name : **Prof. Kiyoshi KITA**
Affiliation : **School of Tropical Medicine and Global Health, Nagasaki University**
E-mail address : **kitak@nagasaki-u.ac.jp**

Education:

University: The University of Tokyo (Bachelor of Pharmaceutical Science) March 1974
Ph. D : The University of Tokyo (Doctor of Pharmaceutical Science) March 1980

Position held:

1980 - 1983 : Assistant Professor of The University of Tokyo (Department of Botany, Faculty of Science)
1983 - 1987 : Assistant Professor of Juntendo University (Department of Parasitology, School of Medicine)
1987 - 1990 : Lecturer of Juntendo University (Department of Parasitology, School of Medicine)
1991 - 1998 : Associate Professor of The University of Tokyo (Department of Parasitology, The Institute of Medical Science)
1998 - 2016 : Professor of The University of Tokyo (Department of Biomedical Chemistry, Graduate School of Medicine)
2011- 2015 : Vice Dean of Graduate School of Medicine
2015- : Dean Nagasaki University (School of Tropical Medicine and Global Health)

2002-2007 : Treasurer of FAOBMB (2002-2007).
1994-1996, 2010-: Eexecutive board of Japanese Society of Tropical Medicine
2003-2006 : President of Japanese Society of Parasitologist
2009-2011 : President of Japanese Biochemical Society

2010- : Program Officer of SATREPS (Science and Technology Research Partnership for Sustainable Development : http://www.jst.go.jp/global/english/index.html)
2013- : Scientific Advisory Committee member of DNDi (Drugs for Neglected Diseases initiative: http://www.dndi.org/about-dndi/our-people/team/geneva/)
2013- : Selection Committee member of GHIT (Global Health Innovative Technology Fund : https://ghitfund.org/)

Plenary 1 - Exploration of bioresources for drug development

Session 1

The Role of Microbial Culture Collection in Drug Discovery

Atit Kanti
Indonesia Culture Collection, Indonesian Institute of Science
e-mail: atit.kanti@lipi.go.id

The number of natural products, discovered from various living organisms including plants, animals and microbes, to date exceeds 1 million, with the majority (40–60%) derived from terrestrial plants. Of these natural products, 20–25% possess various bioactive properties including antibacterial, antifungal, antiprotozoal, antinematode, anticancer, antiviral and anti-inflammatory activities.

Plants and plant extracts have been used for the treatment of human diseases for millennia, and their use has been recorded in the most ancient archaeological sources. In contrast, the exploration of microorganisms as producers of therapeutical agents only began in the 20th century. However, despite this relatively short history, nearly 10% of all currently known biologically active natural products are of microbial origin. These include the majority of antibiotics, clearly demonstrating the potential of microorganisms as an emerging source for the production of biologically active products. Indeed, by the 20th century microbially derived bioactives had become the foundation of modern pharmaceuticals. For example, the production of antimicrobials is observed in 30–80% of actinomycete and fungal strains screened in various studies.

Moreover, mathematical models predict that the number of undiscovered antibiotics from actinomycetes could be in the order of 107. An emerging source of new bioactives may result from the many recent studies of microbial diversity in the terrestrial environment, particularly those microbes associated with plants and animals. Several studies have demonstrated that “living surfaces” represent an environment rich in epibiotic microorgansims that produce bioactives.

Nevertheless, the vast biotechnological potential of epibiotic microorgansims remains mostly unexplored. This presentation discusses the importance of exploring new sources potentially rich in bioactives, and highlights the significance of considering the chemical ecology of marine microorganism-host associations for the targeted isolation of bioactive producing microorganisms. InaCC (Indonesian Culture Collection) in LIPI has been playing important role in drug discovery. Many national and international collaboration have been established to explore the rich microbial diversity of tropical ecosystem. The intensive microbial survey has been started from 2000 at which the research partnership with NBRC-NITE was introduced to explore the richness of tropical microorganism. From this project more than 6000 valid microbial collection are deposited as RD collection and general collection. The microbial survey was also conducted through collaboration with University of California – USA. The focus of the exploration was Sulawesi. The Indonesian government through Bioresources Exploration program conducted microbial survey through out Indonesia and success on isolation many potensial microbe for secondary metabolite production.

The SATREPS-InaCC was further improve research facilities and depository of microorganism to meet internationally standardized culture collection. Currently InaCC have met the International standard. All the collections are developed, managed and maintained by highly trained, dedicated staff who work in accordance with internationally recognized quality standards including certification to ISO 9001:2015. Cultures from the collections are used by scientists who need to reassure themselves and others that the materials they are using are authentic, so the conclusions to their studies are valid and relevant. This is particularly important where research may lead to peer-reviewed publications, for example, in drug discovery and vaccine efficacy studies. Authenticated reference strains are also of paramount importance for clinical diagnostic testing, food, water and environmental microbiology testing and validation studies. Due to this success story the research collaboration is established with BPPT and Tsukuba University to exploit the potential use of the collection for antimalaria. We believe culture collection can be good source for bioprospecting studies for many industrial applications.

Curriculum Vitae

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Education

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1993	Biology	Bachelor	Padjajaran University
2000	Microbial Taxonomy	Master	Tokyo University of Agriculture
2014	Microbial Taxonomy	PhD	Bogor Agricultural University

Publication

Atit Kanti and I Made Sudiana. Carboxymethyl cellulose as a C-source for lipid accumulation by the oleaginous yeast Candida orthopsilosis. Current Research in Environmental & Applied Mycology 5 (4): 344–351(2015) ISSN 2229-2225. Doi 10.5943/cream/5/4/4.

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Plenary 1 - Exploration of bioresources for drug development

Session 2

Analysis of Eponemycin (α'β' epoxyketone) Analog Compound from *Streptomyces hygroscopicus* subsp. *hygroscopicus* and Its Antiplasmodial Activity *in vivo* and *in vitro* through Inhibition of Ubiquitin-proteasome System

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Epenomycin is a secondary metabolite of *Streptomyces hygrocopicus* subsp. *Hygroscopicus* that is known to possess 20S proteasome inhibitory activities in ubiquitin-proteasome system (UPS), and may have antimalarial activity. This study aimed to analyze eponemycin analog in *Streptomyces hygroscopius* subsp. Hygroscopicus extract and whether its metabolite extract can inhibit UPS function of *Plasmodium berghei* and decrease the viability of *Plasmodium falciparum* 3D7 *in vitro.*
Isolate of *S.hygroscopicus* was macerated using ethyl acetate: International *Streptomyces* Project 4/ISP4 medium (1:1 v/v) and analyzed using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). *In vivo* study was done using *P.berghei*-infected mice that were grouped into a group of non-treated control and three group trated with various dosages of *S.hygroscopicus*. We also conducted *in vitro* study using *P.falciparum* 3D7 culture containing trophozoite and schizont stages that incubated with increasing concentration of the *S.hygroscopicus* metabolite extract. Parasite degree and changes in parasite morphology were observed under microscopic by Giemsa-stained both *in vivo* and *in vitro* studies. *In vivo*, the accumulation level of *polyubiquitin* was measured using *Western blot* and ELISA method. *In vitro* DNA parasite density was measured using flowcytometry.
TLC analysis showed a spot with refractory factor (Rf) 0.7 and HPLC demostrated 3.768% and 5.796% Dyidroeponemycin in two samples. *In vivo* treatment with this compound at the dosage of 2600 μg/kgBW reduced the degree of parasite on almost all days and there was a strong accumulation of *polyubiquitinated* protein in the group treated with this dosage. An increasing dose of extract followed by an increasing of inhibition parasite growth (r=0.850). Probit analysis showed that ED50 was 9.418 μg/kgBW. At 8 hours of *in vitro* incubation there was a significant decrease in DNA parasite density in parasite culture exposed to more than 0.02 mg/ml of the extract ($p<0.001$). There was a significant inverse correlation between the concentration of extract and the degree of parasite (r=-0.772, $p<0.001$). *In vivo* and *in vitro* studies showed that metabolite extract of *S.hygroscopicus* affected the morphology of all parasite asexual stages. It can be concluded that Eponemycin analog in crude metabolite extracts of *S.hygroscopicus* subsp. Hygroscopicus is a potential candidate for a new antimalarial drug by inhibiting UPS function of the parasite and cause stress and dead of the parasite.

Keywords: *Streptomyces hygroscopicus* subsp. Hygroscopicus, TLC, HPLC, *Plasmodium*, morphology, DNA parasite, parasite degree

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Educational background

Year	Dicipline	Degree	Institution
1990	Medicine	M.D. (dr.)	Faculty of Medicine, Brawijaya University
1997	Tropical medicine	Master (M.Kes)	Postgraduate program, Gadjah Mada University
2004	Biomedical	PhD (Dr.)	Postgraduate program, Brawijaya University
2004	Clinical parasitology	Specialist (Sp.ParK)	Collegiums Parasitology

Research subjects

- Immunology malaria
- Drug development (anti-malaria)
- Tropical medicine

Recent publication

Fitri LE, Cahyono AW, Rivo YBN, Alkarimah A, Ramadhani NN, Laksmi DA, Triaty L, Noviyanti R. 2017. Analysis of eponemycin (α'β' Epoxyketone) analog compound from *Streptomyces hygroscopicus* subsp. Hygroscopicus extracts and its antiplasmodial activity during *Plasmodium berghei* infection. *Biomedical Research* 2017; 28(1):164-172.

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Plenary 2 - Utilization of Natural Resources for Drug Development

Session 3

Role and Potency of Marine Biodiversity on Drug Development

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The oceans, which cover almost two-thirds of the Earth's surface, have been the habitat of various living creatures, including algae, sponges, cnidarians, molluscs, bryozoans, ascidians and echinoderms as well as microorganisms. This species diversity makes the marine environment one of the most prolific sources of natural products. Many of these marine creatures produce unique and biologically active compounds which may not be found in the terrestrial ecosystem. These metabolites may be produced as a means of self-defence against predation, since many marine organisms have no spine or protective shell.

Our previous study on marine samples had revealed the presence of terpenoid and aromatic metabolites with anticancer and antimicrobial activities. The current study has focussed on the screening of marine sponges from Barrang Lompo Island, Makassar as anticancer and anti hepatitis C. The ethyl acetate extracts of *Diacarnus debeauforti*, *Haliclona amboinensis*, and *Agelas cavernosa* were screened against T47D and He La cancer cell lines using MTT method. The results showed that all three sponges gave anticancer activity against T47D cancer cell line, with the lowest IC_{50} of 18.2 µg/mL given by extract of *A. cavernosa*. In the screening against He La cancer cell line, extract of D. debeauforti gave the highest potency with IC_{50} of 15.7 µg/mL. Ethyl acetate extracts from marine sponges were prepared and screened for antiviral activity using JFH1a-Huh7it cell culture system. Extracts of *Stylissa flabelliformis*, *Homaxinella tanitai* and *Microxina subtilis* showed the highest inhibitory effect against HCV with IC_{50} values of 8.23, 27.12 and 40.50 µg/ml. Bioassay guided isolation of the samples revealed the presence of fatty acid metabolites in the active fractions. Identification of the fatty acids were carried out by 1H NMR and GC MS. The findings has revealed the potency of Indonesian marine sponges for the development of anticancer and antiviral agents.

Keywords: marine sponges, anti hepatitis C, anticancer, fatty acids

Curriculum Vitae

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Education:

	Bachelor	Master	Doctor
Institution	Universitas Airlangga	The University of Queensland, Australia	The University of Queensland, Australia
Major	Pharmacy	Natural Product Chemistry	Natural Product Chemistry
Year of graduation	2003	2008	2013
Tesis title	Optimasi Suhu dan pH pertumbuhan *Streptomyces griseus* ATCC 10137 dalam Media yang Mengandung Ampas Tahu	Secondary Metabolites and Acetylcholinesterase Inhibitors from *Fagraea* spp. and *Pandanus* spp.	From the Sea to the Jungle: The Search for Bioactive Metabolites
Supervisors	Dr. Isnaeni, MS. Apt Prof. Dr. Sudjarwo, MS. Apt	Prof. Mary Garson Dr Benjamin Ross	Prof. Mary Garson Dr James Fraser

Research Interest: Chemistry and Bioactivity of Marine organisms.

Plenary 2 - Utilization of Natural Resources for Drug Development

Session 4

Exploration of bioactive compound from marine organisms for drug discovery

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Located in in the center of coral triangle, Indonesia is blessed with second largest biodiversity in the world after Brazil. The presence of marine volcanoes, deep- sea, trenches, etc., makes Indonesia's sea are rich with unique chemical diversity that have high potential benefits for the drug discovery and other industrial needs. This paper presents recent progress on exploration result on marine biodiversity to isolate marine bioactive from seaweed, sea cucumber, sponge and microbes that can be contributed to drug discovery such as for cancer and other diseases. Problems in up scalling researches and its commercialization will also be discussed.

Keywords: marine organism, bioactive, drug discovery, Indonesia

Curriculum Vitae

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Degree & non degree trainings :

Year	Dicipline	Degree	Institution
1984	Agricultural Product Processing & Food Science	Ir (BSc Hon)	Faculty of Agricultural Technology, Gadjah Mada University
1991	Food Science	MSc.	Dept. of Food Science and Nutrition, University of Rhode Island
2004	Food Science	PhD	The Graduate School of Bogor Agricultural University
2006-2007	Marine Biodiscovery	(Postdoctoral & Visiting scientist)	Australian Institute of Marine Science (AIMS)

Professional experience :

- Leader of Marine Biotechnology Research group, R&D Center for Marine and Product Processing and Biotechnology (2008 – present)
- Chief editor of Squalen, Scientific bulletin/journal (2010 – present)
- Reviewer scientific journals focusing in fish processing technology and marine biotechnology
- Member of TP2I KKP
- Assessor of ISO/IEC 17025

Recent publication

Januar H. I, Zamani N.P., Soedarma D., Chasanah E. and Wright , A.D. 2017. Tropical coral reef coral patterns in Indonesian shallow water areas close to underwater volcanic vents at Minahasa Seashore, and Mahengetang and Gunung Api Islands. *Marine Ecology* 2017;00; e12415;

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Plenary 3 - Recent update of technology for drug development

Session 5

Development of animal model for pre-clinical studies of anti-protozoan agents

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Animals are essential to the development of drugs for veterinary and human medicine. Animal model often mimics human in its anatomy, physiology, and response to pathogens and therefore, they are highly valuable in biomedical research as the results can be extrapolated to human medicine. In drug development, animals hold an important role in various stages of the process – from basic research, efficacy study of potential compounds, pharmacokinetic and pharmacodynamics evaluation, up to the safety testing prior to application in clinical trials. In this presentation, an overview of animal model relevant to drug development process, particularly in regards to antiparasitic drugs, will be discussed. Emphasis will be put on the key aspect in the selection of animals for the different stages of the drug development, example of relevant guidelines pertaining to the use of animals for pre-clinical studies, and the ethical considerations in performing research and testing using laboratory animals.

Keywords: *in vivo* study, animal model, laboratory animals, pre-clinical testing, drug development

Curriculum Vitae

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Fitriya Nur Annisa Dewi earned her Doctor of Veternary Medicine (Drh.) from Bogor Agricultural University, Bogor, Indonesia (1997), her Ph.D. in Molecular Pathology and Comparative Medicine at Wake Forest University, North Carolina, USA (in 2013), and the Certificate in Laboratory Animal Medicine from the University of Guelph, Ontario, Canada (in 2017).

Currently, she is the head of the biomedical research program at the Primate Research Center at Bogor Agricultural University (IPB-PRC), wherein she also serves as the Chair of the Animal Care and Use Committee. Dr. Dewi is also the Deputy Research Manager at PT. Bimana Indomedical, a Contract Research Organization located in Bogor, Indonesia.

She is an active member of several organizations such as the American Association for Cancer Research, Indonesian Laboratory Animal Veterinarians Association, and Indonesian Association for Laboratory Animal Science. She also serves as a committee member in the National Board of Indonesian Veterinary Medical Association since 2014. Additionally, she has recently been appointed as an *ad-hoc* specialist for AAALAC International, an organization that performs accreditation and assessment on institutions that use animals in research, teaching and testing.

Dr. Dewi has published numbers of papers in international scientific journals, and have received several awards throughout her career, including the fellowship from Association from International Education Japan (AIEJ) to conduct one-year of research fellowship at the University of Miyazaki, Japan in 2014, the L'oreal-UNESCO for Women in Science National Fellowship in 2014, and Her World Indonesia Women of the Year Award in 2016.

Her area of knowledge related to animal care and research includes primate medicine, laboratory animal medicine, and animal model for human diseases.

Plenary 3 - Recent update of technology for drug development

Session 6

Development of screening system for anti-toxoplasmolysis agents

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Toxoplasmosis is caused by the obligate apicomplexan intracellular protozoan *Toxoplasma gondii* (*T. gondii*). It is an important cause of miscarriage or adverse fetal effects, including neurological and ocular diseases, and may also have sequelae later in life for an infected neonate. This parasite can infect most genera of warm-blooded animals and is estimated to infect 30–50% of the global human population. The prevalence of *T. gondii* in Indonesia, one of the strongly affected countries, is estimated to be around 50%. The current anti-Toxoplasma drugs have limited efficacy for eliminating *T. gondii* and also carry severe side effects. Therefore, the development of novel efficacious drugs is urgently needed. Utilization of natural resources is one of strategy for controlling the toxoplasmosis. We have established in vitro screening system and several mouse infection models to evaluate potential drug candidates. Here, I will introduce our research activity to identify new anti-toxoplasmosis drugs.

Curriculum Vitae

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2001: Ph.D., The University of Tokyo
2001-2003: Research Fellow in Yale University School of Medicine,
2003-2005: Senior Research Biochemist in Toray Industries, Inc.
2005-present: Associate Professor in Obihiro University of Agriculture and Veterinary Medicine

Plenary 4 - Current status in parasitic diseases

Session 7

Antimalarial drug-resistant parasite in Indonesia

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Efforts to control malaria are currently hindered by the rapid emergence of parasite resistance to antimalarial drugs, mosquito resistance to insecticides and the failure to develop a suitable malaria vaccine. Artemisinin-based combination therapy (ACT) was recommended by WHO for uncomplicated malaria since 2000. The emergence of artemisinin-resistant parasite in 2009 posed a grave concern as it will render no 'alternative treatments for malaria in Indonesia, the ACT was first recommended in 2004 after resistance to chloroquine and sulphadoxine pyrimethamine was found in many malaria endemic areas.

Since 2009, Eijkman Institute has been conducting therapeutic efficacy studies (TES) to monitor the efficacy of ACTs in 10 sentinel sites in Indonesia and the existence of Single Nucleotide Polymorphisms (SNPs) in the K-13 gene among the *P. falciparum* isolates. The recent results revealed that the burden of malaria is still high in Papua, Sumba and North Sulawesi. No delay in the parasite clearance was observed following Dihydroartemisinin-Piperaquine (DHP) treatment, the first line drug for uncomplicated malaria. Late treatment failures were observed in days 28-42, in 3 sites, Southwest Sumba, Flores and Minahasa Tenggara. The results showed that none of SNPs of the K13 gene in any of the *P. falciparum* isolates examined were found. In conclusion, TES studies in Indonesia revealed no artemisinin-resistant parasites were detected. Late treatment failures that may associated with the partner drug, piperaquine, have been found. The findings indicate that DHP has selected for drug-resistant parasite in several areas of Indonesia. The use of other ACT should be anticipated to delay or prevent the resistance to DHP.

Keywords: *Plasmodium, K-13 gene SNPs, dihydroartemisinin+piperaquine, insecticide resistance, kdr alleles*

Curriculum Vitae

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Syafruddin was trained in medicine and doctoral degree in the parasite cell biology, he is currently a senior researcher fellow at the Malaria and Vector Resistance Laboratory at the Eijkman Institute for Molecular Biology, Jakarta, and Professor and chairman of the Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

He joined the Eijkman Institute in 1993 as a post doctoral research fellow and was involved in a research project to elucidate the rol of the extrachromosomal DNAs in the assembly of the mitochondrial energy transducing membrane of the malarial parasite. His current activities include molecular mechanisms underlying *Plasmodium* resistance to antimalarial drug, antimalarial drug discovery, molecular taxonomy aof the mosquito vector and the molecular studies on the vector resistance to insecticides. To date, he has publised over dozen of international scientific publications in the relevant fields.

Plenary 4 - Current status in parasitic diseases

Session 8

Genetic diversity of *Plasmodium* sp. in Indonesia and its potential for malaria vaccine development

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Indonesia as a malaria endemic nation harbors diverse parasite genetics. Studies employing microsatellite analysis done by our group[1)] so far showed that *P. falciparum* populations clustered distinctly between West- and East- Indonesia. Whereas *P. vivax* reflects high diversity of isolates between different islands with no clear cluster. Furthermore, with a more high-resolution genotyping using whole genome sequencing approach[2,3)], however, *Plasmodium* isolates from Indonesia demonstrated unique cluster compared to worldwide isolates indicating exclusive parasite lines.

Sequence polymorphisms of *Plasmodium* antigens used in malaria vaccine component have been studied in various extent. The genes encoding parasite antigens involved in parasite invasions into human red cells have been explored. In *P. falciparum*, the family of erythrocyte binding antigens (PfEBAs) and reticulocyte binding-like homologous (PfRhs) are the two important antigens in malaria vaccine development. In *P. vivax*, the Duffy Binding Protein (PvDBP) has also been explored for malaria vaccine development.

The extent of sequence polymorphisms has hampered the development of all-for-one malaria vaccine. The challenges are therefore trying to understand the development of immunity to different malaria antigens. The antigens that potentially correlate with protection will ultimately be very useful for malaria vaccine. The efforts toward malaria vaccine development will be discussed during this presentation.

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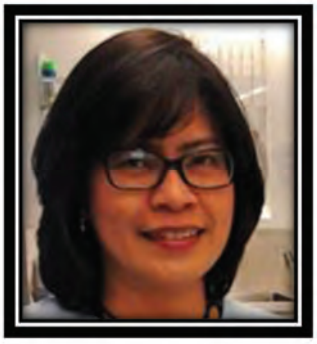
Curriculum Vitae



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Educational background

1987-1992 Faculty of Science, Department of Biology, The University of Indonesia

1997-1998 Post Graduate Diploma in Science, the University of Melbourne

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Recent publication

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Plenary 4 - Current status in parasitic diseases

Session 9
Epidemiology study of malarial parasites in Indonesia

Pretty Multihartina Sasono
Agency for Health Research and Development, Ministry of Health
e-mail:

Curriculum Vitae

Name : **Dr. Pretty Multihartina Sasono**
Affiliation : **Agency for Health Research and Development, Ministry of Health**
E-mail address :

Pretty Multihartina Sasono, is a researcher at the National Institute of Health Research and Development (NIHRD), Ministry of Health (MoH) Republic of Indonesia since 1989. She received Ph.D in Biology from The University of Leeds, UK in 1997. She had been honored a postdoctoral program at Center for Biologics Evaluation and Research (CBER), US-Food and Drug Administration (FDA) within US-NIH Campus from 2008 to 2010. Presently, beginning from February 2013, she is appointed as the Director of Center for Research and Development on Biomedical and Basic Health Technology at the NIHRD, MoH Republic of Indonesia. Recentlzy, she is elected as a President of Indonesian Biorisk Association.

Plenary 5 - Current status of drug discovery

Session 10

Search for new antibiotics from natural resources

Kazuro Shiomi
Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences,
Kitasato University
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Microorganisms have provided many drugs and useful compounds and are rich resources for drug discovery. We focus on discovering useful bioactive compounds, particularly anti-infectious drugs for bacteria, fungi, parasites, and viruses, from these microbial resources. In this symposium, I will talk about achievements of Professor Satoshi Ōmura, Nobel Laureate in Physiology or Medicine. He discovered an anthelmintic antibiotic, avermectin, produced by *Streptomyces avermectinius* with Merck's researchers. Its derivative, ivermectin, proved effective against river blindness and elephantiasis. I will also introduce our recent discovery of new microbial metabolites.

Curriculum Vitae

Name : **Prof. Kazuro Shiomi**
Affiliation : **Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University**
E-mail address : **shiomi@lisci.kitasato-u.ac.jp**

1979	Bachelor of Agriculture, Dept. Agricultural Chemistry, University of Tokyo, Japan
1979-1990	Researcher, Takara Shuzo Co., Ltd., Otsu, Japan
1980-1988	Guest Researcher, Institute of Microbial Chemistry, Tokyo, Japan
1988	Ph.D. in Agriculture, University of Tokyo, Japan
1990-2002	Chief Researcher, The Kitasato Institute, Japan
2002-2005	Associate Professor, School of Pharmaceutical Sciences, Kitasato University, Japan
2005-Present	Professor, Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Japan

Plenary 5 - Current status of drug discovery

Session 11

Searching for Lead Compounds of Antimalarial and Antiamebic Agents by Utilizing Indonesian Bioresources

Danang Waluyo
Laboratory for Biotechnology, Agency for the Assessment and Application of Technology (BPPT)
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In the other side, Indonesia is widely known as the second largest bio-diversity country just after Brazil. It has big potential to produce numerous active compounds for many purposes. To BPPT has collected more than 20,000 microbe isolates from all over Indonesia since 2004, and found that the microbe is quite unique compare to that from Japan. This indicates the potentiality of finding new lead compounds for drug development purposes.

Malaria is one of infectious diseases caused by parasites belonging to the genus *Plasmodium* and infecting more than 214 million people. This leads *Plasmodium* to become the most killing parasites in the world. WHO recommends malaria to be treated with ACT (artemisinin-based combination therapies). Despite of plant *Artimesia annua*, the producer of artemisinin, is regarded as introduced plant in Indonesia and has lower productivity compared to its original habitat, it has been reported that artimisinin resistance had been detected in 5 countries around Mekong's area.

Similar to malaria, amebiasis, a kind of diarrhea (intestinal infection disease) caused by parasite *Entamoeba histolytica*, is regarded as neglected diseases which is triggered by low hygiene and public sanitation environment. With 50 million cases annually, amebiasis becomes the second most killing disease caused by parasite. Metronidazole became the main drug to treat amebiasis, however, resistant to this drug has also been reported.

A project that is aimed to screen these bio-resources, mainly from microbes and plants, for searching novel lead compounds that has anti-malarial and anti-amebic activities is currently enrolled. Exploration of Indonesian bio-resources will be done to enrich the current bio-resource collection. Screening of active compound from extract of bio-resource will be conducted based on parasite-specific enzyme-based and in vitro-cell-based screening. Active compound will be purified from the extract and characterized by elucidating the chemical structure. Efficacy test of the active compound will be tested using animal model.

Curriculum Vitae

Name : **Danang Waluyo, MEng.**
Affiliation : **Laboratory for Biotechnology, Agency for the Assessment and Application of Technology (BPPT)**
E-mail address : **danang.waluyo@bppt.go.id**

Danang Waluyo serves as a Research Scientist at Laboratory for Biotechnology, the Agency for the Assessment and Application of Technology (BPPT), Indonesia. Danang pursued his bachelor (1999) and master (2001) in Department of Biotechnology, Faculty of Engineering, Osaka University. He also pursued his doctoral course at the same department in Osaka University. His dissertation research topic was application of metabolomics for development of high-ethanol producing recombinant yeast. This study employed multi-techniques including genetic engineering, fermentation, analytical chemistry and bioinformatics. He completed the course without degree (2009). He was then employed by his supervisor as research assistance to pursue metabolome analysis of xylose-assimilating recombinant yeast funded by NEDO Japan (2009-2011).

Danang has interested in application of bioengineering by employing multi-biotechniques for his research works, including microbiology, genetic engineering, analytical chemistry, and bioinformatics. In BPPT, he was appointed as PI for several national projects including the project for improvement of vitamin B12 production using genetic engineering approach (2003-2005), improvement of microbial antibiotics (penicillin, tetracycline) production (2013), and development of recombinant hepatitis B vaccine using local strain (2012-2015). Having a strong relationship with many research institutions from Japan, Danang is actively collaborating with several Japanese partners to do applied biotechnology-based research, including exploration and application of Indonesia microbial resources (2012-present), improvement of productivity of industrially important crops (rubber and oil palm, 2013-present), and development of analytical method for chiral drugs (2014-present). Recently, he was assigned as project head of a collaborative research project on searching of anti-parasitic lead compounds from Indonesian bioresources with several local and Japanese research institutions through SATREPS project (funded by JICA) (2015-present).

Plenary 5 - Current status of drug discovery

Session 12

Development of anti-malarial drug from Indonesian Plants

Eti Nurwening Sholikhah
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Several active compounds from medicinal plants have been developed as modern drugs. Artemisinin is an example of modern drug developed from Artemisia annua L, a plant that has been used for a long time in China. Indonesia is a country with a rich natural resources. The biodiversity in Indonesia consists of thousands of plant species. Therefore, the flora and fauna of Indonesia are potential for the development of antimalarial drugs. Several studies have been conducted to find some new compounds that could be developed as antimalarial drug candidates. It needs several steps of testing to develop an active compound to an antimalarial drug, both in vitro and in vivo preclinical study before conducting clinical study in human.

Curriculum Vitae

Name : **Dr. Eti Nurwening Sholikhah, M.D., M.Sc., M.Med.Ed.**
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Education

1. December 1993, Medical doctor, Universitas Gadjah Mada, Yogyakarta
2. May 2000, Master degree, Pharmacology, Universitas Gadjah Mada, Yogyakarta
3. October 2010, Doctoral degree, Pharmacology, Universitas Gadjah Mada, Yogyakarta
4. October 2015, Master degree, Medical Education, Universitas Gadjah Mada, Yogyakarta

Current Position

1. Secretary of Institutional Review Board (Medical & Health Research Ethics Committee) Faculty of Medicine Universitas Gadjah Mada - Dr. Sardjito General Hospital, Yogyakarta
2. Vice chair of Indonesian Pharmacology Association, Branch Yogyakarta
3. Head of Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Gadjah Mada.

Plenary 6 - Promotion of Drug Development Research

Session 13

Strategic planning for natural resources-based drug discovery in Indonesia

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Curriculum Vitae

Name : **Dr. Ira Nurhayati Djarot**
Affiliation : **Ministry of Research, Technology, and Higher Education**
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Education

Year	Dicipline	Degree	Institution
1987	Agronomy	Ir (BSc Hon)	Bogor Agricultural University
1991	Agronomy	MSc.	University of Wisconsin
2000	Plant Sciences	PhD	Australian National University

Training/Course

Name	Period	Place
Plant Gene Technology Workshop	17 – 23 December 1996	Canberra- Australia
Ionizining Radiation Hazard	18 March 1999	Canberra- Australia
Biological Hazard	10 October 1997	Canberra- Australia
Management and Commercialization of IP assests	12 – 13 July 2012	Singapore
Science, Technology and Innovation policy	10 – 28 June 2013	China

Publication in International Journal

Djarot, I. N. & Peterson, D. M. 1991. Seed development in a shrunken endosperm barley mutant. Annals of Botany 68:495-499.

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Plenary 6 - Promotion of Drug Development Research

Session 14

LPDP scheme for Research and Development in Indonesia

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Curriculum Vitae

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Affiliation **: Indonesia Endowment Fund for Education, Ministry of Finance**
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Education

Bachelor : Universitas Pancasila
Master : Brock University Canada

Plenary 6 - Promotion of Drug Development Research

Session 15

Business opportunities in Drug Development Research*

Wahono Sumaryono**
PT. Kimia Farma (Persero), Tbk.
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Research & Development could be directed into two way approach. One approach is conducted from upstream into downstream; which is also familiar as supply-push or technology-push approach while the other is directed from downstream into upstream, which is also familiar as demand-pull or market oriented approach. Any forementioned approach has its characteristics in term of advantages and disadvantages.

Pharma market is projected shifting from "Treating Disease Orientation" into "Balanced Treating & Preventing Disease Management".

Indonesia as a country that has big population, rich of biodiversity, and also belong to Pharma-Emerging Market" is necessarily directed its R&D program on Pharmaceutical Development based on its potentials leading to market orientation. Since disease pattern in Indonesia is still dominated by infectious disease and degenerative disease, therefor any research program for drug development is suggested to focus on both of two group diseases. In addition, research development on antioxidative compounds, immunostimulant, and anti cancer which has selective cytotoxicity are also seem to be prospective to be conducted.

* International Symposium on Natural Resource-based Drug Development, held at BPPT, Building II, Jakarta 21-22 August 2017, BPPT-JICA-AMED

** 1. Professor in Natural Product Chemistry-Faculty of Pharmacy-Univ of Pancasila, Jakarta
2. Commisioner of PT.Kimia Farma (Persero) Tbk.

Curriculum Vitae

Name : **Prof.Dr.rer.nat. Wahono Sumaryono, Apt.**
Affiliation : **PT. Kimia Farma**
E-mail address : **prof.wahono@gmail.com**

Place/date of birth : Solo, 21 January 1954

Education : 1. Faculty of Pharmacy –Airlangga University, Surabaya for Pharmacist Degree (Doctorandus Pharmaciae), 1974-1980 for Professional Pharmacist (Apothecary Level), 1980-1981
2. Technical University Braunschweig Germany for Doctor degree (Dr.rer.nat), 1986-1990

Work Experience :

Research Activities

1. Research staff at BPPT ; 1981-1986
2. Research Associate at Directorate of Life Sciences-BPPT, 1990-1992
3. Principal Researcher at The Center for Pharmaceutical & Medical Technology-BPPT, 1999
4. Research Professor at The Center for Pharmaceutical & Medical Technology-BPPT, 2006
5. Professor in Natural Product Chemistry at Faculty of Pharmacy-University of Pancasila, 2007

Managerial Activities

1. Deputy Director for Life Science BPPT, 1992-1998
2. Director for Pharmaceutical & Medical Technology-BPPT, 1998-2000
3. Deputy Chairman of BPPT for Agroindustry & Biotechnology, 2000-2010
4. Dean of Faculty of Pharmacy-University of Pancasila, 2011-2014
5. Comissioner for PT.Kimia Farma (Persero) Tbk, 2012-present
6. Rector of the University of Pancasila, 2014-present

ORGANIZING COMMITTEE

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Accomodation and Transportation	Nurlaila, MSi. - Eka Siska, SSi. - Kristiningrum, SSi. - Kesi Kurnia, SSi. - Wawan

SATREPS SLeCAMA Project

The Project for Searching Lead Compound for Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

International Symposium on

Natural Re ource ba ed Drug Development

August 21-22, 2017

BPPT Build.II, Jakarta

Organized by:

- Agency for the Assessment and Application of Technology (BPPT)
- Japan International Cooperation Agency (JICA)
- Japan Agency for Medical Research and Development (AMED)

Co-organized by:

- Indonesian Institute of Science (LIPI)
- Airlangga University
- The University of Tokyo
- Nagasaki University
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SATREPS SLeCAMA Project

The Project for Searching Lead Compound for Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Program Book

The 2nd International Symposium on

Natural Resources-based Drug Development

October 9th, 2019

Sari Pacific Jakarta, Jl. MH Thamrin 6, Jakarta, Indonesia

Organized by:

Agency for the Assessment and Application of Technology (BPPT)

Japan International Cooperation Agency (JICA)

Co-organized by:

LIPI Indonesian Institute of Science

Airlangga University

東京大学 The University of Tokyo

長崎大学 Nagasaki University

北里大学 Kitasato University

MicroBiopharm Japan

CONTENT

GREETING AND OPENING SPEECH FROM DEPUTY CHAIRPERSON OF BPPT

Mr. Tadayuki MIYASHITA, Minister of Economic Affairs, Embassy of Japan in Indonesia
Mr. Daisuke INOUE, First Secretary, Health Sector, Embassy of Japan in Indonesia
Mr. Shunsuke TAKATOI, Senior Representative, JICA Indonesia Office
Prof. Haruo WATANABE, Program Supervisor, AMED

Dr. Soni Solistia Wirawan, M.Eng.
BPPT Deputy Chairperson

Invited speakers
Distinguished Guests, Ladies and Gentlemen,

Good morning,
Ohayou gozaimasu,
Assalamu'alaikum Wr. Wb.

First of all, I would like to warmly welcome you all in this very special event, the 2th International Symposium on Natural Resources-based Drug Development
In this great occasion, I would like to express my greatest and sincere thanks and appreciation to JICA and all partners in preparing and organizing this special event. I hope that this symposium will be a fruitful and beneficent for BPPT, JICA and all of the partners involved.

Ladies and Gentlemen,
Developing a drug is a long and complex process that requires a large and serious effort. As a country with abundant biological resources to be used as a giant chemical library, it is the perfect time for Indonesia to start utilizing these resources for drug development more seriously. With support from JICA and AMED, BPPT has started through the SATRPES Program 2015-2020, Searching Lead Compound of Anti-Malarial and Anti-Amoebic Agents by Utilizing Diversity of Indonesian Bio-resources (SleCAMA project).
The SATREPS program has been running well and smoothly, and currently the project is entering the final stage of the activities. BPPT and Indonesian counterparts gained significant benefits from this SATREPS program. Capacity building is very crucial for Indonesia to discover lead compounds from natural resources. It requires a strong and solid concept and systematic process to obtain promising compounds for drug. Many aspects are needed to be strengthened to realize our dream, which is to develop a drug from our natural resources by our hands. I am very happy that the SATREPS program through SeCLAMA project has made this become closer to be realized. In addition, not only improvement in term of lab capacity such as equipment, this activity has also provided significant reinforcement to increase the capacity of human resources, especially the ability to design and conduct research toward drug discovery.

Ladies and Gentlemen,

At today's symposium, I hope there is sharing of scientific information and strategic policies to strengthen drug discovery activities, especially in Indonesia. Communication and information exchange between researchers followed by collaboration between research institutions is very important. Through its role as technology intermediator, BPPT will play an active role in activities and collaborative research in field of drug development. Indonesia requires an accelerated mastery of technology to utilize biological resources as a source of developing active ingredients for medicines.

I am very happy to hear that the invited speakers in this symposium are from several key institutions for drug development in Indonesia, such as National Food and Drug Control (or BPOM), National Institute of Health Research and Development of Ministry of Health, LIPI, Universities (UGM, IPB, UI), Ministry of Marine and Fisheries Affairs, and of course from BPPT.

Distinguished Guests, Ladies and Gentlemen,

I wish you are pleased to follow the symposium, enjoy and actively involve in information exchanging. Hopefully today's symposium will strengthen networking among participants, between institutions and between Indonesia and Japan.

Once again on behalf of BPPT I would like to thank JICA, AMED, Japanese and Indonesian partners and to all symposium participants today for your participation in this symposium.

Wassalamu'alaikum wr.wb.

Jakarta, October 9th, 2019

BPPT Deputy Chairperson

Dr.Soni Solistia Wirawan, M.Eng.

BPPT in Brief

Agency for the Assessment and Application of Technology (BPPT) is a non-ministry government institution that is directly responsible to the President of the Republic of Indonesia under the coordination of the Ministry of Research, Technology and Higher Education. BPPT was established in 1978, and Prof. Dr. Ing. Bacharudin Jusuf Habibie was the first Chairperson.

BPPT's vision is "To be a technology center of excellent that prioritizing innovation and technology services for promoting national competitiveness and sovereignity". BPPT has mission to assess and apply technology to create innovation and technology services in field of technology for food, health, electricity, fuel, ICT, transportation, security and defense, material, machinery, disaster reduction, natural resources and marine, environment, and innovation system.

BPPT has governmental duties in the assessment and application of technology in accordance with the provisions of applicable laws. BPPT does not only play a role as an intermediary agency that bridges the interests of customers and technology providers, but also serves to provide approval to the key technologies that will be used in Indonesia. The role of BPPT as a clearing house agency is realized here. Other roles performed by BPPT are technology assessment (engineering) and technology audit including providing technology solutions. Its entire activities are aimed to provide innovation and technology services to support the improvement of the people's welfare. Its technology services comprise recommendation, advocacy, technology transfer, consultancy, testing, operation services, pilot project, pilot plant, prototype, surveys, technical reference, technology audit, and technology-based startups.

These roles of BPPT should be able to provide value proposition to the beneficiaries of BPPT's output in the improvement of competitiveness and sovereignity in technology mastery through technology transfer as well as the acquisition of the latest technology. These roles are implemented by BPPT through principle secretary and 5 deputies: Deputy for Technology Policy Assessment, Deputy for Natural Resource Development Technology, Deputy for Agroindustrial Technology and Biotechnology, Deputy for Information, Energy and Material Technology, and Deputy for Industrial Technology, Design and Engineering.

Currently, BPPT has more than 3000 employees (engineers, researchers, administrative staffs) and advanced laboratories to support its technology innovation and service activities. BPPT also has wide collaboration and networking with numerous local and international research institutes and industries.

Deputy of Agroindustrial Technology and Biotechnology-BPPT in Brief

Deputy of Agroindustrial Technology and Biotechnology (TAB) is one of technical deputy in BPPT that has duties of planning and implementation of policies in field of agroindustrial technology and biotechnology. There are 4 centers and 2 implementing units under TAB: Center for Agroindustrial Technology, Center for Agricultural Production Technology, Center for Bioindustry, Center for Pharmaceutical and Medical Technology, Center for Starch Technology, and Laboratory for Biotechnology.

TAB has been largely contributed to create innovation and services in field of technology for food and health for promoting national competitiveness and sovereignity.

Gelatin from seaweed: alternatif for halal ingedient

Standardized herbal medicine *Neurat* for lowering blood uric acid

Pharmaceutical grade salt (currently manufactured by local pharmaceutical industry)

Black garlic, a health supplementary food with stong antioxidant activity

Rice made from cassava flour for food diversification

Rice and noodle products made from local row material such as sago and corn

Development of salt water-tolerance Tilapia

Ex-vitro propagation technology for pepper seedlings production

Complete feed for grower cattle qualified from oil palm waste

Laboratory for Biotechnology-BPPT in Brief

Laboratory for Biotechnology (previously known as Biotech Center, BC) is one of implementing unit in Deputy of Agrotechnology and Biotechnology, BPPT. It was inaugurated by President Soeharto in December 29, 1995. Located in Puspiptek, South Tangerang, the laboratory has vision "To be center of excellent of Biotechnology that prioritizing biotechnological innovation and services for promoting industrial competitiveness and national sovereignity".

BC enroles two major competencies, industrial biotechnology and agricultural biotechnology, which contributes to BPPT's mission in field of technology for health and food. BC has 106 employees and more than half of them are govermental officials as engineers, researchers, and administrative staffs. Equipped with advanced laboratory facilities, including pilot-scale fermentation and recovery facility and pilot-scale *in-vitro* and *ex-vitro* plant propagation facility, BC has been developed numerous bio-based technology innovation that are useful for industries and communities. Accreditated by National Accreditation Committee as internationally standardized testing laboratory (ISO17025) and strong networking with both local and international research institutes and industries, as well as with local government, BC has diseminated numbers of technologies developed, as well as assessed, in BC to numerous stake holders.

Plant propagation technology

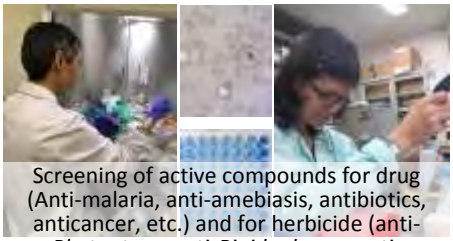
Plant Productivity and Field Improvement

Bitumman (self-growing seedling), a biofertilizer-coated seedling for revegetation of ex-mining field

Technofert, a biofertilizer containing microbial consorsia (*Mycorrhiza arbuscular, Corynebacterium* sp.) for increasing nutrient adsorption from soil

Technology for Drug Development

Microbial collection: field sampling, isolation, identification, preservation (focus on Fungi and Actinomycetes)



Screening of active compounds for drug (Anti-malaria, anti-amebiasis, antibiotics, anticancer, etc.) and for herbicide (anti-*Phytoptora*, anti-*Rigidophorus*, anti-*Fusarium*, etc)

Fermentation, purification, and characterization of active compounds (lab and pilot scale fermenter up to 2500 L, lab and pilot scale down stream process, mass spectrometry, etc.)

Laboratory for Biotechnology
(Biotech Center)
Building 630, Puspiptek Area, Setu,
South Tangerang 15314, Banten, Indonesia
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AGENDA

The 2nd International Symposium on Natural Resources-based Drug

Wednesday, October 9th,2019	
08.30 – 09.00	Registration
Opening Session	
09.00 – 09.30	Opening remark by Minister, Embassy of Japan in Indonesia
	Opening remark by Chief Representative, JICA Indonesia Office
	Opening remark by Project Director (BPPT Deputy Chairperson)
	Photo session
Keynote speech **Moderator: Dr. Tomoyoshi Nozaki** (Professor, The University of Tokyo)	
09.30 – 10.00	Microbial diversity: exploration and utilization of microbial resources for drug discovery **Kenichiro SUZUKI** (Professor, Tokyo Agriculture University)
10.00 – 10.30	Screening of new antifungal compounds based on morphological change of fungi **Hiroyuki OSADA** (Director, Chemical Biology Research Group RIKEN Center for Sustainable Resource Science)
Plenary 1 – Promoting Drug Discovery Research in Indonesia **Moderator: Dr. Agung Eru Wibowo** (Director, Laboratory for Biotechnology, BPPT)	
10.30 – 10.50	Session 1: Regulatory Framework for Supporting Drug Research and Development in Indonesia **Siti Asfijah Abdoellah** (National Agency of Drug and Food Control)
10.50 – 11.10	Session 2: Concept and Direction of Health Research in Indonesia **Siswanto** (National Research Council/Head of Agency for Health Research and Development)
Plenary 2 – Exploration of bioresources for drug development **Moderator: Dr. Daniel Ken Inaoka** (Nagasaki University)	
11.10 – 11.30	Session 3: (Fish Pathogenic Microbial Collection) **Woro Nur Endang Sariati** (Ministry of Marine Affairs and Fisheries)
11.30 – 11.50	Session 4: Community Based Exploration of Local Ethnomedicine Knowledge and Medicinal Plants in Indonesia **Yuli Widiyastuti** (Center for Research and Development of Medicinal Plant and Traditional Medicine, Ministry of Health, Indonesia)
11.50 – 12.10	Session 5: Utilization of Culture Collection for Drug Discovery Resources **Gayuh Rahayu** (IPBCC, IPB University)
12.10 – 13.30	Lunch
Plenary 3 – Target Development and Screening of Active Compound **Moderator: Dr. Takaya Sakura** (Nagasaki University)	
13.30 – 13.50	Session 6: Anticancer Properties of Curcumin and Its Analogs Targeted on ROS Metabolizing Enzymes **Edy Meiyanto** (Cancer Chemoprevention Research Center, Gadjah Mada University)
13.50 – 14.10	Session 7: Searching of Antibiotic from Indonesian Endophytic Fungi **Andria Agusta** (Research Center for Biology, LIPI)
14.10 – 14.30	Session 8: Searching Lead Compounds of Anti-Amebic Agent by Utilizing Diversity of Indonesian Bio-Resources **Myrna Adianti** (Institute for Tropical Disease, Airlangga University)
Plenary 4 – Fermentation Technology and Structure Modification **Moderator: Dr. Chaidir (BPPT)**	
14.30 – 14.50	Session 9: Modification Design of Cinnamic Acid Derivates as Dipeptidyl Peptidase-IV Inhibitor Using In Silico Fragment-Based Method **Arry Yanuar** (Biomedical Computation and Drug Design Laboratory, Faculty of Pharmacy, Universitas Indonesia)

14.50 – 15.10	Session 10: Strategy for Optimization of Lead Compounds as Drug Candidate from Natural Resources **Muhammad Hanafi** (Research Center for Chemistry, LIPI)
15.10 – 15.30	Session 11: Pilot Scale Fermentation of Cephalosporin C **Anis Herliyati Mahsunah** (Laboratory for Biotechnology, BPPT)
15.30 – 16.00	Coffee Break and Poster Session
Plenary 5 – General Discussion	
16.00 – 17.00	Chair: **Agung Eru Wibowo** (Laboratory for Biotechnology, BPPT) Topic: - Promoting drug discovery research in Indonesia - Strengthening research network
17.00 – 17.10	Closing Remark **Dr. Soni Solistia Wirawan** (Deputy Chairperson, BPPT)

Abstract and Curriculum Vitae of Invited Speakers

Keynote Speech

Microbial Diversity for Bioprospecting and the Roles of Microbial Resource Center

Kenichiro Suzuki
Dept. Fermentation Sciences, Tokyo University of Agriculture
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Microorganisms with potential for new function and utilization are important resources for bioprospecting. For discovery of such microorganisms, we must make screening from a large number of resources with variety. International cooperation enables access to diverse microbial resources. Generally, microorganisms, especially bacteria, are thought to be cosmopolitan and widely distributed in the world. However, repeated isolation from the samples collected at various places, a certain location is found. Even among the strains classified in the same species, there are diversity in the characteristics. It is of value to collect diverse microbial strains from microbial resource centers (mBRC) for screening source as well as the own isolates. BRC is an infrastructure of biotechnology research and basic sciences as the supplier of (1) supplier of the strains for standardized tests, (2) taxonomic type strains and (3) strains used in the published studies. Microorganisms in mBRC are (1) diverse because they were isolated by many researchers (2) correctly identified with appropriate taxonomic characterization and (3) clear accessibility and limit of use including international transfer in compliance with laws and regulations of the provider countries. Recently whole genome sequences have been determined for many of strains in mBRC. The microbial resources in mBRC are useful for effective screening with available information of the resources.

Curriculum Vitae

Name : **Prof. Dr. Kenichiro Suzuki**
Affiliation : **Tokyo Uiversity of Agriculture**
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Employment record:

- 2014 - 2019 Tokyo University of AgricultureFaculty of Applied Bio-Science, Department of Fermentation Science - Professor
- 2001 - 2014 NBRC Culture Collection – Director General
- 1994 – 2001 Japan Collection Microorganism - Head

Publication:

- Hamada, M., Shibata, C., Nurkanto, A. Ratnakomala, S., Lisdiyanti, P., Tamura, T. and Suzuki, K. Ropicihabitans flavus gen. nov., sp. nov., a new member of the family Cellulomonadaceae.Antonie van Leeuwenhoek 107. 2015.05
- Hamada, M., Shibata, C., Nurkanto, A. Ratnakomala, S., Lisdiyanti, P., Tamura, T. and Suzuki, K. Serinibacter tropicus sp. nov., an actinobacterium isolated from the rhizosphere of a mangrove, and emended description of the genus Serinibacter. Int J Syst Evol Microbiol 65. 2015.04
- Mori, K., Suzuki, K., Yamaguchi, K., Urabe, T. and Hamada, S. Thiogranum longum gen nov., sp. nov., an obligately chemolithoautotrophic, sulfur-oxidizing bacterium of the family Ectothioorhodospiraceae isolated from a deep-sea hydrothermal field, as an emended description of the genus Thiohalomonas. Int J Syst Evol Microbiol 65. 2015.01
- Moon, J-Y., Kim, S-J., Hamada, M., Ahn, J-H., Weon, H-Y., Suzuki, K., Yoon, J-H. & Kwon, S-W. Gryllotalpicola soli sp. nov., isolated from soil. Int J Syst Evol Microbiol 64. 2014.12
- Lim, J-M., Kim, S-J., Hamada, M., Ahn, J-H., Weon, H-Y., Suzuki, K., Ahn, T-Y., Kwon, S-W. Nocardioides daecheongensis sp. nov., isolated from soil. Int J Syst Evol Microbiol 64. 2014.12
- Iino, T., Mori, K., Itoh, T., Kudo, T., Suzuki, K., Ohkuma, M. Mariniphaga anaerophila gen. nov., sp. nov., a facultative aerobic marine bacterium isolated from tidal flat sediment, and proposed reclassification of Draconibacteriaceae into Prolixibacteraceae and the family Marinifilaceae fam. nov. Int J Syst Evol Microbiol 64.2014.
- Lim, J-M., Kim, S-J., Hamada, M., Ahn, J-H., Weon, H-Y., Suzuki, K., Ahn, T-Y. & Kwon, S-W. Oryzihumus terrae sp. nov., isolated from soil and emended description of the genus Oryzihumus. Int J Syst Evol Microbiol 64. 2014.07
- Mori, K., Yamazoe, A., Hosoyama, A., Ohji, S., Fujita, N., Ishibashi, J, Kimura, H. & Suzuki, K. Thermotoga profunda sp. nov. and Thermotoga caldifontis sp. nov., anaerobic thermophilic bacteria isolated from terrestrial hot springs. Int J Syst Evol Microbiol 64. 2014.06

Keynote Speech

Screening of new antifungal compounds based on morphological change of fungi

Hiroyuki Osada
RIKEN Center for Sustainable Resource Science

Several fungicides have been developed to control fungal infection; however, pathogenic fungi often acquire resistance to fungicides. Thus, new antifungal agents need to be developed continuously to suppress the fungal infection. In order to find novel antifungal compounds, we have established screening systems based on the morphology-change induced by the compounds. When we added the compounds isolated from microbial fermentation broths, fungi change their morphology according to the mode of action of the compound added. We have constructed databases linking morphology to drug function, named "MorphoBase", which can be helpful for drug discovery.

For the morphology based screening, we use two fungal strains; one is a plant pathogenic fungus, *Pyricularia oryzae*, which causes rice blast disease. The other one is an opportunistic human pathogen, *Candida albicans*, which causes candidiasis in human. The morphology-change of fungi was captured by a high-content microscope machine and analyzed by deep learning method (NVIDIA DIGITS).

I will present the compounds YO-001A and RK-276A isolated by the screening programs using *P. oryzae* and *C. albicans*, respectively.

CURRICULUM VITAE

Name : **Hiroyuki OSADA**
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Education

1974-1978 : The University of Tokyo,
Department of Agricultural Chemistry
1978-1983 : Doctor Course, The University of Tokyo, Faculty of Agriculture
Dr. Agriculture (1983) The University of Tokyo

Apponitments:

1983-1991 : Scientist, Antibiotics Laboratory, RIKEN
1985-1986 : Fogarty Fellow, National Cancer Institute, NIH, USA.
1992- 2015 : Chief Scientist, Antibiotics Laboratory, RIKEN
1999- present : Visiting Professor, Saitama University
2009- 2013 : Director, Chemical Biology Core Facility, RIKEN ASI
2013- present : Director, Chemical Biology Research Group,
Deputy Center Director, RIKEN Center for Sustainable Resource Science

Academic Activities :

President of the Society for Actinomycetes Japan (2012-2015)
President of the Japanese Association for Molecular Target Therapy of Cancer (2015-2018)
President of the Japanese Society for Chemical Biology (2018-present)
Editorial Board Member;
Assay and Drug Development Technology
Cancer Science
Journal of Antibiotics
Journal of Microbiology and Biotechnology
Oncology Research
Advisory Board Member;
ACS Chemical Biology

Award :

Research Promotion Award of Agricultural Chemical Society of Japan (1991)
Sumiki-Umezawa Memorial Award from Japan Antibiotic Research Association (1996)
Award of the Society for Actinomycetes Japan (2000)

Award of the Minister of Education, Culture, Sports, Science and Technology (2001)
Award of the Bioindustry Association (2007)
Award of Agricultural Chemical Society of Japan (2009)
Significant Achievement Award (S) RIKEN (2010)
Inhoffen Award in Germany (2015)
Special Award of Agricultural Chemical Society of Japan (2016)

Major Recearch Interest

Isolation of new bioactive compounds
Biosynthesis of bioactive microbial metabolites
Molecular target identification of bioactive compounds

Selected papers

1. W. Scott, *et al.* "Using BEAN-counter to quantify genetic interactions from multiplexed barcode sequencing experiments", **Nature Protocols,** 14:415-440 (2019)**.**
2. JS Piotrowski, *et al.* "Functional annotation of chemical libraries across diverse biological processes", **Nature Chemical Biology,** 13: 982-993 (2017).
3. F Hasebe, K Matsuda, T Shiraishi, T Yashiro, Y Futamura, M Hara, N Nakano, T Nakano, T Tomita, K Ishigami, H Taka, T Fujimura, C Nishiyama, H Osada, T Kuzuyama & M Nishiyama. "Amino group carrier protein-mediated secondary metabolite biosynthesis in *Streptomyces*", **Nature Chemical Biology** 12: 967–972 (2016).
4. L Ray, T Valentic, T Miyazawa, DM Withall, L Song, JC Milligan, H Osada, S Takahashi, SC Tsai & GL Challis. "A crotonyl-CoA reductase-carboxylase independent pathway for assembly of unusual alkylmalonyl-CoA polyketide synthase extender units", **Nature Communications** 7: 13609 (2016).
5. CS Yun, T Motoyama & H Osada. "Biosynthesis of the mycotoxin tenuazonic acid by a fungal NRPS-PKS hybrid enzyme", **Nature Communications** 6: 8758 (2015).
6. Y Soeda, M Yoshikawa, OF Almeida, A Sumioka, S Maeda, H Osada, Y Kondoh, A Saito, T Miyasaka, T Kimura, M Suzuki, H Koyama, Y Yoshiike, H Sugimoto, Y Ihara & A Takashima. "Toxic tau oligomer formation blocked by capping of cysteine residues with 1,2-dihydroxybenzene groups", **Nature Communications** 6: 10216 (2015).
7. S Takahashi, A Toyoda, Y Sekiyama, H Takagi, T Nogawa, M Uramoto, R Suzuki, H Koshino, T Kumano, S Panthee, T Dairi, J Ishikawa, H Ikeda, Y Sakaki & H Osada. "Reveromycin A biosynthesis uses RevG and RevJ for stereospecific spiroacetal formation", **Nature Chemical Biology** 7: 461-468 (2011).
8. Y Sun, F Hahn, Y Demydchuk, J Chettle, M Tosin, H Osada & PF Leadlay. "*In vitro* reconstruction of tetronate RK-682 biosynthesis", **Nature Chemical Biology** 6: 99-101 (2010).
9. I Miyazaki, S Simizu, H Okumura, S Takagi & H Osada. "A small-molecule inhibitor shows that pirin regulates migration of melanoma cells", **Nature Chemical Biology** 6: 667-673 (2010).
10. A Yano, S Tsutsumi, S Soga, MJ Lee, J Trepel, H Osada & L Neckers. "Inhibition of Hsp90 activates osteoclast c-Src signaling and promotes growth of prostate carcinoma cells in bone", **Proceedings of the National Academy of Sciences, USA.** 105: 15541-15546 (2008).

11. M Kawatani, H Okumura, K Honda, N Kanoh, M Muroi, N Dohmae, M Takami, M Kitagawa, Y Futamura, M Imoto & H Osada. "The identification of an osteoclastogenesis inhibitor through the inhibition of glyoxalase I", **Proceedings of the National Academy of Sciences, USA** 105: 11691-11696 (2008).
12. J-T Woo, M Kawatani, M Kato, T Shinki, T Yonezawa, N Kanoh, H Nakagawa, M Takami, KH Lee, PH Stern, K Nagai & H Osada. "Reveromycin A, an agent for osteoporosis, inhibits bone resorption by inducing apoptosis specifically in osteoclasts", **Proceedings of the National Academy of Sciences, USA.** 103: 4729-4734 (2006).
13. N Watanabe, H Arai, J-I Iwasaki, M Shiina, K Ogata, T Hunter & H Osada. "Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways", **Proceedings of the National Academy of Sciences, USA.** 102: 11663-11668 (2005).
14. N Watanabe, H Arai, Y Nishihara, M Taniguch, N Watanabe, T Hunter & H Osada. "M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by $SCF^{B\text{-}TrCP}$", **Proceedings of the National Academy of Sciences, USA.** 101: 4419-4424 (2004).
15. S Simizu & H Osada. "Mutations in the Plk gene lead to instability of Plk protein in human tumor cell lines", **Nature Cell Biology.** 2: 852-854 (2000).

Plenary 1 – Promoting Drug Discovery Research in Indnoesia

Session 1

Regulatory Framework for Supporting Drug Research and Development in Indonesia

Siti Asfijah Abdoellah, SSi, Apt, MmedSc
National Agency of Drug and Food Control

As mandated in Presidential Instruction No. 6 Year 2016, regarding Acceleration on Pharmaceutical and Medical Device Industries Development, which is aimed to: toward independency of pharmaceutical and medical devices industry and its competitiveness, Badan POM (Indonesia FDA) is one of government institutions that has been included in. In specifically, concerning of drug development, Badan POM is instructed to facilitate drug development to support access and availability of medical product to improve public health care services in term of National Health Insurance (Jaminan Kesehatan Nasional atau JKN).

Badan POM, as National Regulatory Authority (NRA) has already some regulations and guidances to support the research and development of drug or medical product as references for researchers, pharmaceutical industry, and clinical research organization. Badan POM also provides and facilitates mechanism to consult with, through regulatory assistance program and activities, in order to be in line with the standard and requirements for safety, efficacy and quality of medical product and ready to be marketed and used for fulfilling access for public health.

CURRICULUM VITAE

Siti Asfijah Abdoellah, a pharmacist, graduated from **Faculty of Pharmacy, Airlangga University in Surabaya, Indonesia in 1996**. Took a **master degree in Clinical Epidemiology (specialization on Pharmacoepidemiology) from 2004 – 2006 in Faculty of Health, Center of Clinical Epidemiology and Biostatistic, University of New Castle, Australia. The title for the master degree is Master in Medical Science (MMedSc)**

Starting her career as a staff in Badan POM (Indonesia FDA) since 1998. There were several positions had been assigned for, namely: staff of Cosmetic and Medical Devices Evaluation Division (1998-2000); staff of Medical Devices Evaluation (2000 – 2002); Head of Section of Non Electrical and Low Risk Medical Devices Evaluation (2002-2004); Head of Section of Surveillance of Therapeutic Products (2006 -2012); and Deputy Director of Surveillance and Risk Analysis of Therapeutic Product (2012 – 14 Feb 2018).

Starting 15 February 2018, she has been assigned as **Deputy Director of Clinical Trial and Special Access Evaluation, under Directorate of Drug Registration.** During her career, she has attended some trainings, workshops, seminars, conferences, meetings as participant/facilitator/speaker/ source person; become member of some committees/working groups (national and international).

Plenary 1 - Exploration of bioresources for drug development

Session 2

Policy and Direction of Health Research in Indonesia

Siswanto
DG, National Institute of Health Research and Development
Ministry of Health, Republic of Indonesia

Based on Indonesian Burden of Disease Study, Indonesia is facing an epidemiologic transition. The Proportion of Disability Adjusted Life Years (DALY) lost caused by Non Communicable Diseases (NCDs) is rising from 40% in 1990 to 70% in 2017. The proportion of DALY lost caused by Communicable Diseases (CDs) is declining from 51% in 1990 to 24% in 2017. The rest of DALY lost is caused by injuries that has shown a small decline from 9% in 1990 to 6.5% in 2017. The major NCDs as the leading causes of death are cerebrovascular diseases, heart diseases, diabetes, cirrhosis, COPD, cancer, and maternal and neonatal death. While, the major CDs are tuberculosis, diarrhea, and lower respiratory tract infection (pneumonia).

In terms of risk factors, they can be categorized into metabolic factors, behavior, and environment. The leading causes of metabolic factors are hypertension, dietary risks, high fasting plasma glucose, high body mass index, and malnutrition. The major behavior risks are smoking, occupational risks, low physical activity, and alcohol use. While, the environmental risks are air pollution, water and sanitation, and other environmental risks (climate change).

By combining health sector review analysis and burden of diseases data, the five-year midterm plan for health 2020-2024 (RPJMN 2020-2024) has determined 5 strategic priorities, i.e. reducing maternal dan infant mortality rate, reducing prevalence of stunting, improving diseases prevention and control, institutionalizing healthy behavior to community, and improving access and quality of health care.

From the above priorities, health research will be focused on providing solutions of the problems. In terms of operational research, research will be focused on strengthening health system as well as multi-sectoral collaboration to tackle the problems. The priorities of research themes would be: effective management, effectiveness of interventions, innovation of interventions (including the use of IT), surveillance, as well as, policy framework and regulation. While in terms of health products, it should be developed the effective modalities to address the major burden of diseases, i.e. biological therapies (stem cell therapy, cell therapy, biosimilar, monoclonal antibodies), vaccines, rapid test for diagnostics, phytomedicines (phytofarmaca), drugs for NCD (hypertension, cerebrovascular diseases, cancers, COPD), and new drugs for major CDs (tuberculosis, malaria, dengue, HIV).

For operational research, the best approach is by conducting Client Oriented Research Approach (CORA), while for products development is by ABGC synergy, i.e. the synergy and collaboration between Academicians (researchers), Business (Industries), Government (Regulatory), dan Community (Users). One of the methods for synergizing ABGC is by developing research consortium or research cluster that is capable of enabling sharing of resources and avoiding un-necessary overlap.

Keywords*:* Indonesian Burden of Diseases, Epidemiologic transition, research priority, operational research, health products, synergy, consortium

Curriculum Vitae

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Place and date of Birth : **August, 1954**
Present Position : **Director General of National Institute of Health Research and Development, Ministry of Health**
Office Address : **Jl. Percetakan Negara 29, Jakarta**
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Education

- Diploma of Tropical Medicine, Nagasaki University, Japan (2001)
- Master of Health Planning, University of New South Wales, Sydney (1998)
- Fakultas Kedokteran Unair, Surabaya (1987)

Professional History

- Ketua Komisi Saintifikasi Jamu Nasional
- Kepala Pusat Teknologi Terapan Kesehatan dan Epidemiologi Klinik, Badan Litbang Kesehatan
- Kepala Pusat Penelitian dan Pengembangan Gizi dan Makanan, Badan Litbang Kesehatan
- Kepala Bidang Pelayanan Penelitian, Pusat Penelitian dan Pengembangan Sistem dan Kebijakan Kesehatan, Badan Litbang Kesehatan
- Kepala Bidang Program dan Kerjasama, Pusat Penelitian dan Pengembangan Sistem dan Kebijakan Kesehatan, Badan Litbang Kesehatan
- Kepala Seksi Yankesmas, Kantor Departemen Kesehatan, Kabupaten Kulonprogo
- Kepala Puskesmas Panjatan, Kantor Departemen Kesehatan, Kabupaten Kulonprogo
- Kepala Puskesmas Pengasih, Kantor Departemen Kesehatan, Kabupaten Kulonprogo
- Dokter Puskesmas Samigaluh, Kantor Departemen Kesehatan, Kabupaten 1 Kulonprogo

Scientific publication

- Saintifikasi Jamu Sebagai Upaya Terobosan Untuk Mendapatkan Bukti Ilmiah Tentang Manfaat dan Keamaman Jamu. Buletin Penelitian Sistem Kesehatan. April 2012.
- Asean Common Guideline of Research on Traditional Herbal Medicine. Asean Secretariat. Co-author.
- Reviving health posts as an entry point for community

- development: a case study of the Gerbangmas movement in Lumajang district, Indonesia, Social determinants approaches to public health, from concept to practice, World Health Organization, 2011
- Trade-off analysis of Indonesian Health Reform, Journal of Health Service Management. Sep-Dec 2010
- Political methapor as magerial approach Journal of Health Administration and Policy, Vol. 4, No.3, Sep-Dec 2006.
- Political Approach as A Strategy in Health Development Advocacy (The Indonesian Journal of Health Service Management)

Seminar Articles

- Jamu Scientification, A breakthrough for eveidence-based health care. FAPA, Bali, 2012.
- The Development of Medicinal Plant and Traditional Medicine. Life Science Inovation Forum (LSIF). APEC Jakarta 2013.
- Meeting Health System Needs to Achieve Sustainability and Quality of Healthcare, (A Concept for a better amalgam between hospital care and primary health care as well as between conventional care and Traditional Complementary Medicine). SUM 3 APEC Bali 2013.
- Utilizing T&CM for Better Achieving Universal Health Coverage. Health Working Group (HWG). APEC Beijing. 2014
- The Use of Traditional Medicines and Functional Foods for Wellness and Nutrition Problems. Life Science Innovation Forum (LSIF). SOM 1 APEC Philippines. 2015

Plenary 2 - Exploration of bioresources for drug development

Session 3

Culture Collection Role in a Reliable Fish Quarantine Measure and Inspection

Dr. Ir. Woro Nur Endang Sariati, MP.
Standard Examination Laboratory (SEL), Fish Quarantine and Inspection Agency (FQIA)
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E mail: woronuresa@gmail.com

Indonesia, a maritime coutry which well-known to have a massive number of live aquatic biodiversity. The country also plays a vital role as one of the biggest fishery product exporters around the globe. The trend of world fish consumption, which shows increase values year by year as well as domestic demand, has become an opportunity and challenge for the Indonesian fishery sector. Another significant impact of the high demand for fishery products is that it will stimulate the rise of transboundary trade. International traffic of commodities across the border simultaneously allows the transfer of pathogen from one place to another, as well as one island to another and furthermore one continent to another. Some of the transferred pathogens can cause mass havoc in the fishery industry of destination country and the other playas foodborne disease; for example, Indonesia experienced to have Koi Herpesvirus (KHV) outbreak in 2002-2003, which killed million koi and carp resulting a significant economic loss. The virus was then known to come together with a particular commodity through an importation activity. Therefore, the rapid and accurate quarantine measures and inspection is essential to detect and diagnose transboundary pathogen and determine effective and efficient measures and or policy. In the technical level of the implementation of quarantine measures and inspection policy, positive control of pathogen is very crucial as a scientific justification that strongly determines the eligibility of detection as well diagnosis method and result. For those considerations, Fish Quarantine and Inspection Agency has an interest in the development of culture collection to support and strengthen the duty and prevent and minimize the introduction of the dangerous transboundary pathogen into Indonesian territory for the sustainability of Indonesian aquatic biodiversity.

Curriculum Vitae

Plenary 2 - Utilization of Natural Resources for Drug Development

Session 4

Community Based Exploration of Local Ethnomedicine Knowledge and Medicinal Plants in Indonesia

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Community-Based Exploration of Ethnomedicine Local Knowledge and Medicinal Plants in Indonesia, hereinafter referred to RISTOJA, was a mapping study of traditional knowledge in the use of plant-based medicines by ethnic or tribes conducted by the National Institute of Health Research and Development in 2012, 2015, and 2017. The research was carried out to address the needs of information related to the data of traditional medicinal plants and herbs used by every ethnicity in Indonesia. RISTOJA aims to establish ethnomedicine knowledge databases, traditional medicine (TM) and medicinal plants (MP) in Indonesia.

Data collected include characteristics of traditional healers, symptoms and diseases, plant species, plant use in medicine, part of the plant used, herbs, how to prepare and how to utilize for treatment, local wisdom in the management and utilization of the medicinal plants and the data of the environment. RISTOJA was held in the first time in 2012, covered of 26 provinces across Indonesia except Java and Bali Islands. This research was explored 209 tribes in collaboration with 25 universities in Indonesia. The second stage of Ristoja comprised of 96 tribes group out of 22 Provinces which was carried out in 2015. The third stage or the last Ristoja held in 2017 was explored 100 ethnics from 10 Provinces.

The number of informants (traditional healers) who have interviewed were 3.384 traditional healers, of which 95.2% live in rural areas; 41.9% more than 61 years old; 18.3% had no formal education and 55.8% do not meet the 9-year basic education program. Refer to the result, it was demonstrated that traditional healers knowledge is still original that been handed down from generations, and less affected by external knowledge. The healing practice is supported by a resource in a rural residence with limited access and information.

There are 32.014 herbal preparations used by 405 ethnic in Indonesia, the main diseases/symptoms that are treated by traditional healers such as fever, headache, sore skin and abdominal pain, are also symptoms/diseases associated with metabolic or degenerative diseases such as diabetic, cancer/tumor and high blood pressure. Medicinal plants which are used in the treatment reach to 50.874 information (based on local name), of which 38.732 of them could be identified to species level comprising 2.848 species/types and 211 plant family. Medicinal plants are frequently used in herbal preparations among others *Curcuma domestica* Val., *Piper betle* L., *Cocos nucifera* L., *Zingiber officinale* Roscoe, *Annona muricata* L. and *Jatropa curcas* L.

Key words: community base, exploration, ethnomedicine, medicinal plants.

Curriculum Vitae

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Institution : **Medicinal Plant and Traditional Medicine Research and Development Centre, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia.**

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Education :

- Bachelor Degree in Agriculture at Sebelas March University, 1990
- Master Degree in Agriculture at Sebelas March University, 2004
- Ph.D in Biotechnology at Gadjah Mada University, 2017

Position :

- Assistance Researcher in Medicinal Plant Research Unit, 1991-1998
- Researcher in Medicinal Plant Research Unit, 1998-2006
- Head of Medicinal Plant Research Unit, 2004-2006
- Head of Program, Networking and Information Division, Medicinal Plant and Traditional Research and Development Centre, 2007-2009
- Head of Research Services Division, in Medicinal Plant and Traditional Research and Development Centre, 2009-2012.
- Researcher in Medicinal Plant and Traditional Research Centre, 2012-now

Organization

- Member of Researcher Association on Natural Product
- Advisory Board Member of National Working Group of Indonesia Medicinal Plant
- Member of International Society of Ethnopharmacology
- Member of Komisi Nasional Saintifikasi Jamu

International activity :

1. Indonesia Delegate on the Govermental Forum on Traditional Medicine, Beijing, PR China.
2. Indonesia Delegate on ASEAN Forum of Traditional Medicine, Bangkok
3. Indonesia Delegate of WHO Consultation Meeting on finalization draft of medicinal plant conservation, Japan.

4. Indonesia Delegated on the Asean Workshop of The Development of Common Guideline on Establishment of Medicinal Plants Garden in Asean Countries.
5. Indonesia Delegate on Policy Dialog of Traditional Medicine, the SOM3-APEC.
6. Ivited Speaker on the Seventh Meeting on Indigenous Indigenous Medicine in The Greater Mekong Basin, Thailand
7. Partcipant of International Training Workshop on Development and Conservation of Traditional Ethnomedicine, Kunming, China
8. Invited expert on the APEC Workshop on the Development of Herbal Medicine Database in Asia Pacific Region, Manila Philiphine

Related Research Experience

1. Principle Investigator of Consortium Research on The Development of Senna siamea as new antimalarial drug.
2. Research coordinator of the Development of Artemisia annua cultivation to support the independency of antimalaria drug raw materials

Plenary 2 - Utilization of Natural Resources for Drug Development

Session 5

Utilization of Culture Collection for Drug Discovery Resources

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Exploration on Indonesian biodiversity has recently increased the number of collected microbial cultures. Microbial expedition for culture collection is usually a project-based and done by a few numbers of institutions. Up to now, Indonesia has 18 registered microbial culture collections with various standard preservation levels. These culture collections focused on different aspects, but mostly dealt with the preservation of bacteria, yeast and fungi of agricultural importance. The bio-prospective use of these genetic resources is discussed emphasizing on drug discovery. Depending on the purpose of the establishment of the culture collection, only a few collections have been used by internal researchers as a source of active substances screening for drug prospectives, such as antidiabetic, antimicrobial and antimalarial. These collections have been stated as potential agents for the production of those active substances through either *in vitro* or *in vivo* screening. External access to the microbial cultures for such screening is also noted, even though microbiologists are likely to collect their own microbial culture for their research. When the research completed, their collections may either be deposited in culture collection or kept as private collections. However, information on the drug derived from those culture collections cannot be tracked down indicating that the research is still in a preliminary stage of drug development.

Curriculum Vitae

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Education

1982 Bachelor Bogor Agriculture University Plant Protection

1992 Doktor University of New England, Australia Botany

Publication

- **Rahayu G**, Thamrin JAD, Rianti P. 2019. Community Structure of *Fusarium oxysporum* f. sp. *cubense* in Java and Sumatra Based on *Cryphonectria parasitica* Vic Primer. Proceeding on The 3rd International Conference on Biosciences (Submitted)
- **Rahayu G**, I Maulana, and Widodo. 2019. Endobacterial symbiont of *Fusarium oxysporum* f.sp. *cubense* and the pathogenicity of their symbiosis towards banana plantling. Proceeding on The 3rd International Conference on Biosciences (Submitted)
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Plenary 3 – Target Development and Screening of Active Compound

Session 6

Anticancer Properties of Curcumin and Its Analogs Targeted on ROS Metabolizing Enzymes

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Cancer is a group of diseases with a physiological complex aberration due to the accumulation of genes mutation and/or the alteration of genes expression that make it difficult to be cured completely. Among those of the various molecular marks of cancer, the ROS metabolism plays a pivotal role in cancer development. ROS is a reactive species identified as radical Oxygen or radical containing molecules which is maintained under ROS metabolizing enzymes. Hence, ROS metabolizing enzymes are highly expressed in cancer cells but very low expression in normal cells. The development of chemicals that targets on ROS metabolizing enzymes hopefully can increase intra-cellular ROS level which leads to induce senescence and apoptosis. Curcumin performed cytotoxic potential to K562 cell, a Leukemic cancer cell, in a reversible manner that differ to Gleevec which exhibit reversible characteristic. Curcumin inhibited tumor development in vivo of K562. Curcumin induced apoptosis and senescence in correlation with the increasing of intracellular ROS over the threshold. This phenomenon was correlated with the inhibitory characteristic of curcumin to many of ROS metabolizing enzymes, such as GSTP1, CBR1, AKR1C1, PRDX1, GLO1, NQO1, and NQO2. A curcumin analog, namely CCA 1.1 showed more cytotoxic potential to 4T1 cells compared to curcumin. The CCA 1.1 also performed tumor inhibitory effect in vivo better than curcumin. This compound also shares the binding protein targets of ROS metabolizing enzymes with curcumin such as AKR1C1, PRDX1, GLO1, NQO1, and NQO2 but not to CBR1. The binding characteristic of these compounds to the ROS metabolizing enzymes in addition to the others essential targets is believed to its safety properties to normal cells. The ROS metabolizing enzymes are the good targets for anticancer screening with high selectivity against cancer and normal cells.

Keywords: Curcumin and its analog, ROS metabolizing enzymes, Leukemic cancer, Breast cancer

Curriculum Vitae

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Education:

Bachelor	Faculty of Pharmacy/Universitas Gadjah Mada	1986
Professional	Faculty of Pharmacy/Universitas Gadjah Mada	1987
Master	Faculty of Pharmacy/Universitas Gadjah Mada	1995
Doctor	Nara Institute of Science and Technology (NAIST), Japan	2001

Research area:

I established a research center in the faculty of pharmacy, UGM, namely Cancer Chemoprevention Research Center (CCRC) (http://ccrc.farmasi.ugm.ac.id) focusing on the development of agents (synthetic and natural origin) to identify, prevent, inhibit, modulate, and reverse of developing cancer and/or cell proliferation and differentiation. CCRC has plenty collaborative researches with other institutions (universities and other research groups) and funding resources from industries and government. CCRC research activities are particularly in cancer chemoprevention focuses on *in vitro*, *in vivo* , and *in silico* experiments. The *in vitro* researches included observing cytotoxicity of Indonesian plant's extract, metabolites and curcumin analogues in several cancer cell lines, such as breast cancer cell line (T47D, MCF-7, 4T1, HCC), cervical cancer cell line (HeLa), and colon cancer cell line (WiDr), either single or in combination with chemotherapeutical agents. Besides cyctotoxicity, CCRC also investigates the chemopreventive, antiproliverative, and apoptosis effect of agents using double staining, immunohistochemistry, AgNOR staining, FACS analyses (cell cycle, apoptosis, ROS), as well as gene expression studies to establish a targeted therapy of cancer. CCRC also use RAW 264.7 macrophage cell line as the model of cell differentiation and immunomodulatory studies. CCRC also works *in vivo* i.e. implant cell method; investigating immunostimulatory effect in rat treated by cytostatic agent, and estrogenic activity using ovariectomized rat. In addition CCRC also develop *in silico* studies covering bioinformativs research to explore drug-receptor interaction using MOE and PLANT programs.

Publication:

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7. Edy Meiyanto, Beni Lestari, Raisatun Nisa Sugiyanto, Riris Istighfari Jenie, Rohmad Yudi Utomo, Ediati Sasmito, Retno Murwanti, 2018, Caesalpinia sappan L. heartwood ethanolic extract exerts genotoxic inhibitory and cytotoxic effects, Oriental Pharmacy and Experimental Medicine, 19(1), 27-36
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Plenary 3 – Target Development and Screening of Active Compound

Session 7

Searching of Antibiotic from Indonesian Endophytic Fungi

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Endophytic fungi have been known as a potential source for old and new biologically active metabolites. Since 2006, totally more than 1600 isolates of endophytic fungi were obtained from several medicinal plants collected around the Indonesian archipelago. All of isolated endophytic fungi are deposited at Indonesian Culture Collection. Furthermore, all of isolated endophytic fungi are cultivated and their culture extract were subjected on screening assays for antibacterial and antioxidant. The screening test showed that the active extracts were approximately 10 % for antibacterial for both gram positive and negative bacterial tested. While 6 % extracts are show selective activity against gram positive or gram-negative bacteria. Only 2 % extract were shown activity as antioxidant. Among of these tested extracts, many secondary active metabolites have been isolated and characterized. The most promising antibacterial metabolite was found produced by the endophytic fungus Diaporthe sp. GNBP-10, epicytoskyrin A. This metabolite can inhibit the growth of many kinds of pathogenic bacteria in-vitro and in-vivo, especially Staphylococcus aureus. In a mouse model, epicytoskyrin A can inhibit an ulcer formation induced by S. aureus suspension. The secondary metabolites from the endophytic fungus Diaporthe sp. GNBP-10 are also show a promising activity as anti-TB.

Key words: Endophytic fungi, active metabolites, epicytoskyrin A, antibacterial.

Curriculum Vitae

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Job Experiences

1994 - present Natural Product Chemistry Laboratory, Research Center for Biology, The Indonesian Institute of Sciences.

2006 – present Head of Natural Product Chemistry Laboratory, Research Center for Biology.

2011 – 2014 Coordinator for LIPI Competitive Research Program on sub theme of Exploration and Utilization of Indonesian Biodiversity, Indonesian Institute of Science.

Educations

2003 – 2006 PhD degree in Natural Product Chemistry, Fukuyama University, Hiroshima, Japan.

2001 – 2003 Master degree in Natural Product Chemistry, Fukuyama University, Hiroshima, Japan.

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Scholarship

2001 – 2006 Monbukagakusho Scholarship from Japanese Government

Training

1995 Computer-Assisted Chemical Education and Research, Faculty of Sciences, Chulalongkorn University, Bangkok, Thailand (three months).

Books

1. Agusta A. 2009. Biology and chemistry of endophytic fungi. ITB Press, Bandung. ISBN. 978-979-1344-42-5. 120 pp (in Indonesian).
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8. Anita S., **Agusta A**., Farida WR., Nugroho N., and Wulansari D. 2017. A Preliminary Study of Aphrodisiac Property from Porcupine Tail Meat Ethanol Extract in Male Mice. Zoo Indonesia 26 (1), 52-58.
9. Purwantoro RS., Siregar HM., Sudarmono dan Agusta A. 2017. Antibacterial potency of leaves extract of Lasianthus plant against *Pseudomonas aeroginosa*. Jurnal Jamu Indonesia, 1(3); 6-11.
10. Wulansari D., Putra AP., Ilyas M., Praptiwi, Fathoni A., Palupi KD. dan **Agusta A**. 2017, Skrining beberapa Jamur Endofit Tumbuhan Dari Pulau Enggano, Bengkulu Sebagai Antibakteri Dan Antioksidan. Berita Biologi, 15(3), 227-235.
11. Praptiwi, Palupi KD., Fathoni A., Keim AP., Royani MF., Effendi O. dan **Agusta A**. 2017. Evaluasi Antibakteri dan Antioksidan Ekstrak *Simllax* spp. dari Pulau Enggano. Berita Biologi, 15(3), 227-235.
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Plenary 3 – Target Development and Screening of Active Compound

Session 8

Searching Lead Compounds of Anti-Amebic Agent by Utilizing Diversity of Indonesian Bio-Resources

Myrna Adianti[1,3], Defi Kartika Sari[3], Lidya Tumewu[3], Hilkatul Ilmi[3], Aty Widyawaruyanti[2,3], Achmad Fuad Hafid[2,3]

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Institute of Tropical Disease, Universitas Airlangga

Background: Amoebic dysentery caused by ingestion of *Entamoeba histolytica* trophozoites which can damage the intestinal mucosa and causes amoebic colitis. In some cases, amoeba can penetrate the mucosal lining, spread to the liver and cause abscesses. Indonesia has the second largest tropical forest in the world after Brazil. These vast biological resources could be the sources to develop a new drugs to various diseases such as amebiasis infection.

Objective: To conduct screening of Indonesian Biological resources (microorganism and plants) to develop anti-amebic drug candidate lead compounds.

Methods: More than 16 000 extracts of microorganism were tested for anti-amebic activities. Screening of anti-amebic activities using in vitro cell *E. histolytica* cell culture, and enzymatic assay of CS3 and CS3/SAT1 coupled assay and NADKinase/NO1 coupled assay.

Results: The results of CS3 enzymatic assay showed two hit extract of *Penicillum* and *Aspergillus neoflavipes*. The in vitro screening showed one hit extract inhibit *E. histolytica* growth. These extracts have been confirmed to contain antiamebic compounds other than Fumagilin or Citrinin.

Conclusion: From screening of Indonesia biological resources (microorganism) showed three extracts with anti-amebic activities which are not a Fumagilin or Citrinin. The further identification of the compound from active extracts of in vitro screening will be continued.

Curriculum Vitae

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Academic Education and Qualifications

KOBE UNIVERSITY

April 2010-2014 **PhD in Medical Science**

Thesis Title: Anti-hepatitis C virus compounds obtained from *Glycyrrhiza uralensis* and other *Glycyrrhiza* species

Supervisor: Professor Hak Hotta

UNIVERSITAS AIRLANGGA

September 2005-April 2008 **Master of Health (M.Kes.)**

Major subject: Basic Medical

Thesis Title: Correlation of bacteriological index from slit skin and ear lobe with antibody anti PGL-1 titer in new leprosy patient at Dr.Soetomo public hospital, Surabaya.

Supervisor: Professor Dr.Indropo Agusni,dr,Sp,KK(K)

UNIVERSITAS AIRLANGGA

August 2000-2004 **Bachelor of Science (S.Si.)**

Major subject: Biology

Thesis Title: Amylases enzyme production from tapioca liquid waste using *Aspergillus niger* dan *Aspergillus oryzae*

Supervisor: Dr. Ir. Tini Surtiningsih, DEA.

Publications

No	Title	Journals	Vol/No/ Tahun
1.	High rate of seronegative HCV infection in HIV-positive patients.	Biomedical Reports	2014, 2(1):79-84
2.	Anti-hepatitis C virus compounds obtained from *Glycyrrhiza uralensis* and other Glycyrrhiza species	Microbiology and Immunology	2014, 58:180-187
3.	Activities of *Ficus fistulosa* Leave Extract and Fractions against Hepatitis C Virus	Procedia Chemistry	2016, 18:179-184
4.	AntiHepatitis C Virus Activity of *Alectryon serratus* Leaves Extract	Procedia Chemistry	2016, 18:169-173
5.	Antiviral activity of the dichloromethane extracts from *Artocarpus heterophyllus* leaves against hepatitis C virus	Asian Pacific Journal of Tropical Biomedicine	2017, Volume 7, issue 7, July, pages 633-639
6.	Anti Hepatitis C Virus Activity of Indonesian Mahogany (*Toona sureni*)	Asian Journal of Pharmaceutical and Clinical Research	2018, Vol. 11, Issue 2.

Plenary 4 – Fermentation Technology and Structure Modification

Session 9

Modification Design of Cinnamic Acid Derivates as Dipeptidyl Peptidase-IV Inhibitor Using In Silico Fragment-Based Method

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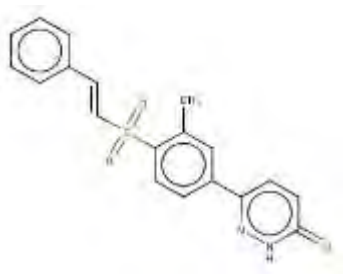
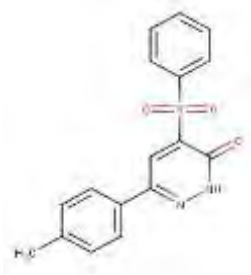
Research on new compounds as DPP-IV inhibitors for treatment diabetes mellitus type 2 have been carried out, including on cinnamic acid derivates. However, the compound could be developed to achieve optimum affinity through structure modification using in silico fragment-based method. The aims of this research are to obtain new compound as DPP-IV inhibitor by fragment modification; to obtain the alternative compound that has potential as DPP-IV inhibitor, based on its similarity with the modified compounds; and to analyze their interactions with DPP-IV; to predict ADME, toxicity, and easiness of synthesis based on binding affinity and inhibition constant. Modified structures come from the fragmentation of structure from cinnamic acid derivates and fragments from Zinc database which previously screened against Rule of Three and Heavy Atom using Knime and docked in each DPP-IV active site. The results are docked again with PyRx and analyzed further based on ADME, toxicity, and interactions was done using SwissADME and ProTox-II. The structures are searched for similarities with compounds in PubChem database based on Tanimoto parameters. The modification result obtained by 133 design structure. Thirteen structures have shown binding affinity close to the value of DPP-IV inhibitor affinity. Based on docking and analyze of ADME, toxicity, and interaction result, structure38 and 15471581 compound are predicted as the best potential DPP-IV inhibitor from cinnamic acid derivatives.



Structure 38



15471581

Keywords: DPP-IV, Fragment based virtual screening, structure modification, cinnamic acid derivates

Curriculum Vitae

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Publication

1. Ahmad, I., Azminah, Mulia, K., **Yanuar, A.**, Mun'im, A. **(2019)** Angiotensin-converting enzyme inhibitory activity of polyphenolic compounds from Peperomia pellucida (L) Kunth: An in silico molecular docking study. Journal of Applied Pharmaceutical Science. 9(8), pp. 25-31
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Plenary 5 – Fermentation Technology and Structure Modification

Session 10
Strategy for Optimization of Lead Compounds as Drug Candidate from Natural Resources

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The Indonesia archipelago is rich natural resources and is number two after Brazil as mega diversity in the word, but until now almost raw material medicine (drugs) still import. There are 8000 species that have been as herbal medicine (jamu) but the product of herbal medicine standardized (OHT, 64) and fitopharmaca (21).Drug discovery mainly from natural product resources as like medicinal plants, marine resources, animals and microbes. The challenge for discovery takes time, high cost and technology also need multidiscipline. The problem also many drugs is already resistant, any side effect, so they still need to find and develop new drugs, from various sources. Some drugs as artemisinin, quinine, campotechine, and taxol produced from plants, lovastatin from *Aspergillus terreus*. Indonesia has many natural sources as medicinal plants and as raw materials for produce lead compounds. We need to choose plants that have major compounds (active), easy to isolate also can use commercial drug to develop to get more active compounds for some diseases target. One of the main fail discovery drugs is ADME and effectivity problem. For this solution, we need to make optimization with the modification the structure and make some analogs base on Lipinski Rule, to control lipophilicity (Log P) and energy interaction between ligand and receptor. Some major compounds as like quinine, cinnamic acid, and citronella oil and others can use to make some derivatives or analogs to increase activities as candidate drugs. The strategies make simple synthesis and short steps reaction.

Curriculum Vitae

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.	University	Faculty - Department	Graduated
Strata - 1	Indonesia University (UI)	Chemistry - FMIPA	1985
Strata - 2	Osaka City University (OCU)	Fac. of Sciences (Dept. of Chemistry)	1993
Strata - 3	Osaka City University (OCU)	Fac. of Science (Dept. of Chemistry)	1996
Prof. riset	Lembaga Ilmu Pengetahuan Indonesia (LIPI)		Nov 2012

Professional Activities:

1. Head of Nat Prod Food and Pharm. Div., RC Chem. LIPI (2005-2014)
2. Coordinator of Research Group- Nat Prod & Pharm Chem. (2015-2016)
3. Lecture - Structure Elucidation by FT-NMR- Magister of Chem., IPB (2003-now)
4. Lecture - Med. Chem. Magister of Pharmacy – Univ. of Pancasila (2005- now)
5. Special Lecture (PhD Prog) : Structure Elucidation & Med. Chem. Pharmacy, UI (2016), FKG – USAKTI 2018- Natural Products Of Chemistry
6. PI : Joints Reseacrh Prog. Osaka City Univ. Japan – Drug Discovery for Anticancer (2007-2012)
7. Princple Investigation : 1997 - now
   - Synthesis Lovastatin Derivatives & Its Bioactivity for Anticholesterol
   - Synthesis Phenazine Derivatives as Anticancer
   - Synthesis Analog UK-3A for Anticancer
   - Synthesis Quinine derivatives for Anticaner and Antimalaria
   - Joint Research : Osaka City Univ., Kobe Univ. and UC Davis, CSIRO Australia
8. Publication: Intnl Journal/Proceeding, National Journal/Proceeding (> 150)
   Google scholar: h-index 17, i-10-index 27; Scopus Index: 13
9. Patens: 33
10. Books: 1. Structure Elucidation by FTR-NMR; 2. Citronella Oil derivative for candidate drug
11. Co-promotor, Reviewer, Supervisor: > 150 students (doctor, master and graduate program)

Plenary 4 – Fermentation Technology and Structure Modification

Session 11

Pilot Scale Fermentation of Cephalosporin C

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Laboratory for Biotechnology BPPT

Cephalosporin C (CPC) is a class of β-lactam antibiotics produced from the fungus *Acremonium chrysogenum* by the process of fermentation in a bioreactor. CPC demand is increasing worldwide because of its enhanced antibacterial spectrum that it can be used to treat diseases and infections caused by Gram positive/or Gram negative bacterial strains.
The use of local raw materials on fermentation media for CPC fermentation can reduce production costs on an industrial scale. The aim of the study was to obtain the best media composition using local raw materials as fermentation media for CPC fermentation on a pilot plant scale. The fermentation was carried out on a 50L bioreactor in a fed-batch fermentation process. Fermentation processes were performed using CC3 media (local raw material modification) for 132 hours. The used local raw materials were CSL (Corn Steep Liquor), Liquid Sago sugar, Urea and Palm oil as sources of nitrogen and carbon. The maximum concentration of CPC in this study was 17.1 g/L.

Keywords: *Acremonium chrysogenum*, Fed-Batch Fermentation; Cephalosporin C, Local Raw Materials

Curriculum Vitae

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Education

Ph.D., Separation Sciences, Saarland University, Germany, 2007
M.Sc., Bioprocess Engineering, Technical University of Hamburg-Harburg, Germany, 2001
B.Sc., Technical Chemistry, University of Applied Sciences, Nuremberg, Germany, 1998

Research Experiences

2015-2019: Production Technology of Cephalosporin C and Its Derivatives

2016-2019: Purification and Structure Elucidation of Anti Malarial Bioactive Compounds

2010-2014: Production Technology of Penicillin G, 6-Aminopenicillanic Acid (6-APA) and Amoxicillin

2008-2009: Purification and structure identification of bioactive compounds produced by Indonesian microbes

Publications

HS Permana, Rudiyono, Nurhayadi, D Dewi, S Wulyoadi, A Marasabessy, G Heryanto, Suyanto, NB Nugroho, **AH Mahsunah**, Use of Local Raw Materials for Fermentation Medium of Cephalosporin C Production on a Pilot Plant Scale, Poster Presentation, The 10th International Seminar of the Indonesian Society for Microbiology (*ISISM*) *2019,* August 29th-30th *2019*. Surakarta

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SATREPS SLeCAMA Project

The Project for Searching Lead Compound for Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

The 2nd International Symposium on

Natural Resources-based Drug Development

October 9th, 2019

Sari Pacific Jakarta, Jl. MH Thamrin 6, Jakarta, Indonesia

Organized by:

- Agency for the Assessment and Application of Technology (BPPT)
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Project Design Matrix (PDM) (Version 0)

Date: October 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development

Project Duration: 5 years after the date indicated on the Record of

Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Implementing Agencies:

【Indonesia】 Biotech Center of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)

【Japan】 University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia

Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)

Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievements	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minities of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			
3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10,000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents			

Activities	Inputs		Pre-conditions
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	Japan	Indonesia	1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project.
1-1 To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.
1-2 To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-3 In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-4 To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-5 To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			↓
1-6 To determine chemical structures of the lead compound candidates.			Issues and Countermeasures
1-7 To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			
1-8 To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			
2-1	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc).			
2-2	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-3	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-4	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.			
2-5	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			
2-6	To determine chemical structures of the lead compound candidates.			
2-7	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.			
2-8	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
3	**Technologies and research system for drug discover y using biological recourses are established at the Indonesian research institutes.**			
3-1	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

[Abbreviations]DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Project Monitoring Sheet I (Revision of Project Design Matrix)

Version 01
Dated March 31, 2016

Project Design Matrix (PDM) (Version 1)
Project Title: The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebaic Agents by Utilizing Diversity of Indonesian bio-Resources in the Republic of
Period of Project: From April 01, 2015 to March 31, 2020
Implementing Agencies:
【Indonesia】Center for Pharmaceutical and Medical Technologies of the Agency for Assessment and Application of Technology (PTFM-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.
Target Area: the Republic of Indonesia
Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievement	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minutes of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents		1. This indicator is expected to be achieved by the time of the end of the Project. - Two compounds with anti-malarial activity had already been isolated and purified. The chemical structure of these compounds were also been elucidated. - Efficacy test using animal experiment will be conducted in 2018 2. This indicator is expected to be achieved by the time of the end of the Project. - First screening of 5200 microbial extracts revealed that more than 30 extracts showed anti-amebic activity. - Efficacy test using animal experiment will be conducted in 2018 3. This indicator is expected to be achieved by the time of the end of the Project. - A scientific paper about screening, isolation, and structure elucidation of 2 anti-malarial compounds is being prepared (the paper are expected to be submitted to peer-reviewed journal in Q3 of 2016)	
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.	1-1. The indicator has been achieved - Three (3) compounds with anti-malarial had been isolated and purified - More than 100 (one hundred) active extracts were obtained from the 1st screening (cell- and enzyme-based screening) employing more than 1700 extracts. The activity of these extracts will further be verified and objected to 2nd screening. - Compound from active extracts that shows significant inhibitory activity will be isolated and purified. 1-2. The indicator has been achieved - The chemical structure of two (2) compounds with anti-malarial activity had been elucidated. - The chemical structure of other isolated and purified active compound from the result of screening activity will also be elucidated. 1-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents		2-1. The indicator is expected to be achieved by the Mid-term Review. - More than 5500 extracts (including old-prepared extracts) were objected to enzyme- and cell-based screening for anti-amebic activity, resulting more than 35 hits were achieved. - Compound from active extracts that shows significant inhibitory activity will be isolated and purified. 2-2. The indicator is expected to be achieved by the time of Terminal Evaluation. - The chemical structure of isolated and purified active compound from the result of [illegible] 2-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	
3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of Plasmodium falciparum and Entamoeba histolytica are established at the Indonesian research institute by the end of the 3rd year of the Project.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents		3-1. The indicator is expected to be achieved by the end of 3rd year of the Project. - Currently, more than 1400 of microbial extracts were newly prepared, and more than 700 microbes were newly isolated during the 1st year of the project. All extracts and microbes were registered in [illegible] 3-2. The indicator is expected to be achieved by the end of 2nd year of the Project. - Microbial extracts had been started to be prepared by BTC-BPPT from the beginning of the project. - Enzymes needed for enzyme-based screening are being prepared and expected to be available in April 2016. - Red blood and blood plasma needed for anti-malarial cell-based screening are expected to be supplied by local Red Cross start from Q2 of 2016 (currently, BPPT is negotiating with local Red Cross for supply of blood and plasma). - Equipment are expected to be installed and available to be used in May 2016. 3-3. The indicator is expected to be achieved by the end of the 3rd year of the Project. - Both parasite cells are already preserved in BPPT. E.histolytica clone 6 culture is currently maintained using currently available equipment. P.falciparum 3D7 is currently preserved as a frozen stock, and will be revived and maintained when the equipment are installed in BTC-BPPT. - Cell-based evaluation system will be established after the equipment are	

		3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.			3-4. The indicator is expected to be achieved by the time of the Terminal Evaluation. - Equipment needed for isolation and purification of compounds are expected to be installed and available to be used in May 2016 - Laboratory space for isolation and purification of compounds was prepared in BTC-BPPT. - Training on isolation and purification of compounds had already been done in Kitasato University. Two (2) researchers from BTC-BPPT were participated in this	
		3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.			3-5. The indicator is expected to be achieved by the time of the Terminal Evaluation. - Training on chemical structure analysis of compounds had been done in Kitasato University. One (1) researcher from BTC-BPPT was participated in this training. - A computer for structural analysis of compounds is being installed in BTC-BPPT. - Survey to laboratories who has NMR was conducted. RCChem of LIPI (Puspiptek) and AU (Surabaya) had similar type of	
		3-6. International symposiums are held for drug discovery for two(2) times at least.			3-6. The indicator is expected to be achieved by the time of the end of the project. - The symposium are expected to be held in	

Activities	Inputs				
	Japan	**Indonesia**	**Important Assumptions**		
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	**Pre-conditions** 1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.		
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).				1-1. 1.440 extracts were screened by enzyme-basedly in Japan 1-1. 320 extracts were screened by cell-basedly in Japan	1-1. In 2016, totally 5,000 extracts are planned to be screened by enzyme and cell basedly in Indonesia
1-2. To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.					
1-3. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of P*lasmodium falciparum* under the condition of *in vitro* culture system.					
1-4. To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.				1-4. Three (3) compounds were isolated and purifeid in Japan	1-4. Ten(10) active compouds will be purified in Indonesia
1-5. To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target			<Issues and countermesures>		
1-6. To determine chemical structures of the lead compound candidates.			0-1. Equipment Provision 2015 imported from Japan delayed due to new rules of import restriction. Now UT&JICA are arranging mean of the importation to clear the Indonesian regulation.	1-6. Chemical structure of two(2) anti-malarial compounds were elucidated in Japan	1-6. Five(5) active compounds' structure were edetermined in Indonesia
1-7. To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			0-2. Costs on Consumables Estimated annual cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).The cost was calculated based on the annual working plan of each working teams, the working plan was planned to meet the requirements to implement 5,000 extracts annually in line with the expected output of the Project Design Matrix (PDM). Japanese side understands the status and allocates the budget for the those consumables as well as equipment and trainings in Japan. On the other hand, Indonesian institutes will propose more budget for coming years in future.		
1-8. To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target					
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**					
2-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).				2-1. 5,200 extracts were screened by enzyme-basedly in Japan 2-1. 320 extracts were screened by cell-basedly in Japan	2-1. In 2016, totally 5,000 extracts are planned to be screened by enzyme and cell basedly in Indonesia
2-2. To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro*					
2-3. In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.					
2-4. To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.					2-4. Ten(10) active compouds will be purified in Indonesia
2-5. To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target					
2-6. To determine chemical structures of the lead compound candidates.				2-6. Five(5) active compounds' structure were determined in Indonesia	
2-7. To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.					
2-8. To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.					
3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**					

3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.				3-1. In 2016, total 500 newly collected microbes are planned to be isolated in Indonesia
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.				3-2. Equipment are expected to be installed and available to be used in May 2016.
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.				3-3. Cell-based evaluation system will be established after the equipment are installed
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.				3-4.Training on isolation and purification of compounds had already been done in K U. Two researchers BPPT participated
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.				3-5. Training on chemical structure analysis of compounds had been done in Kitasato University. One (1) researcher BPPTparticipated
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.				3-6. The symposium are expected to be held in 2017 and 2019.

【Abbreviations】DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Project Monitoring Sheet I （Revision of Project Design Matrix）

Version 02

Dated September 30, 2016

Project Design Matrix (PDM)（Version 2）
Project Title: The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebaic Agents by Utilizing Diversity of Indonesian bio-Resources in the Republic of Indonesia
Period of Project: From April 01, 2015 to March 31, 2020
Implementing Agencies:
【Indonesia】Center for Pharmaceutical and Medical Technologies of the Agency for Assessment and Application of Technology (PTFM-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.
Target Area: the Republic of Indonesia
Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievement	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minutes of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents		1. This indicator is expected to be achieved by the time of the end of the Project. - More than 5000 extracts were objected for first screening against DHODH and MQO. - Cytotoxicity test of 34 active extracts that showed inhibitory activity against DHODH and MQO was performed resulting 14 active - Fourteen extracts were prepared in larger scale - Two more compounds with anti-malarial activity are being purified in this semester. - Efficacy test using animal experiment will be started in 2018 2. This indicator is expected to be achieved by the time of the end of the Project. - First screening of more than 2200 microbial extracts were done against CS3 and SAT1 assay, as well as against E.histolytica, resulting in 48 active extracts. - Purification of active compound from 4 active extracts that have inhibitory activity against CS3 enzyme are currently conducting - Large scale extract preparation of 4 more extracts that had inhibitory activity against proliferation of E.histolytica had been prepared and will be purified in next semester. - Efficacy test using animal experiment will be conducted in 2018 3. A scientific paper about screening, isolation, and structure elucidation of anti-malarial compounds is being prepared (the paper are expected to be submitted to peer-reviewed journal in Q4 of 2016)	
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.	1-1. The indicator has been achieved (3 compounds with anti-malarial had been isolated and purified) - More than 5000 of microbial extracts and 100 of plant extracts were objected for 1st screening resulting more than 78 active extracts that showed inhibitory activity against DHODH and MQO. - Confirmation of inhibitory activity of 21 active extracts has been done resulting in 9 active extracts. - Toxicity test of these confirmed 9 active extracts against 4 kinds of mammalian cell has been done resulting in 9 active extracts. These extracts were then proposed to be purified. - Purification of 2 active extracts are currently being performed 1-2. The indicator has been achieved (The chemical structure of two (2) compounds with anti-malarial activity had been elucidated) - Purification of other 2 active extracts are currently being performed 1-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents		2-1. The indicator is expected to be achieved by the Mid-term Review. - More than 2000 extracts were objected to enzyme- and cell-based screening for anti-amebic activity, resulting more than 130 hits were achieved. - Confirmation of inhibitory activity of 48 active extracts from cell-based screening has been done resulting in 5 active extracts. - Purification of active compound from 4 active extracts that have inhibitory activity against CS3 enzyme are currently conducting 2-2. The indicator is expected to be achieved by the time of Terminal Evaluation. - The chemical structure of isolated and purified active compound from the result of screening activity will be elucidated. 2-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of Plasmodium falciparum and Entamoeba histolytica are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents		3-1. The indicator is expected to be achieved by the end of 3rd year of the Project. - Currently, more than 5000 of microbial extracts and 119 of plant extracts were newly prepared from January 2016. More than 1000 microbes were newly isolated from soil sample that was taken from Biak Island in June 2016. All extracts and microbes were registered in the in-house biological resource libraries. 3-2. The indicator is expected to be achieved by the end of 2nd year of the Project. - Equipment have already installed and available to be used in August 2016 - Enzymes needed for enzyme-based screening (DHODH, MQO, CS3, SAT1) have been prepared and characterized - Enzyme-based screening for extracts with anti-malarial, as well as anti-amebic, activity has been started and established at BTC and AU. - Cell-based screening for extracts with anti-amebic activity has been started and established at AU. Cell-based assay for anti-amebic activity has been started at BTC as well. - Maintenance of parasite cell (Entamoeba) has been conducted at BTC and AU - Maintenance of mammalian cell (4 type of cells) has been conducted at BTC - Cell cytotoxicity test of active extracts against mammalian cells have been started and established. - Cell-based screening of extracts against Plasmodium cells will be started after establishment of Plasmodium cell culture at BTC. 3-3. The indicator is expected to be achieved by the end of the 3rd year of the Project. - E.histolytica clone 6 culture is currently maintained and cultured at BTC and AU. - E.histolytica cell-based evaluation system are established and implemented at both BTC and AU. - Establishment of culture and evaluation system using P.falciparum 3D7 will be started in next semester. 3-4. The indicator is expected to be achieved by the time of the Terminal Evaluation. - Equipment needed for isolation and purification of compounds were installed in August 2016. - Two experts from Japan visited BTC to give training on purification of active compounds. - Isolation and purification of 4 active compounds with inhibitory activity against CS3 and 2 active compounds with inhibitory activity against DHODH is currently being conducted.	

	3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.			3-5. The indicator is expected to be achieved by the time of the Terminal Evaluation. - NMR data of an active compound with inhibitory activity against DHODH that was taken in last semester is being analyzed at BTC. - NMR analysis of other active compound with inhibitory activity against DHODH has been conducted at Kitasato U, but need to be re-analyzed due to low amount of the sample. 3-6. The indicator is expected to be achieved by the time of the end of the project. - The symposium are expected to be held in 2017 and 2019.	

Activities	Inputs				
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	**Japan**	**Indonesia**	**Important Assumptions**		
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	**Pre-conditions** 1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.	1-1. 1.440 extracts were screened by enzyme-basedly in Japan 1-1. 320 extracts were screened by cell-basedly in Japan	1-1. In 2016, totally 5,000 extracts are planned to be screened by enzyme and cell basedly in Indonesia
1-2. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of P*lasmodium falciparum* under the condition of *in vitro* culture system.					
1-3. To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.				1-4. Three (3) compounds were isolated and purifeid in Japan	1-4. Ten(10) active compouds will be purified in Indonesia
1-4. To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			<Issues and countermesures>		
1-5. To determine chemical structures of the lead compound candidates.			0-1. Equipment Provision 2015 imported from Japan delayed due to new rules of import restriction. UT&JICA rearranged mean of the importation to clear the Indonesian regulation. Then it was imported in Jun 2016. 0-2. Costs on Consumables	1-6.. Chemical structure of two(2) anti-malarial compounds were elucidated in Japan	1-6. Five(5) active compounds' structure were edetermined in Indonesia
1-6. To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.					
1-7. To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.					
1-8.					

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**				
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).			2-1. 5,200 extracts were screened by enzyme-basedly in Japan 2-1. 320 extracts were screened by cell-basedly in Japan	2-1. In 2016, totally 5,000 extracts are planned to be screened by enzyme and cell basedly in Indonesia
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.				
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.				
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.				2-4. Ten(10) active compouds will be purified in Indonesia
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.				
2-6.	To determine chemical structures of the lead compound candidates.			2-6. Five(5) active compounds' structure were determined in Indonesia	
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.				
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.				
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**				
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			3-1. In 2016, total 500 newly collected microbes are planned to be isolated in Indonesia	
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			3-2. Equipment are expected to be installed and available to be used in May 2016.	
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			3-3. Cell-based evaluation system will be established after the equipment are installed	
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			3-4.Training on isolation and purification of compounds had already been done in K U. Two researchers BPPT participated	
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			3-5. Training on chemical structure analysis of compounds had been done in Kitasato University. One (1) researcher BPPTparticipated	
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			3-6. The symposium are expected to be held in 2017 and 2019.	

Estimated annual cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).The cost was calculated based on the annual working plan of each working teams, the working plan was planned to meet the requirements to implement 5,000 extracts annually in line with the expected output of the Project Design Matrix (PDM).
Japanese side understanded the status and allocated the budget for the those consumables as well as equipment and trainings in Japan for 2016.
On the other hand, Indonesian institutes will propose more budget for coming years in future.

【Abbreviations】DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Project Monitoring Sheet I （Revision of Project Design Matrix）

Version 03

As of March 31, 2017

Project Design Matrix (PDM)（Version 3）
Project Title: The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebaic Agents by Utilizing Diversity of Indonesian bio-Resources in the Republic of Indonesia
Period of Project: From April 01, 2015 to March 31, 2020
Implementing Agencies:
【Indonesia】Laboratory for Biotechnology of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tsukuba, Kitasato University, Nagasaki University, University of Tokyo, MicroBiopharm Japan Co., Ltd.
Target Area: the Republic of Indonesia
Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievement	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minutes of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents		1. This indicator is expected to be achieved by the time of the end of the Project. - More than 6000 extracts were objected for first screening against DHODH and MQO. - Cytotoxicity test of 93 active extracts that showed inhibitory activity against DHODH and MQO was performed resulting 77 non-toxic active extracts - Sixteen compounds with anti-malarial activity are being purified in this semester. - Efficacy test using animal experiment will be started in 2018 2. This indicator is expected to be achieved by the time of the end of the Project. - First screening of more than 2200 microbial extracts were done against CS3 and SAT1 assay, as well as against E.histolytica, resulting in 98 active extracts. - Purification of active compound from 8 active extracts that have inhibitory activity against CS3 enzyme are currently conducting - Efficacy test using animal experiment will be conducted in 2018 3. A scientific paper about screening, isolation, and structure elucidation of anti-malarial compounds is being prepared (the paper are expected to be submitted to peer-reviewed journal in Q3 of 2017)	
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.	1-1. The indicator has been achieved - More than 6,000 of microbial extracts and 100 of plant extracts were objected for 1st screening resulting active extracts that showed inhibitory activity against DHODH and MQO as much as 139 and 89 hits, respectively. - Confirmation of inhibitory activity of 110 active extracts has been done resulting in 21 active extracts. - Toxicity test of these confirmed 93 active extracts against DLD-1 cell has been done resulting in 77 non-toxic active extracts. - Purification of 16 active extracts are currently being performed 1-2. The indicator has been achieved - Currently, purification of active extract are being perfomed. 1-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	2-1. The indicator is expected to be achieved by the Mid-term Review. - More than 2200 extracts were objected to enzyme- and cell-based screening for anti-amebic activity, resulting more than 98 hits were achieved. - Confirmation of inhibitory activity of 48 active extracts from cell-based screening has been done resulting in 5 active extracts. - Purification of active compound from 8 active extracts that have inhibitory activity against CS3 enzyme and proliferation of E.histolytica cell are currently conducting. 2-2. The indicator is expected to be achieved by the time of Terminal Evaluation. - The chemical structure of isolated and purified active compound from the result of screening activity will be elucidated. 2-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of Plasmodium falciparum and Entamoeba histolytica are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents	3-1. The indicator is expected to be achieved by the end of 3rd year of the Project. - On 2016, more than 8000 of microbial extracts and 119 of plant extracts were newly prepared. More than 800 microbes were newly isolated from soil sample that was taken from Biak Island in June 2016. All extracts and microbes were registered in the in-house biological resource libraries. 3-2. The indicator is expected to be achieved by the end of 2nd year of the Project. - Equipment have already installed and available to be used in August 2016 - Enzymes needed for enzyme-based screening (DHODH, MQO, CS3, SAT1) have been prepared and characterized - Enzyme-based screening for extracts with anti-malarial, as well as anti-amebic, activity has been started and established at BTC and AU. - Cell-based screening for extracts with anti-amebic activity has been started and established at AU. Cell-based assay for anti-amebic activity has been started at BTC as well. - Maintenance of parasite cell (Entamoeba) has been conducted at BTC and AU - Maintenance of mammalian cell (4 type of cells) has been conducted at BTC - Cell cytotoxicity test of active extracts against mammalian cells have been started and established. - Cell-based screening of extracts against Plasmodium cells will be started after establishment of Plasmodium cell culture at BTC. 3-3. The indicator is expected to be achieved by the end of the 3rd year of the Project. - E.histolytica clone 6 culture is currently maintained and cultured at BTC and AU. - E.histolytica cell-based evaluation system are established and implemented at both BTC and AU. - Establishment of culture and evaluation system using P.falciparum 3D7 are established in BTC, and will be implemented in next semester. 3-4. The indicator is expected to be achieved by the time of the Terminal Evaluation. - Equipment needed for isolation and purification of compounds were installed in August 2016. - Two experts from Japan visited BTC to give training on purification of active compounds. - Isolation and purification of 4 active compounds with inhibitory activity against CS3 and 2 active compounds with inhibitory activity against DHODH is currently being conducted.	

	3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.			3-5. The indicator is expected to be achieved by the time of the Terminal Evaluation. - NMR data of an active compound with inhibitory activity against DHODH that was taken in last semester is being analyzed at BTC. - NMR analysis of other active compound with inhibitory activity against DHODH has been conducted at Kitasato U, but need to be re-analyzed due to low amount of the sample.	
	3-6. International symposiums are held for drug discovery for two(2) times at least.			3-6. The indicator is expected to be achieved by the time of the end of the project. - The symposium are expected to be held in 2017 and 2019.	

Activities	Inputs		
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	**Japan**	**Indonesia**	**Important Assumptions**
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.		**Pre-conditions** 1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT. ↓ <Issues and countermesures> 0-1. Equipment Provision 2015 imported from Japan delayed due to new rules of import restriction
1-2. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of P*lasmodium falciparum* under the condition of *in vitro* culture system.			
1-3. To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-4. To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			
1-5. To determine chemical structures of the lead compound candidates.			
1-6.			

1-7.	To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.		to new rules of import restriction. UT&JICA rearranged mean of the importation to clear the Indonesian regulation. Then it was imported in Jun 2016. 0-2. Costs on Consumables Estimated annual cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).The cost was calculated based on the annual working plan of each working teams, the working plan was planned to meet the requirements to implement 5,000 extracts annually in line with the expected output of the Project Design Matrix (PDM). Japanese side understanded the status and allocated the budget for the those consumables as well as equipment and trainings in Japan. On the other hand, Indonesian institutes are trying to allocate more budget for coming years in future.	
1-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).			
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.			
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			
2-6.	To determine chemical structures of the lead compound candidates.			
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.			
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**			
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			

3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

【Abbreviations】DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Project Monitoring Sheet I （Revision of Project Design Matrix）

Version 04

As of September 30, 2017

Project Design Matrix (PDM)（Version 4）
Project Title: The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebaic Agents by Utilizing Diversity of Indonesian bio-Resources in the Republic of Indonesia
Period of Project: From April 01, 2015 to March 31, 2020
Implementing Agencies:
【Indonesia】Laboratory for Biotechnology of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tokyo, Kitasato University, Nagasaki University, MicroBiopharm Japan Co., Ltd.
Target Area: the Republic of Indonesia
Project Implementers: Approximately 32 researchers engaged in the Project (22 from BPPT, 6 from AU and 4 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievement	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minutes of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents		1. This indicator is expected to be achieved by the time of the end of the Project. - More than 5000 extracts were objected for first screening against DHODH and MQO. - Cytotoxicity test of 34 active extracts that showed inhibitory activity against DHODH and MQO was performed resulting 14 active - Fourteen extracts were prepared in larger scale - Two more compounds with anti-malarial activity are being purified in this semester. - Efficacy test using animal experiment will be started in 2018 2. This indicator is expected to be achieved by the time of the end of the Project. - First screening of more than 2200 microbial extracts were done against CS3 and SAT1 assay, as well as against E.histolytica, resulting in 48 active extracts. - Purification of active compound from 4 active extracts that have inhibitory activity against CS3 enzyme are currently conducting - Large scale extract preparation of 4 more extracts that had inhibitory activity against proliferation of E.histolytica had been prepared and will be purified in next semester. - Efficacy test using animal experiment will be conducted in 2018 3. A scientific paper about screening, isolation, and structure elucidation of anti-malarial compounds is being prepared (the paper are expected to be submitted to peer-reviewed journal in Q4 of 2016)	
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.	1-1. The indicator has been achieved (3 compounds with anti-malarial had been isolated and purified) - More than 5000 of microbial extracts and 100 of plant extracts were objected for 1st screening resulting more than 78 active extracts that showed inhibitory activity against DHODH and MQO. - Confirmation of inhibitory activity of 21 active extracts has been done resulting in 9 active extracts. - Toxicity test of these confirmed 9 active extracts against 4 kinds of mammalian cell has been done resulting in 9 active extracts. These extracts were then proposed to be purified. - Purification of 2 active extracts are currently being performed 1-2. The indicator has been achieved (The chemical structure of two (2) compounds with anti-malarial activity had been elucidated) - Purification of other 2 active extracts are currently being performed 1-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents		2-1. The indicator is expected to be achieved by the Mid-term Review. - More than 2000 extracts were objected to enzyme- and cell-based screening for anti-amebic activity, resulting more than 130 hits were achieved. - Confirmation of inhibitory activity of 48 active extracts from cell-based screening has been done resulting in 5 active extracts. - Purification of active compound from 4 active extracts that have inhibitory activity against CS3 enzyme are currently conducting 2-2. The indicator is expected to be achieved by the time of Terminal Evaluation. - The chemical structure of isolated and purified active compound from the result of screening activity will be elucidated. 2-3. The indicator is expected to be achieved by the end of the project period. - According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of Plasmodium falciparum and Entamoeba histolytica are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents		3-1. The indicator is expected to be achieved by the end of 3rd year of the Project. - Currently, more than 5000 of microbial extracts and 119 of plant extracts were newly prepared from January 2016. More than 1000 microbes were newly isolated from soil sample that was taken from Biak Island in June 2016. All extracts and microbes were registered in the in-house biological resource libraries. (how about Togeans'?) 3-2. The indicator is expected to be achieved by the end of 2nd year of the Project. - Equipment have already installed and available to be used in August 2016 - Enzymes needed for enzyme-based screening (DHODH, MQO, CS3, SAT1) have been prepared and characterized - Enzyme-based screening for extracts with anti-malarial, as well as anti-amebic, activity has been started and established at BTC and AU. - Cell-based screening for extracts with anti-amebic activity has been started and established at AU. Cell-based assay for anti-amebic activity has been started at BTC as well. - Maintenance of parasite cell (Entamoeba) has been conducted at BTC and AU - Maintenance of mammalian cell (4 type of cells) has been conducted at BTC - Cell cytotoxicity test of active extracts against mammalian cells have been started and established. - Cell-based screening of extracts against Plasmodium cells will be started after establishment of Plasmodium cell culture at BTC. 3-3. The indicator is expected to be achieved by the end of the 3rd year of the Project. - E.histolytica clone 6 culture is currently maintained and cultured at BTC and AU. - E.histolytica cell-based evaluation system are established and implemented at both BTC and AU. - Establishment of culture and evaluation system using P.falciparum 3D7 will be started in next semester. 3-4. The indicator is expected to be achieved by the time of the Terminal Evaluation. - Equipment needed for isolation and purification of compounds were installed in August 2016. - Four experts from Japan visited BTC many times to give training on purification of active compounds. - Isolation and purification of 4 active compounds with inhibitory activity against CS3 and 2 active compounds with inhibitory activity against DHODH is currently being conducted.	

	3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.			3-5. The indicator is expected to be achieved by the time of the Terminal Evaluation. - NMR data of an active compound with inhibitory activity against DHODH that was taken in last semester is being analyzed at BTC. - NMR analysis of other active compound with inhibitory activity against DHODH has been conducted at Kitasato U, but need to be re-analyzed due to low amount of the sample.
	3-6. International symposiums are held for drug discovery for two(2) times at least.			3-6. The indicator is expected to be achieved by the time of the end of the project. - The International symposium was held in August 2017, and another symposium in Japan will be organized in 2019.

Activities	Inputs		
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	**Japan**	**Indonesia**	**Important Assumptions**
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	<u>Experts</u> (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises <u>Training in Japan</u> (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises <u>Equipment and materials</u> Necessary equipment for research activities in the Project <u>Local costs</u> Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	<u>Counterparts</u> (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities <u>Facilities, equipment and materials</u> (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI <u>Local costs</u> Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	**Pre-conditions** 1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT. ↓ <Issues and countermesures> 0-2. Costs on Consumables Estimated annual cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).The cost was calculated based on the annual working plan of each working
1-2. To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-3. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of P*lasmodium falciparum* under the condition of *in vitro* culture system.			
1-4. To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-5. To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for <u>enhancing productivity of the target compounds.</u>			
1-6. To determine chemical structures of the lead compound candidates.			
1-7. To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			
1-8. To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			teams, the working plan was planned to meet the requirements to implement 5,000 extracts annually in line with the expected output of the Project Design Matrix (PDM). Japanese side understanded the status and allocated the budget for the those consumables as well as equipment and trainings in Japan. On the other hand, Indonesian institutes are trying to allocate more budget for coming years in future.	
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).				
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.				
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.				
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.				
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.				
2-6.	To determine chemical structures of the lead compound candidates.				
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.				
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.				
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**				
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.				
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.				
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.				
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.				
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.				
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.				

【Abbreviations】DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Project Monitoring Sheet I （Revision of Project Design Matrix）

Version 05

As of March 31, 2018

Project Design Matrix (PDM)（Version 5）

Project Title: The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebaic Agents by Utilizing Diversity of Indonesian bio-Resources in the Republic of Indonesia

Period of Project: From April 01, 2015 to March 31, 2020

Implementing Agencies:

【Indonesia】Laboratory for Biotechnology of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)

【Japan】University of Tokyo, Kitasato University, Nagasaki University, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia

Project Implementers: Approximately 40 researchers engaged in the Project (28 from BPPT, 7 from AU and 5 from LIPI)

Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievement	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minutes of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			

	2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.			
3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of Plasmodium falciparum and Entamoeba histolytica are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents		

Activities	Inputs		
	Japan	**Indonesia**	**Important Assumptions**
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**			
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI	
1-2. To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			

1-3.	In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of P*lasmodium falciparum* under the condition of *in vitro* culture system.	(3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises <u>Equipment and materials</u> Necessary equipment for research activities in the Project <u>Local costs</u> Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	<u>Local costs</u> Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	**Pre-conditions** 1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT. ↓
1-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-5.	To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for <u>enhancing productivity of the target compounds.</u>			<Issues and countermesures>
1-6.	To determine chemical structures of the lead compound candidates.			0-2. Costs on Consumables Estimated annual cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).The cost was calculated based on the annual working plan of each working teams, the working plan was planned to meet the requirements to implement 5,000 extracts annually in line with the expected output of the Project Design Matrix (PDM). Japanese side understanded the status and allocated the budget for the those consumables as well as equipment and trainings in Japan. On the other hand, Indonesian institutes are expected to allocate more budget for reagents & consumables.
1-7.	To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			
1-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).			
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.			
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			
2-6.	To determine chemical structures of the lead compound candidates.			
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.			

2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**			
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

【Abbreviations】DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Project Monitoring Sheet I （Revision of Project Design Matrix）

Version 07

As of March 31, 2019

Project Design Matrix (PDM)（Version 7）
Project Title: The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebaic Agents by Utilizing Diversity of Indonesian bio-Resources in the Republic of Indonesia
Period of Project: From April 01, 2015 to March 31, 2020
Implementing Agencies:
【Indonesia】Laboratory for Biotechnology of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tokyo, Kitasato University, Nagasaki University, MicroBiopharm Japan Co., Ltd.
Target Area: the Republic of Indonesia
Project Implementers: Approximately 40 researchers engaged in the Project (28 from BPPT, 7 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievement	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minutes of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents		This indicator is expected to be achieved by the time of the end of the Project. □ About 17500 of microbial extracts and 128 of plant extracts were objected for 1st screening against DHODH and MQO in cumulative. □ More than 950 reconfirmation extracts and 57 extracts for purification in cumulative □ About 11000 extracts have been objected into malarial cell-based screening in cumulative. □ Optimization of cell-based screening system was performed. □ Additional 5 antimalarial compounds were purified and structure elucidated. □ Large scale production of antimalarial active compound for efficacy test is being conducted	
	2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy.			This indicator is expected to be achieved by the time of the end of the Project. □ More than 5300 extract were screened against EhCS3, 2200 extracts against EhSAT1, and 10000 extracts against parasite in cumulative. □ Enzymatic screening using newly introduced target EhNAD Kinase/NO1 was done using 7000 extracts resulting 90 hit. □ About 10 extracts with enzymatic inhibition activity and 30 extracts with cell proliferation inhibition activity were reconfirmed to be active. □ Three active extracts are being purified, and 4 other extracts are being prepared for large scale production. □ Efficacy test using animal experiment will be conducted in 2019	
	3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.			This indicator is partly achieved, and will be completely achieved by the time of the end of the Project. □ A scientific paper about screening system using target PfMQO written by Indonesian researcher as first author was published in peer-reviewed journal. □ A scientific paper about new fungal species is being prepared.	
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.	The indicator has been achieved (10 compounds with anti-malarial had been isolated and purified) □ More than 11000 extracts have been objected into malarial cell-based screening in cumulative. □ One active compound with antiplasmodial activity were isolated and structure elucidated within the semester.	1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards.
	1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation.			The indicator has been achieved (The chemical structure of 9 compounds with anti-malarial activity had been elucidated). □ One active compound with antiplasmodial activity were isolated and structure elucidated.	2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
	1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.			The indicator is expected to be achieved by the end of the project period. □ Large scale production of antimalarial active compound for efficacy test is being prepared	

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	The indicator has been achieved. (1 compound with antiamebic activity was isolated and purified) □ More than 5300 extract were screened against EhCS3, 2200 extracts against EhSAT1, and 10000 extracts against parasite in cumulative. □ Enzymatic screening using newly introduced target EhNAD Kinase/NO1 was done using 7000 extracts resulting 90 hit. □ About 10 extracts with enzymatic inhibition activity and 30 extracts with cell proliferation inhibition activity were reconfirmed to be active. □ 1 compound with antiamebic activity was isolated and purified. The indicator has been achieved (1 compound with antiamebic activity was structurally elucidated) □ 1 compound with antiamebic activity was structurally elucidated The indicator is expected to be achieved by the end of the project period. □ According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.	
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of Plasmodium falciparum and Entamoeba histolytica are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents	The indicator is already achieved. More than 17000 extracts for first screening have been produced from newly-obtained and existing microorganisms and plants. All of them have been registered. A new species of fungi was identified from the collection and being further investigated. The indicator has been achieved. Enzyme- and cell-based screening systems have been established and implemented in BTC and AU. □ Equipment have already installed and available to be used in August 2016 □ Enzymes needed for enzyme-based screening (DHODH, MQO, CS3, SAT1, and newly added NDH2 and NADKinase/NO1) have been prepared and characterized □ Enzyme-based screening for extracts with anti-malarial, as well as anti-amebic, activity has been started and established at BTC and AU. □ Cell-based screening for extracts with anti-amebic activity has been started and established at AU. Cell-based assay for anti-amebic activity has been started at BTC as well. □ Maintenance of parasite cell (Entamoeba) has been conducted at BTC and AU □ Maintenance of mammalian cell (5 type of cells) has been conducted at BTC □ Cell cytotoxicity test of active extracts against mammalian cells have been started and established. □ Cell-based screening of extracts against Plasmodium cells will be started after establishment of Plasmodium cell culture at BTC. The indicator has been achieved. Both P.falciparum and E.histolytica culture and evaluation system, as well as mammalian cell culture for counter assay, have been established at BTC and AU. □ E.histolytica clone 6 culture is currently maintained and cultured at BTC and AU. □ E.histolytica cell-based evaluation system are established and implemented at both BTC and AU. □ Culture and evaluation system using P.falciparum 3D7 are established at BTC. □ Mammalian cell culture and evaluation system are established at BTC and AU. The indicator is expected to be achieved by the time of the Terminal Evaluation. □ Equipment needed for isolation and purification of compounds were installed in August 2016. □ Pre-extraction test to ensure the extract remained active was introduced. □ Dereplication method for avoiding obtaining of fatty acids as active compound with PfMQO inhibitory activity was introduced. □ Dereplication method for avoiding obtaining frequent hit produced by fungi and actinomycetes by examining extract activity against gram positif bacteria was introduced. □ Dereplication method for avoiding obtaining frequent hit with antiamebic activity by excluding Aspergillus fumigatus from the list of the producer of those hits.	

	3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.			The indicator is expected to be achieved by the time of the Terminal Evaluation. □ Fatty acids as frequent hit as PfMQO inhibitory agents were determined based on result of purification and structure elucidation. □ Structure prediction method using Natural Product Dictionary was introduced. The indicator has been partially achieved. International symposium was held on August 2017 in Jakarta. □ The 2nd international symposium is expected to be held on October 8, 2019.

Activities	Inputs		
	Japan	**Indonesia**	**Important Assumptions**
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**			
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	<u>Experts</u> (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises <u>Training in Japan</u> (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises <u>Equipment and materials</u> Necessary equipment for research activities in the Project <u>Local costs</u> Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	<u>Counterparts</u> (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities <u>Facilities, equipment and materials</u> (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI <u>Local costs</u> Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	**Pre-conditions** 1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT. ↓ <Issues and countermesures> 0-2. Costs on Consumables Estimated annual cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).The cost was calculated based on the annual working plan of each working
1-2. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of P*lasmodium falciparum* under the condition of *in vitro* culture system.			
1-3.			
1-4. To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-5. To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for <u>enhancing productivity of the target compounds.</u>			
1-6. To determine chemical structures of the lead compound candidates.			
1-7. To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			
1-8. To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			teams, the working plan was planned to meet the requirements to implement 5,000 extracts annually in line with the expected output of the Project Design Matrix (PDM). Japanese side understanded the status and allocated the budget for the those consumables as well as equipment and trainings in Japan. On the other hand, Indonesian institutes are expected to allocate more budget for reagents & consumables.	
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).				
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.				
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.				
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.				
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.				
2-6.	To determine chemical structures of the lead compound candidates.				
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.				
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.				
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**				
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.				
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.				
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.				
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.				
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.				
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.				

【Abbreviations】DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Project Monitoring Sheet I （Revision of Project Design Matrix）

Version 08

As of September 30, 2019

Project Design Matrix (PDM)（Version 8）

Project Title: The Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebaic Agents by Utilizing Diversity of Indonesian bio-Resources in the Republic of Indonesia

Period of Project: From April 01, 2015 to March 31, 2020

Implementing Agencies:

【Indonesia】Laboratory for Biotechnology of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)

【Japan】University of Tokyo, Kitasato University, Nagasaki University, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia

Project Implementers: Approximately 40 researchers engaged in the Project (28 from BPPT, 7 from AU and 5 from LIPI)

Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievement	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minutes of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents		This indicator is expected to be achieved by the time of the end of the Project. □ More than 550 microbes were newly isolated from sample taken in West Jawa during field trip in April 2019. These microbes had been registered into microbial library. Total microbes isolated from the beginning of this project are more than 3500 isolates, and the total microbes in the collection reached 27 thousands isolates. □ About 20000 of microbial extracts and 128 of plant extracts were objected for 1st screening against DHODH and MQO in cumulative. □ About 2000 reconfirmation extracts and 130 extracts for purification in cumulative were prepared. □ About 11000 extracts have been objected into malarial cell-based screening in cumulative. □ Optimization of cell-based screening system was performed. □ Additional 5 antimalarial compounds were purified and structure elucidated. □ Large scale production of 2 antimalarial active compounds for efficacy test were conducted. Total amount of prepared compound was 200 mg. □ Efficacy test of 1 antimalarial active compound is currently conducted. This indicator is expected to be achieved by the time of the end of the Project. □ More than 16000 extract were screened against amebic target enzyme EhSAT1, EhSAT1/CS3, and EhNADK/NO1, and against E.histolytica cell in cumulative. □ Anti amebic active compounds were isolated and purified, and most of them were known as citrinin and fumagilin. □ 3 active extracts that were not containing citrinin and fumagilin were selected and prepared to be objected for purification process. □ Efficacy test using animal experiment will be conducted in 2020. This indicator is partly achieved, and will be completely achieved by the time of the end of the Project. □ A scientific paper about screening system using target PfMQO written by Indonesian researcher as first author was published in peer-reviewed journal. □ A scientific paper about new fungal species is being prepared. □ Another scientific paper about the use of Indonesian microbes as resource for antimalarial drug discovery was submitted and being reviewed.	
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.	The indicator has been achieved (12 compounds with anti-malarial had been isolated and purified) □ More than 12000 extracts have been objected into malarial cell-based screening in cumulative. □ Two active compounds with antiplasmodial activity were isolated and structure elucidated within the semester. The indicator has been achieved (The chemical structure of 11 compounds with anti-malarial activity had been elucidated). □ Two active compounds with antiplasmodial activity were isolated and structure elucidated. The indicator is expected to be achieved by the end of the project period. □ Efficacy test an active anti-malarial compound is currently being conducted in Brawijaya University.	1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	The indicator has been achieved. (2 compound with antiamebic activity was isolated and purified) □ More than 16000 extract were screened against amebic target enzyme EhSAT1, EhSAT1/CS3, and EhNADK/NO1, and against E.histolytica cell in cumulative. The indicator has been achieved (2 compound with antiamebic activity was structurally elucidated) □ Anti amebic active compounds were isolated and purified, and most of them were known as citrinin and fumagilin. □ 3 active extracts that were not containing citrinin and fumagilin were selected and prepared to be objected for purification process. The indicator is expected to be achieved by the end of the project period. □ Efficacy test using animal experiment will be conducted in 2020.	
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of Plasmodium falciparum and Entamoeba histolytica are established at the Indonesian research institute by the end of the 3rd year of the Project.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents	The indicator is already achieved. More than 550 newly isolated microbes were isolated, identified and registered into microbial library. More than 20000 extracts for first screening have been produced from newly-obtained and existing microorganisms and plants. All of them have been registered. A new species of fungi was identified from the collection and being further investigated. The indicator has been achieved. Enzyme- and cell-based screening systems have been established and implemented in BTC and AU. □ Equipment have already installed and available to be used in August 2016 □ Enzymes needed for enzyme-based screening (DHODH, MQO, CS3, SAT1, and newly added NDH2 and NADKinase/NO1) have been prepared and characterized □ Enzyme-based screening for extracts with anti-malarial, as well as anti-amebic, activity has been started and established at BTC and AU. □ Cell-based screening for extracts with anti-amebic activity has been started and established at AU. Cell-based assay for anti-amebic activity has been started at BTC as well. □ Maintenance of parasite cell (Entamoeba) has been conducted at BTC and AU □ Maintenance of mammalian cell (5 type of cells) has been conducted at BTC □ Cell cytotoxicity test of active extracts against mammalian cells have been started and established. □ Cell-based screening of extracts against Plasmodium cells will be started after establishment of Plasmodium cell culture at BTC. □ A new anti-malarial screening system targeted on PfDPCK enzyme is being prepared to be introduced in BTC. The indicator has been achieved. Both P.falciparum and E.histolytica culture and evaluation system, as well as mammalian cell culture for counter assay, have been established at BTC and AU. □ E.histolytica clone 6 culture is currently maintained and cultured at BTC and AU. □ E.histolytica cell-based evaluation system are established and implemented at both BTC and AU. More than 16 thousands extracts had been screened. □ Culture and evaluation system using P.falciparum 3D7 are established at BTC. □ Mammalian cell culture and evaluation system are established at BTC and AU.	

	3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.			The indicator is expected to be achieved by the time of the Terminal Evaluation. □ Equipment needed for isolation and purification of compounds were installed in August 2016. □ Pre-extraction test to ensure the extract remained active was introduced. □ Dereplication method for avoiding obtaining of fatty acids as active compound with PfMQO inhibitory activity was introduced. □ Dereplication method for avoiding obtaining frequent hit produced by fungi and actinomycetes by examining extract activity against gram positif bacteria was introduced. □ Dereplication method for avoiding obtaining frequent hit with antiamebic activity by excluding Aspergillus fumigatus from the list of the producer of those hits. □ A new dereplication method based on HPLC profile of extracts was introduced in BTC.
	3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation.			The indicator is expected to be achieved by the time of the Terminal Evaluation. □ Fatty acids as frequent hit as PfMQO inhibitory agents were determined based on result of purification and structure elucidation. □ Structure prediction method using Natural Product Dictionary was introduced. □ Prediction system of active compounds in active extracts based on HPLC profiles was introduced
	3-6. International symposiums are held for drug discovery for two(2) times at least.			The indicator has been partially achieved. International symposium was held on August 2017 in Jakarta. □ The 2nd international symposium is expected to be held on October 8, 2019.

Activities	Inputs		
	Japan	**Indonesia**	**Important Assumptions**
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**			
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	**Pre-conditions** 1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.
1-2. To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-3. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of P*lasmodium falciparum* under the condition of *in vitro* culture system.			

1-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.	
1-5.	To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.	
1-6.	To determine chemical structures of the lead compound candidates.	
1-7.	To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.	
1-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.	
2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).	
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.	
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.	
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.	
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.	
2-6.	To determine chemical structures of the lead compound candidates.	
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.	
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.	
3	**Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.	
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.	

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<Issues and countermesures>

0-2. Costs on Consumables
Estimated annual cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).The cost was calculated based on the annual working plan of each working teams, the working plan was planned to meet the requirements to implement 5,000 extracts annually in line with the expected output of the Project Design Matrix (PDM).
Japanese side understanded the status and allocated the budget for the those consumables as well as equipment and trainings in Japan.
On the other hand, Indonesian institutes are expected to allocate more budget for reagents & consumables.

3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

【Abbreviations】DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

MINUTES OF MEETINGS
BETWEEN
THE JAPANESE DETAILED PLANNING SURVEY TEAM
AND
THE AUTHORITIES CONCERNED OF
THE GOVERNMENT OF THE REPUBLIC OF INDONESIA ON JAPANESE TECHNICAL COOPERATION FOR
THE PROJECT FOR UTILIZATION OF INDONESIAN BIORESOURCE FOR ANTI-MALARIAL AND ANTI-AMEBIC DRUG DEVELOPMENT

Japan International Cooperation Agency (hereinafter referred to as "JICA") organized the Detailed Planning Survey Team (hereinafter referred to as "the Team"), headed by Dr. Kaname KANAI, which visited the Republic of Indonesia from 2 October to 10 October, 2014 for the purpose of discussing the framework of the technical cooperation project entitled "Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development" (hereinafter referred to as "the Project").

During their stay in the Republic of Indonesia, the Team had a series of discussions and exchanged views on the Project with the Indonesian authorities.

As a result of the discussions, the Team and the Indonesian authorities concerned agreed on the matters referred to in the document attached hereto.

Jakarta, 10 October 2014

Dr. Kaname KANAI
Team Leader
Detailed Planning Survey Team
Japan International Cooperation Agency
Japan

Dr. Listyani Wijayanti
Deputy Chairperson
Agency for the Assessment and
Application of Technology (BPPT)
Republic of Indonesia

Witnessed by:

Prof. Dr. Kazuro SHIOMI
Professor
Kitasato Institute for Life Sciences,
Kitasato University
Japan

Prof. Dr. Sulaiman Yusuf
Representing Deputy Chairman for Life Sciences
Indonesian Institute of Sciences, LIPI
Republic of Indonesia

Prof. Dr. Soetjipto Vice Rector
Airlangga University
Republic of Indonesia

THE ATTACHED DOCUMENT

I. OBJECTIVES OF THE DETAILED PLANNING SURVEY

The objectives of the survey were to confirm background and contents of the request from the Government of the Republic of Indonesia and to make a cooperation plan (project design) through discussions with the Indonesian authorities concerned. The Team also collected and analyzed necessary information for ex-ante evaluation.

The contents of the survey were as follows:

1. To confirm the contents of the request from the Republic of Indonesia and the research plan of the University of Tsukuba (hereinafter referred to as "UT") and to harmonize the two;
2. To have discussions with the Indonesian authorities concerned on the project design including, Project Design Matrix (hereinafter referred to as "PDM"), a tentative Plan of Operation (hereinafter referred to as "PO"), inputs and implementing structure, and to reach an agreement;
3. To confirm actions and schedule up to the Project's commencement; and
4. To exchange the Minutes of Meetings (hereinafter referred to as "M/M") containing the project design and the draft Record of Discussions (hereinafter referred to as "R/D"), which is to be signed before commencement of the Project as a token of confirmation of result of the discussions.

II. BASIC FRAMEWORK OF THE PROJECT

1. Project Implementation Scheme

Both sides confirmed that the Project should be implemented under the "Science and Technology Research Partnership for Sustainable Development (SATREPS)*" promoted by JICA in collaboration with the Japan Science and Technology Agency (hereinafter referred to as "JST").

JICA will take necessary measures for the technical cooperation such as dispatch of experts, provision of equipment and training of personnel, and other supports related to the Project in Indonesia. JST will support UT and other members of the Japanese research team for the project activities implemented in Japan.

The Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT"), as the Indonesian counterpart, will take necessary measures for technical cooperation, such as preparation of research facilities, equipment and materials, personnel, utilities and other support related to the Project.

* "SATREPS" aims to develop new technology and its applications, and also aims at capacity development of researchers and research institutions in both countries.

2. **Project Title**

It is appropriate to modify the title of the Project from the one indicated in the application entitled "The Project for Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development" to "The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources" so that the agreed contents of the Project are accurately reflected.

Both parties agreed the above change and will propose the title modification to the authorities concerned of each government and, if approved, the title will be changed officially through diplomatic procedure.

3. **Term of Cooperation**

The duration of the Project will be five (5) years from the date, which will be indicated in the R/D.

4. **Implementation Structure of the Project**

4-1. Administration

Both sides agreed that the administration of the Project would be organized as shown in Annex I as follows:

There will be:

(1) Project Director (who will bear overall responsibility for the administration and implementation of the Project);
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

(2) Project Manager (who will be responsible for the managerial and technical matters of the Project);
Head, the Center for the Assessment of Biotechnology (hereinafter referred to as "Biotech Center") of BPPT

(3) Project Co-manager (who will be responsible for the managerial and technical matters of the Project in collaboration with the Project Manager);
- Head of Technological Services Division, Biotech Center of BPPT
- Director, Institute of Tropical Disease, Airlangga University (hereinafter referred to as "AU")
- Director, Research Center for Biotechnology, Indonesian Institute of Sciences (hereinafter referred to as "LIPI")

(4) Indonesian counterpart researchers, as shown in Annex II;

(5) Japanese Chief Advisor (who will provide necessary recommendations and advice to the Project Director and the Project Manager on any matters pertaining to the implementation of the Project): Professor, Graduate School of Life and Environmental Sciences, UT;

(6) JICA Project Coordinator; and

(7) Other JICA Experts (who will give necessary technical guidance and advice to Indonesian counterpart researchers on technical matters pertaining to the implementation of the Project).

4-2. Joint Coordinating Committee

For the effective and successful implementation of technical cooperation for the Project, a Joint Coordinating Committee will be established whose functions and composition are described as follows:

(1) Functions

1) To formulate and authorize the annual activity plan of the Project;
2) To endorse major achievements and products of the Project;
3) To monitor and review overall progress and supervise the Project; and
4) To review and discuss major issues arising from or concerning the Project.

(2) Composition

1) Chairperson: Project Director or person appointed by the Project Director
2) Members
   a. Indonesian side
      - Project Manager
      - Project Co-Managers
      - Other representative(s) from BPPT
   b. Japanese side
      - Japanese Chief Advisor
      - JICA Project Coordinator
      - Representative(s) from the JICA Indonesia Office
3) Observers

   Stakeholders appointed by the Chairperson such as the Embassy of Japan, JST and/or other relevant organizations

4-3. Scientific Meeting

In order to ensure effective monitoring of the research progress and timely feedback of the technical advice from the experts, researchers and personnel engaged in the Project will have opportunities for exchanging and monitoring research outcomes as well as administrative matters at least once a year. Reports and/or minutes of meetings will be prepared in English and will be shared with the relevant researchers and personnel.

5. Project Design Matrix and Tentative Plan of Operation

The basic framework of the Project is as shown in the PDM in Annex III. The tentative PO is as shown in Annex IV.

6. Inputs

The inputs from each side are as follows:

6-1. Japanese side

(1) Chief Advisor;
(2) Project Coordinator;
(3) Research scientists and staff;
(4) Project local staff, including assistant(s) and driver(s);
(5) Training in Japan for several Indonesian counterpart personnel; and
(6) Necessary equipment for research and development activities, as shown in Annex V.

6-2. Indonesian side

(1) Research scientists and staff;

(2) Office space and laboratory space;

(3) Existing equipment; and

(4) Available data, information and specimens related to the Project.

7. Special Issues

7-1. Memorandum of Understanding between research institutes

Both sides agreed that UT and BPPT should reach an agreement to execute the collaborative research in accordance with the project design immediately after signing R/D. The document (e.g. Memorandum of Understanding) will contain the following items of the collaborative research:

a. Objective and Plan;
b. Implementation;
c. Confidentiality and Intellectual Property Rights;
d. Access to Genetic Resources;
e. Publication of Results;
f. Dispute Resolution;
g. Duration of the Agreement;
h. Compliance with Laws and Regulations; and
i. Other items concerning both sides.

7-2. Intellectual Property Rights

Both sides confirmed that matters related to intellectual property rights should follow the Memorandum of Understanding.

7-3. Research Approvals

Both sides agreed that research approvals from the relevant institutions of Indonesia will be obtained.

7-4. Material Transfer

Both sides agreed that clearance of material transfer from relevant ministry/authority should be obtained. The materials may include pathogens, microorganisms, plants, extracts, substances, etc.

7-5. Compliance of Rules

Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards.

7-6. Biosafety

Both sides agreed that all laboratory activities should follow the international biosafety regulations.

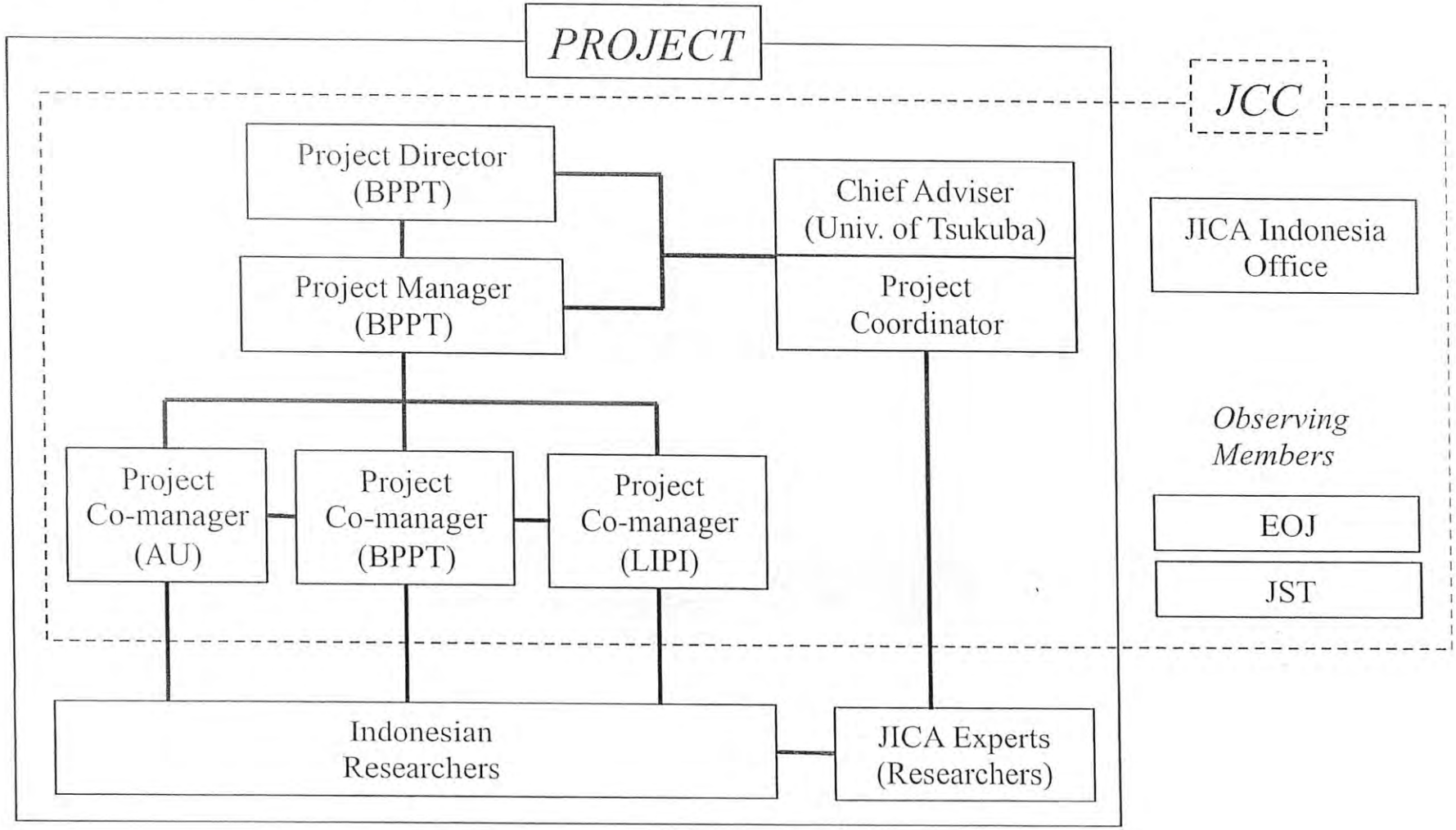
III. WAY FORWARD

1. Based on this M/M and the draft R/D as shown in Annex VI, the Indonesian and the Japanese side will prepare the final version of the R/D.
2. Based on the mutual agreement reached, the R/D should be signed by both sides as soon as possible aiming at the end of January 2015, but no later than the end of February 2015.
3. Memorandum of Understanding between UT and BPPT will be finalized by the end of January 2015.
4. The Project is expected to start in April 2015.
5. The schedule is subject to change in accordance with approval processes of the Project.

LIST OF ANNEXES

Annex I	Project Implementation Structure
Annex II	List of Researchers
Annex III	PDM version 0
Annex IV	Tentative PO Version 0
Annex V	Tentative List of Equipment
Annex VI	Draft R/D

Project Implementation Structure



Annex II

List of Researchers

Research Subject	The Indonesian Side	The Japanese Side
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).		
1.1 Primary screening for inhibitory activity of extracts to the plasmodium-derived recombinant enzyme	• Erwahyuni E Prabandari (BPPT) • Endah Dwi HartU.Tokyoi (BPPT)	• Daniel Ken Inaoka (U.Tokyo)
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of Plasmodium falciparum	• Astutiati Nurhasanah (BPPT) • Nuralih (BPPT) • Mutia Hardhiyuna (BPPT) • Siska Andrina Kusumastuti (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Keisuke Komatsuya (U. Tokyo)
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1 and 1-2	• Astutiati Nurhasanah (BPPT) • Nuralih (BPPT) • Mutia Hardhiyuna (BPPT) • Siska Andrina Kusumastuti (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Keisuke Komatsuya (U. Tokyo)
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT) • Presetyawan Yunianto (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU)
1.5 Establishment of mass production system of the lead compound candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)
1.6 Determination of chemical structures of the lead compound candidates	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU)

	• Eka Siska (BPPT) • Rudiyono (BPPT) • Presetyawan Yunianto (BPPT)	
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	• Agung Eru Wibowo (BPPT) • Kurnia Agustini (BPPT)	• Daniel Ken Inaoka (U.Tokyo) • Keisuke Komatsuya (U.Tokyo)
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .		
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica*-derived site-specific recombinant enzyme	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	• Achmad Fuad Hafid (AU) • Myrna Adianti (ITD-AU) • Ratna Wahyuni (ITD-AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BTC-BPPT) • Presetyawan Yunianto (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
2.5 Establishment of mass production system of the lead compound candidates	• Diana Dewi (BPPT) • Suyanto (BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)

	• Anna Safarrida (BPPT) • Dyah Noor Hidayati (BPPT)	
2.6 Determination of chemical structures of the lead compound candidates	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT) • Presetyawan Yunianto (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
2.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.		
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	• Achmad Dinoto (LIPI) • Puspita Lisdiyanti (LIPI) • Rifgiyah Nur Umami (LIPI) • Eris Septiana (LIPI) • Muhammad Ilyas (LIPI) • Dyah Noor Hidayati (BPPT)	• AUTko MaUTmoto (KU) • Ken-ichi Nonaka (KU) • Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ) • Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (U. Tokyo)
3.2 Establishment of screening systems	• Erwahyuni E Prabandari (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (U. Tokyo)
3.3 Establishment of culture and evaluation systems	• Astutiati Nurhasanah (BPPT)	• Tomoyoshi Nozaki (UT)

	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Daniel Ken Inaoka (U. Tokyo)
3.4 Introduction of technologies of isolation and purification	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
3.5 Introduction of technologies of chemical structure elucidation	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
3.6 Establishment and enhancement of a research network in Indonesia	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Ahmad Fuad (AU) • Puspita Lisdyanti (LIPI)	• Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (U. Tokyo) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)

Institution abbreviation:

- BPPT: Agency for the Assessment and Application of Technology
- AU: Institute for Tropical Diseases, Airlangga University
- LIPI: Biotechnology Research Institute, Indonesia Institute of Science
- U.Tokyo: University of Tokyo
- KU: Kitasato University
- MBJ: MicroBiopharm Japan, Co., Ltd.
- UT: University of Tsukuba
- NIID: National Institute of Infectious Diseases of Japan

Project Design Matrix (PDM) (Version 0)

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development

Date: October 10, 2014
Project Duration: 5 years after the date indicated on the Record of Discussion

Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Implementing Agencies:

【Indonesia】 Biotech Center of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】 University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia

Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievements	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Munities of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			

3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents			

Activities	Inputs		Pre-conditions
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	**Japan**	**Indonesia**	1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project.
1-1 To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities	2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.
To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.		Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI	
1-2 In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system	Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises	Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	
1-3	Equipment and materials Necessary equipment for research activities in the Project		
	Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.		

1-4	To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.
1-5	To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.
1-6.	To determine chemical structures of the lead compound candidates.
1-7.	To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.
1-8	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.
2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).
2-2	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.
2-4	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.
2-5	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.
2-6.	To determine chemical structures of the lead compound candidates.
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.
2-8	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.

↓

Issues and Countermeasures

3	**Technologies and research system for drug discover y using biological recourses are established at the Indonesian research institutes.**			
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

[Abbreviations] DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Tentative PO version 0

Version 0
Dated Oct. 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development
(Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources)

Inputs	Year	1st Year				2nd Year				3rd Year				4th Year				5th Year				6th Year				7th Year				8th Year				9st Year				Remarks	Monitoring	
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV		Issue	Solution
Expert																																								
Chief Advisor/Tropical Medicine Researches	Plan																																							
	Actual																																							
Project Coordinator	Plan																																							
	Actual																																							
Researcher(s) with expertise in malaria	Plan																																							
	Actual																																							
Researcher(s) with expertise in amebiasis	Plan																																							
	Actual																																							
Researcher(s) with expertise in isolation and purification of chemical compounds	Plan																																							
	Actual																																							
Researcher(s) with expertise in structure analysis of chemical compounds	Plan																																							
	Actual																																							
Other researcher(s) with necessary expertise for project research activities as necessity arises	Plan																																							
	Actual																																							
Equipment																																							
Instruments and related equipment for protozoal recombinant enzyme	Plan																																							
	Actual																																							
Instruments and related equipment for culture of protozoa	Plan																																							
	Actual																																							
Instruments and related equipment for chemical compound isolation	Plan																																							
	Actual																																							
Instruments and related equipment for mass production system of the lead compound	Plan																																							
	Actual																																							
Training in Japan																																							
Culture techniques of microorganisms and protozoa	Plan																																							
	Actual																																							
Screening techniques for inhibitory activity	Plan																																							
	Actual																																							
Techniques for Isolation and purification of chemical compounds	Plan																																							
	Actual																																							
Techniques for structure analysis of chemical compounds	Plan																																							
	Actual																																							
Techniques for mass production of chemical compounds	Plan																																							
	Actual																																							
Other training necessary for project research activities as necessity arises	Plan																																							
	Actual																																							
In-country/Third country Training	Plan																																							
	Actual																																							

Activities / Sub-Activities	Year	1st Year				2nd Year				3rd Year				4th Year				5th Year				6th Year				7th Year				8th Year				9st Year				Responsible Organization		Achievements	Issue & Countermeasures
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	Japan	GOI		
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).																																							
1.1 Primary screening for inhibitory activity of extract to the plasmodium-derived recombinant enzyme	Plan																																				U.Tokyo	BPPT		
	Actual																																						
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan																																				U.Tokyo	BPPT		
	Actual																																						
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum* , in parallel with Activity 1-1 and 1-2	Plan																																				U.Tokyo	BPPT		
	Actual																																						
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan																																				KU U.Tokyo	BPPT		
	Actual																																						
1.5 Establishment of mass production system of the lead compound candidates	Plan																																				KU MBJ	BPPT		
	Actual																																						
1.6 Determination of chemical structures of the lead compound candidates	Plan																																				KU	BPPT		
	Actual																																						
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	Plan																																				U.Tokyo	BPPT		
	Actual																																						
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan																																				ALL	ALL		
	Actual																																						
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)																																							
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan																																				UT	AU (BPPT)		
	Actual																																						
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan																																				UT	AU (BPPT)		
	Actual																																						
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica* , in parallel with Activity 2-1 and 2-2	Plan																																				UT	AU (BPPT)		
	Actual																																						
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan																																				KU UT	BPPT (AU)		
	Actual																																						
2.5 Establishment of mass production system of the lead compound candidates	Plan																																				KU MBJ	BPPT		
	Actual																																						
2.6 Determination of chemical structures of the lead compound candidates	Plan																																				KU	BPPT		
	Actual																																						
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan																																				UT	AU (BPPT)		
	Actual																																						
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan																																				ALL	ALL		
	Actual																																						

Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.

3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	Plan / Actual	KU MBJ	BPPT LIPI		
3.2 Establishment of screening systems	Plan / Actual	UT U.Tokyo	BPPT AU		
3.3 Establishment of culture and evaluation systems	Plan / Actual	UT U.Tokyo	BPPT AU		
3.4 Introduction of technologies of isolation and purification	Plan / Actual	KU	BPPT		
3.5 Introduction of technologies of chemical structure elucidation	Plan / Actual	KU	BPPT		
3.6 Establishment and enhancement of a research network in Indonesia	Plan / Actual	ALL	ALL		
Duration / Phasing	Plan / Actual				

Monitoring Plan	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Issue	Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Monitoring													
Joint Coordinating Committee	Plan	•	•	•	•	•							
	Actual												
Scientific Meeting	Plan	▲	▲	▲	▲	▲							
	Actual					▲	▲						
Set-up the Detailed Plan of Operation	Plan	▲	▲	▲	▲								
	Actual				▲								
Submission of Monitoring Sheet	Plan	■ ■	■ ■	■ ■	■ ■	■ ■							
	Actual												
Monitoring Mission from Japan	Plan			■		■							
	Actual												
Post Monitoring	Plan									■			
	Actual												
Reports/Documents													
Project Completion Report	Plan												
	Actual						▲						
Public Relations													
Establishment and Operation of Web Site	Plan	▲											
	Actual												
International symposiums are held for drug discovery	Plan			▲		▲							
	Actual												

Tentative List of Equipment

Category	Name
Microbial isolation/extract preparation	Freezer -30°C
	Freezer -30°C
	250 ml Flask holder (for large scale shaker incubator)
Microbial storage	Deep freezer -80°C, double compressor
Plant extract	Rotary evaporator/concentrator
Enzyme preparation	UV-vis spectrophotometer
	Electrophoresis system (for protein)
Enzyme-based screening	96-plate reader
Hit analysis	Analytical HPLC with DAD detector
	Semi-preparative HPLC (flow rate <20ml/min with UV-vis c
	Photodiode detector for UPLC (waters)
Cell-based screening	Safety cabinet class 2
	Autoclave
	Ultracentrifuge
	Ultracentrifuge Rotors
	CO_2/O_2 incubator
	Incubator
	Refrigerated centrifuge, table top
	Centrifuge Rotors, swing and angle
	Liquid nitrogen tank 30L with canister (box storage) as-one
Scale up production	Mini fermentor (3L (or 5L) x5 jar)
	Fermentor 30L
Experimental instruments and others	Server and PC
	Ultrasonic washer
	Sonicator
	Fraction collector, UV (for protein purification)
	Multichannel automatic micropipette 10ml
	Multichannel automatic micropipette 50ml
	Multichannel automatic micropipette 200ml
	Multichannel automatic micropipette 1000ml
	Micropipette set (2-1000ml)
	Refrigerator
	Freezer

[DRAFT]

RECORD OF DISCUSSIONS
ON
THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES
IN
THE REPUBLIC OF INDONESIA
AGREED UPON BETWEEN
AGENCY FOR THE ASSESSMENT AND APPLICATION OF TECHNOLOGY
AND
JAPAN INTERNATIONAL COOPERATION AGENCY

Jakarta, 〈date〉

Mr. Atsushi Sasaki
Chief Representative
Japan International Cooperation Agency
Indonesia Office

Chairperson
Agency for the Assessment and Application of Technology (BPPT)
The Republic of Indonesia

Based on the minutes of meetings on the detailed planning survey on "the Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources (hereinafter referred to as "the Project") signed on October 10, 2014 between Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT") and the Japan International Cooperation Agency (hereinafter referred to as "JICA"), JICA held a series of discussions with BPPT and relevant organizations to develop a detailed plan of the Project.

Both parties agreed the details of the Project and the main points discussed as described in the Appendix 1 and the Appendix 2 respectively.

Both parties also agreed that BPPT, the counterpart to JICA, will be responsible for the implementation of the Project in cooperation with JICA, coordinate with other relevant organizations and ensure that the self-reliant operation of the Project is sustained during and after the implementation period in order to contribute toward social and economic development of the Republic of Indonesia.

The Project will be implemented within the framework of the Colombo Plan Technical Cooperation Scheme between the Government of Japan (hereinafter referred to as "GOJ") and the Government of the Republic of Indonesia (hereinafter referred to as "GOI").

The effectiveness of the record of discussion is subject to the approval of JICA.

Appendix 1: Project Description
Appendix 2: Main Points Discussed
Appendix 3: Minutes of Meetings on the Detailed Planning Survey
Appendices are integral part of the Record of Discussions

APPENDIX 1

PROJECT DESCRIPTION

Both parties confirmed that there is no change in the project description agreed on in the minutes of meetings on the concerning detailed planning survey on the project signed on October 10, 2014 (appendix 3).

I. BACKGROUND

Indonesia is the second largest biodiversity country in the world. The diversity and uniqueness of bioresources is an important capital for Indonesia to increase wealth and prosperity of the people. GOI declared a strategic directive master plan for Indonesian economic development, through which GOI expects increase its economic competitiveness by transformation from bioresources-based comparative economic activities to innovation-based competitive economic activities. BPPT has been consistently developing innovation for utilization of Indonesian bioresources in order to support industries in Indonesia. One of the prioritized research topics is bio-prospecting for health by utilization of microbial and plant biodiversity. Many achievements have been obtained, however, very little of them had been practically used by industries or people. Thus, capacity building on utilization of bioresource for health, especially drug development, is very urgent.

Malaria and amebiasis are regarded as neglected diseases caused by parasites and a considerable concern in Indonesia which is needed to be overcome. Moreover, resistance of presently available drugs to the parasites is unavoidable, thus drug discovery for anti-malarial and anti-amebic compounds is urgently needed.

This technical cooperation is aimed to strengthen the capacity of Indonesian researchers and institutions in drug discovery against tropical infectious diseases including malaria and amebiasis using Indonesia bioresources.

GOI requested JICA to initiate the Indonesia-Japan collaborative project at BPPT and other associated institutions for conducting research on malaria and amebiasis. The joint research by the Indonesian institutes, BPPT and its collaborating institutes in Indonesia, the Indonesian Institute of Sciences (hereinafter referred to as "LIPI") and the Institute of Tropical Disease, Airlangga University (hereinafter referred to as "AU"), and Japanese institutions, University of Tsukuba, Kitasato University, University of Tokyo, and MicroBiopharm Japan Co. Ltd., aim (i) to strengthen capacity building for Indonesian researchers and institutions, (ii) to reinforce international research collaboration, and (iii) to increase added value of Indonesia bioresources especially for anti-malaria and anti-amebic drug discovery.

II. OUTLINE OF THE PROJECT

Details of the Project are described in the Project Design Matrix (hereinafter referred to as "PDM") (Annex I) and the tentative Plan of Operation (hereinafter referred to as "PO") (Annex II)

1. Project Implementation Structure

The project implementation structure is given in the Annex III. The roles and assignments of relevant organizations are as follows:

(1) BPPT

(a) Project Director will be responsible for overall administration and implementation of the Project. The Project Director will be Deputy Chairperson of Agro-industrial Technology and Biotechnology of BPPT;

(b) Project Manager will be responsible for the managerial and technical matters of the Project. The Project Manager will be Head, Biotechnology Application Center of BPPT; and

(c) Project Co-manager will be responsible for the managerial and technical matters of the Project in collaboration with the Project Manager. The Project Co-managers will be Division Head, Biotechnology Application Center of BPPT.

(2) AU

Director, Institute of Tropical Disease, AU will be Project Co-manager.

(3) LIPI

Director, Research Center for Biotechnology, LIPI will be Project Co-manager.

(4) JICA Experts

The JICA Experts will give necessary technical assistance, advice and recommendations to BPPT on any matters pertaining to the implementation of the Project.

(5) Joint Coordinating Committee

Joint Coordinating Committee (hereinafter referred to as "JCC") will be established in order to facilitate inter-organizational coordination. JCC will be held at least once a year and whenever deems it necessary. JCC will approve an annual work plan, review overall progress, conduct monitoring and evaluation of the Project, and discuss and take necessary measures to major issues that arise during the Project. Outline and a list of proposed members of JCC are shown in the Annex IV.

2. Project Sites and Beneficiaries

(1) Project Sites : Indonesia

(2) Beneficiaries : Indonesian Institutes engaged in the Project

3. Duration

The duration of the Project will be five (5) years starting on April 1, 2015.

4. Reports

Indonesian side and JICA experts will jointly prepare the following reports in English:

(1) Monitoring sheet at every six (6) months until the project completion; and

(2) Project Completion Report at the time of project completion

5. Environmental and Social Considerations

BPPT, AU, LIPI and University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd. agreed to abide by 'JICA Guidelines for Environmental and Social Considerations' in order to ensure that appropriate considerations will be made for the environmental and social impacts of the Project.

III. UNDERTAKINGS OF BPPT

1. BPPT will take necessary measures to:
   (1) ensure that the knowledge and experience acquired by the personnel of the Republic of Indonesia from technical training as well as the equipment provided by JICA will be utilized effectively in the implementation of the Project;
   (2) grant privileges, exemptions and benefits to the JICA experts referred to in ANNEX I and their families, which are no less favorable than those granted to experts of third countries performing similar missions in the Republic of Indonesia under the Colombo Plan Technical Cooperation Scheme;

2. BPPT will take necessary measures to:
   (1) provide security-related information as well as measures to ensure the safety of the JICA experts;
   (2) permit the JICA experts to enter, leave and sojourn in Indonesia for the duration of their assignments therein and exempt them from foreign registration requirements and consular fees;
   (3) exempt the JICA experts from taxes and any other charges on the equipment, machinery and other material necessary for the implementation of the Project; and
   (4) exempt the JICA experts from income tax and charges of any kind imposed on or in connection with any emoluments or allowances paid to them and/or remitted to them from abroad for their services in connection with the implementation of the Project.

3. BPPT, AU and LIPI will bear claims, if any arises, against the JICA experts resulting from, occurring in the course of, or otherwise connected with, the discharge of their duties in the implementation of the Project, except when such claims arise from gross negligence or willful misconduct on the part of the JICA experts.

IV. EVALUATION

JICA and the BPPT will jointly and regularly monitor the progress of the Project through the Monitoring Sheets based on PDM and PO. The Monitoring Sheets shall be reviewed every six (6) months.

Also, Project Completion Report shall be drawn up one (1) month before the

termination of the Project.

V. PROMOTION OF PUBLIC SUPPORT

For the purpose of promoting support for the Project, BPPT will take appropriate measures to make the Project widely known to the people of the Republic of Indonesia.

VI. MISCONDUCT

If JICA receives information related to suspected corrupt or fraudulent practices in the implementation of the Project, BPPT and relevant organizations shall provide JICA with such information as JICA may reasonably request, including information related to any concerned official of the government and/or public organizations of the BPPT and relevant organizations shall not, unfairly or unfavorably treat the person and/or company which provided the information related to suspected corrupt or fraudulent practices in the implementation of the Project.

VII. MUTUAL CONSULTATION

JICA and BPPT will consult each other whenever any major issues arise in the course of project implementation.

VIII. AMENDMENTS

The record of discussions may be amended by the minutes of meetings between BPPT and JICA. The minutes of meetings will be signed by authorized persons of each side who may be different from the signers of the record of discussions.

ANNEX I	PDM version 0 (M/M Annex III)
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ANNEX V	Goods / Services

Note: ANNEX I ,II , and III will be attached when the record of discussions is signed.

ANNEX IV

LIST OF PROPOSED MEMBERS OF JOINT COORDINATING COMMITTEE

1. Functions

The JCC will be held at least once a year and whenever deems it necessary. The functions of JCC are as follows:

(a) To facilitate inter-organizational coordination concerning the Project

(b) To approve an annual work plan of the Project

(c) To review overall progress, conduct monitoring and evaluation of The Project, and

(d) To exchange opinions on major issues that arise during the Project and to take necessary measures.

2. Chairperson: Project Director or person appointed by the Project Director
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

3. Members

(a) The Indonesian side

- Project Manager: Head, the Center for the Assessment of Biotechnology;
- Project Co-Managers: Head of Technological Services Division, Biotech Center of BPPT, Director, Institute of Tropical Disease, AU, and Director, Research Center for Biotechnology, LIPI; and
- Other representative(s) from BPPT.

(b) The Japanese side

- Japanese Chief Advisor;
- JICA Project Coordinator; and
- Representative(s) from the JICA Indonesia Office.

(c) Observers

Stakeholders appointed by the Chairperson such as the Embassy of Japan, JST and/or other relevant organizations.

ANNEX V

GOODS / SERVICES

Both sides confirmed that the Project is categorized as "goods / services" stipulated in article 42 (1) c of Government Regulation no. 10/2011. In accordance with regulation of Minister of Finance no. 191/ PMK.05 /2011, BPPT shall submit the handover delivery certificate of goods/services (BAST) to the Ministry of Finance of Indonesia. In order to secure the accuracy of BAST, JICA Indonesia office will provide BPPT with data on semester basis as follows:

1. Goods: name and price (in effective currency) per item of equipment handed over during last six months

2. Services: total expenditure (in effective currency) of last six months for expert, training, and mission

BPPT will make and sign BAST based on the data provided by JICA, and after obtaining JICA's confirmation, submit it to the Ministry of Finance.

APPENDIX 2

MAIN POINTS DISCUSSED

1. Biosafety

   Both sides agree that all laboratory activities should follow the international biosafety regulations.

2. Interectual Property Rights

   Both sides agree that matters related to intellectual property rights should follow the Memorandum of Understanding between University of Tsukuba and BPPT.

RECORD OF DISCUSSIONS

ON

THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES

IN

THE REPUBLIC OF INDONESIA

AGREED UPON BETWEEN

AGENCY FOR THE ASSESSMENT AND APPLICATION OF TECHNOLOGY

AND

JAPAN INTERNATIONAL COOPERATION AGENCY

Jakarta, 17 February 2015

Mr. Atsushi Sasaki
Chief Representative
Japan International Cooperation Agency
Indonesia Office

Dr.Ir. Unggul Priyanto, MSc.
Chairperson
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   (3) exempt the JICA experts from taxes and any other charges on the equipment, machinery and other material necessary for the implementation of the Project; and
   (4) exempt the JICA experts from income tax and charges of any kind imposed on or in connection with any emoluments or allowances paid to them and/or remitted to them from abroad for their services in connection with the implementation of the Project.
   (5) meet taxes and any other charges on the equipment, machinery and other material, referred to in ANNEX III, necessary for the implementation of the Project.

3. BPPT, AU and LIPI will bear claims, if any arises, against the JICA experts resulting from, occurring in the course of, or otherwise connected with, the discharge of their duties in the implementation of the Project, except when such claims arise from gross negligence or willful misconduct on the part of the JICA experts.

IV. MONITORING AND EVALUATION

JICA and the BPPT will jointly and regularly monitor the progress of the Project through the Monitoring Sheets based on PDM and PO. The Monitoring Sheets shall be reviewed every six (6) months.

Also, Project Completion Report shall be drawn up one (1) month before the termination of the Project.

JICA will conduct the following evaluations and surveys to mainly verify sustainability and impact of the Project and draw lessons. The BPPT is required to provide necessary support for them.

1. Ex-post evaluation three (3) years after the project completion, in principle; and

2. Follow-up surveys on necessity basis

V. PROMOTION OF PUBLIC SUPPORT

For the purpose of promoting support for the Project, BPPT will take appropriate measures to make the Project widely known to the people of the Republic of Indonesia.

VI. MISCONDUCT

If JICA receives information related to suspected corrupt or fraudulent practices in the implementation of the Project, BPPT and relevant organizations shall provide JICA with such information as JICA may reasonably request, including information related to any concerned official of the government and/or public organizations of the GOI.
BPPT and relevant organizations shall not, unfairly or unfavorably treat the person and/or company which provided the information related to suspected corrupt or fraudulent practices in the implementation of the Project.

VII. MUTUAL CONSULTATION

JICA and BPPT will consult each other whenever any major issues arise in the course of project implementation.

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The record of discussions may be amended by the minutes of meetings between BPPT and JICA. The minutes of meetings will be signed by authorized persons of each side who may be different from the signers of the record of discussions.

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ANNEX V	List of Proposed Members of Joint Coordinating Committee
ANNEX VI	Goods / Services

Annex I

Project Design Matrix (PDM) (Version 0)

Date: October 10, 2014
Project Duration: 5 years after the date indicated on the Record of

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development

Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Implementing Agencies:

【Indonesia】Biotech Center of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia

Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievements	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Munities of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			
3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10,000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents			

Annex I

Activities	Inputs		Pre-conditions
	Japan	Indonesia	
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**			1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	
1-2. To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-3. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-4. To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-5. To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			↓ Issues and Countermeasures
1-6. To determine chemical structures of the lead compound candidates.			
1-7. To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			
1-8. To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			

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2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc.).			
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.			
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			
2-6.	To determine chemical structures of the lead compound candidates.			
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.			
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
3	**Technologies and research system for drug discover y using biological recourses are established at the Indonesian research institutes.**			
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5.	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

[Abbreviations] DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

Tentative PO version 0

Version 0
Dated Oct. 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development
(Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources)

Inputs	Year	1st Year				2nd Year				3rd Year				4th Year				5th Year				6th Year				7th Year				8th Year				9st Year				Remarks	Monitoring: Issue	Monitoring: Solution
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV			
Expert																																							
Chief Advisor/Tropical Medicine Researches	Plan																																						
	Actual																																						
Project Coordinator	Plan																																						
	Actual																																						
Researcher(s) with expertise in malaria	Plan																																						
	Actual																																						
Researcher(s) with expertise in amebiasis	Plan																																						
	Actual																																						
Researcher(s) with expertise in isolation and purification of chemical compounds	Plan																																						
	Actual																																						
Researcher(s) with expertise in structure analysis of chemical compounds	Plan																																						
	Actual																																						
Other researcher(s) with necessary expertise for project research activities as necessity arises	Plan																																						
	Actual																																						
Equipment																																						
Instruments and related equipment for protozoal recombinant enzyme	Plan																																						
	Actual																																						
Instruments and related equipment for culture of protozoa	Plan																																						
	Actual																																						
Instruments and related equipment for chemical compound isolation	Plan																																						
	Actual																																						
Instruments and related equipment for mass production system of the lead compound	Plan																																						
	Actual																																						
Training in Japan																																						
Culture techniques of microorganisms and protozoa	Plan																																						
	Actual																																						
Screening techniques for inhibitory activity	Plan																																						
	Actual																																						
Techniques for Isolation and purification of chemical compounds	Plan																																						
	Actual																																						
Techniques for structure analysis of chemical compounds	Plan																																						
	Actual																																						
Techniques for mass production of chemical compounds	Plan																																						
	Actual																																						
Other training necessary for project research activities as necessity arises	Plan																																						
	Actual																																						
In-country/Third country Training	Plan																																						
	Actual																																						

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Activities / Sub-Activities	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Responsible Organization: Japan	Responsible Organization: GOI	Achievements	Issue & Countermeasures
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV				
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).														
1.1 Primary screening for inhibitory activity of extract to the plasmodium-derived recombinant enzyme	Plan										U Tokyo	BPPT		
	Actual													
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan										U Tokyo	BPPT		
	Actual													
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum* , in parallel with Activity 1-1 and 1-2	Plan										U Tokyo	BPPT		
	Actual													
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan										KU U Tokyo	BPPT		
	Actual													
1.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBU	BPPT		
	Actual													
1.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	Plan										U Tokyo	BPPT		
	Actual													
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)														
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan										UI	AU (BPPT)		
	Actual													
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan										UI	AU (BPPT)		
	Actual													
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica* , in parallel with Activity 2-1 and 2-2	Plan										UI	AU (BPPT)		
	Actual													
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan										KU UI	BPPT (AU)		
	Actual													
2.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBU	BPPT		
	Actual													
2.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan										UI	AU (BPPT)		
	Actual													
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													

Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.			
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	Plan / Actual	KU MBJ	BPPT LIPI
3.2 Establishment of screening systems	Plan / Actual	UI U Tokyo	BPPT AU
3.3 Establishment of culture and evaluation systems	Plan / Actual	UI U Tokyo	BPPT AU
3.4 Introduction of technologies of isolation and purification	Plan / Actual	KU	BPPT
3.5 Introduction of technologies of chemical structure elucidation	Plan / Actual	KU	BPPT
3.6 Establishment and enhancement of a research network in Indonesia	Plan / Actual	ALL	ALL
Duration / Phasing	Plan / Actual		

Monitoring Plan	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Issue	Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Monitoring													
Joint Coordinating Committee	Plan / Actual												
Scientific Meeting	Plan / Actual												
Set-up the Detailed Plan of Operation	Plan / Actual												
Submission of Monitoring Sheet	Plan / Actual												
Monitoring Mission from Japan	Plan / Actual												
Post Monitoring	Plan / Actual												
Reports/Documents													
Project Completion Report	Plan / Actual												
Public Relations													
Establishment and Operation of Web Site	Plan / Actual												
International symposiums are held for drug discovery	Plan / Actual												

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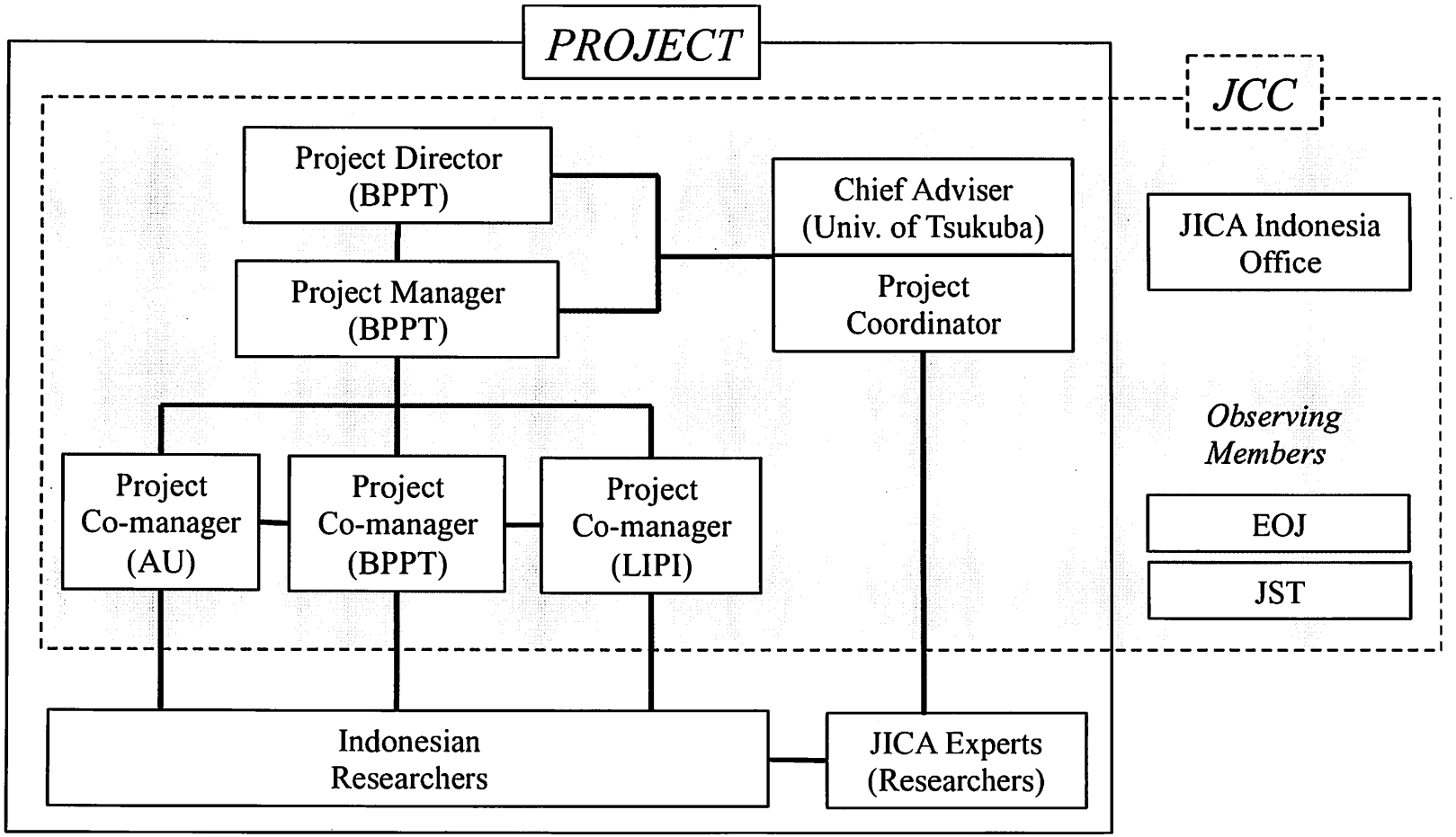
ANNEX III

List of Equipment

Category	Name
Microbial isolation/extract preparation	Freezer -30°C Freezer -30°C 250 ml Flask holder (for large scale shaker incubator)
Microbial storage	Deep freezer -80°C, double compressor
Plant extract	Rotary evaporator/concentrator
Enzyme preparation	UV-vis spectrophotometer Electrophoresis system (for protein)
Enzyme-based screening	96-plate reader
Hit analysis	Analytical HPLC with DAD detector Semi-preparative HPLC (flow rate <20ml/min with UV-vis detector) Photodiode detector for UPLC (waters)
Cell-based screening	Safety cabinet class 2 Autoclave Ultracentrifuge Ultracentrifuge Rotors CO_2/O_2 incubator Incubator Refrigerated centrifuge, table top Centrifuge Rotors, swing and angle Liquid nitrogen tank 30L with canister (box storage) as-one
Scale up production	Mini fermentor (3L (or 5L) x5 jar) Fermentor 30L
Experimental instruments and others	Server and PC Ultrasonic washer Sonicator Fraction collector, UV (for protein purification) Multichannel automatic micropipette 10ml Multichannel automatic micropipette 50ml Multichannel automatic micropipette 200ml Multichannel automatic micropipette 1000ml Micropipette set (2-1000ml) Refrigerator Freezer

ANNEX Ⅳ

Project Implementation Structure



ANNEX V

LIST OF PROPOSED MEMBERS OF JOINT COORDINATING COMMITTEE

1. Functions

The JCC will be held at least once a year and whenever deems it necessary. The functions of JCC are as follows:

(a) To facilitate inter-organizational coordination concerning the Project

(b) To approve an annual work plan of the Project

(c) To review overall progress, conduct monitoring and evaluation of The Project, and

(d) To exchange opinions on major issues that arise during the Project and to take necessary measures.

2. Chairperson: Project Director or person appointed by the Project Director
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

3. Members

(a) The Indonesian side

- Project Manager: Head, the Center for the Assessment of Biotechnology;
- Project Co-Managers: Head of Technological Services Division, Biotech Center of BPPT, Director, Institute of Tropical Disease, AU, and Director, Research Center for Biotechnology, LIPI; and
- Other representative(s) from BPPT.

(b) The Japanese side

- Japanese Chief Advisor;
- JICA Project Coordinator; and
- Representative(s) from the JICA Indonesia Office.

(c) Observers

Stakeholders appointed by the Chairperson such as the Embassy of Japan, JST and/or other relevant organizations.

ANNEX VI

GOODS / SERVICES

Both sides confirmed that the Project is categorized as "goods / services" stipulated in article 42 (1) c of Government Regulation no. 10/2011. In accordance with regulation of Minister of Finance no. 191/ PMK.05 /2011, BPPT shall submit the handover delivery certificate of goods/services (BAST) to the Ministry of Finance of Indonesia. In order to secure the accuracy of BAST, JICA Indonesia office will provide BPPT with data on semester basis as follows:

1. Goods: name and price (in effective currency) per item of equipment handed over during last six months

2. Services: total expenditure (in effective currency) of last six months for expert, training, and mission

BPPT will make and sign BAST based on the data provided by JICA, and after obtaining JICA's confirmation, submit it to the Ministry of Finance.

APPENDIX 2

MAIN POINTS DISCUSSED

1. Biosafety

   Both sides agree that all laboratory activities should follow the international biosafety regulations.

2. Interectual Property Rights

   Both sides agree that matters related to intellectual property rights should follow the Memorandum of Understanding between University of Tsukuba and BPPT.

Amendments on 2nd February 2016

RECORD OF DISCUSSIONS
ON
THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES
IN
THE REPUBLIC OF INDONESIA
AGREED UPON BETWEEN
AGENCY FOR THE ASSESSMENT AND APPLICATION OF TECHNOLOGY
AND
JAPAN INTERNATIONAL COOPERATION AGENCY

Jakarta, 17 February 2015

Mr. Atsushi Sasaki
Chief Representative
Japan International Cooperation Agency
Indonesia Office

Dr.Ir. Unggul Priyanto, MSc.
Chairperson
Agency for the Assessment and Application of Technology (BPPT)
The Republic of Indonesia

Based on the minutes of meetings on the detailed planning survey on "the Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources (hereinafter referred to as "the Project") signed on October 10, 2014 between Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT") and the Japan International Cooperation Agency (hereinafter referred to as "JICA"), JICA held a series of discussions with BPPT and relevant organizations to develop a detailed plan of the Project.

Both parties agreed the details of the Project and the main points discussed as described in the Appendix 1 and the Appendix 2 respectively.

Both parties also agreed that BPPT, the counterpart to JICA, will be responsible for the implementation of the Project in cooperation with JICA, coordinate with other relevant organizations and ensure that the self-reliant operation of the Project is sustained during and after the implementation period in order to contribute toward social and economic development of the Republic of Indonesia.

The Project will be implemented within the framework of the Colombo Plan Technical Cooperation Scheme between the Government of Japan (hereinafter referred to as "GOJ") and the Government of the Republic of Indonesia (hereinafter referred to as "GOI").

The effectiveness of the record of discussion is subject to the approval of JICA.

Appendix 1: Project Description
Appendix 2: Main Points Discussed
Appendix 3: Minutes of Meetings on the Detailed Planning Survey
Appendices are integral part of the Record of Discussions

APPENDIX 1

PROJECT DESCRIPTION

Both parties confirmed that there is no change in the project description agreed on in the minutes of meetings on the concerning detailed planning survey on the project signed on October 10, 2014 (appendix 3).

I. BACKGROUND

Indonesia is the second largest biodiversity country in the world. The diversity and uniqueness of bioresources is an important capital for Indonesia to increase wealth and prosperity of the people. GOI declared a strategic directive master plan for Indonesian economic development, through which GOI expects increase its economic competitiveness by transformation from bioresources-based comparative economic activities to innovation-based competitive economic activities. BPPT has been consistently developing innovation for utilization of Indonesian bioresources in order to support industries in Indonesia. One of the prioritized research topics is bio-prospecting for health by utilization of microbial and plant biodiversity. Many achievements have been obtained, however, very little of them had been practically used by industries or people. Thus, capacity building on utilization of bioresource for health, especially drug development, is very urgent.

Malaria and amebiasis are regarded as neglected diseases caused by parasites and a considerable concern in Indonesia which is needed to be overcome. Moreover, resistance of presently available drugs to the parasites is unavoidable, thus drug discovery for anti-malarial and anti-amebic compounds is urgently needed.

This technical cooperation is aimed to strengthen the capacity of Indonesian researchers and institutions in drug discovery against tropical infectious diseases including malaria and amebiasis using Indonesia bioresources.

GOI requested JICA to initiate the Indonesia-Japan collaborative project at BPPT and other associated institutions for conducting research on malaria and amebiasis. The joint research by the Indonesian institutes, BPPT and its collaborating institutes in Indonesia, the Indonesian Institute of Sciences (hereinafter referred to as "LIPI") and the Institute of Tropical Disease, Airlangga University (hereinafter referred to as "AU"), and Japanese institutions, University of Tsukuba, Kitasato University, University of Tokyo, and MicroBiopharm Japan Co. Ltd., aim (i) to strengthen capacity building for Indonesian researchers and institutions, (ii) to reinforce international research collaboration, and (iii) to increase added value of Indonesia bioresources especially for anti-malaria and anti-amebic drug discovery.

II. OUTLINE OF THE PROJECT

Details of the Project are described in the Project Design Matrix (hereinafter referred to as "PDM") (Annex I) and the tentative Plan of Operation (hereinafter referred to as "PO") (Annex II)

Director, Center for Pharmaceutical and Medical Technologies

1. Project Implementation Structure

The project implementation structure is given in the Annex IV, The roles and assignments of relevant organizations are as follows:

(1) BPPT

(a) Project Director will be responsible for overall administration and implementation of the Project. The Project Director will be Deputy Chairperson of Agro-industrial Technology and Biotechnology of BPPT;

(b) Project Manager will be responsible for the managerial and technical matters of the Project. The Project Manager will be ~~Head, Biotechnology Application Center~~ of BPPT; and

(c) Project Co-manager will be responsible for the managerial and technical matters of the Project in collaboration with the Project Manager. The Project Co-managers will be ~~Division Head, Biotechnology Application Center~~ of BPPT.

Program Head, Center for Pharmaceutical and Medical Technologies

(2) AU

Director, Institute of Tropical Disease, AU will be Project Co-manager.

(3) LIPI

Director, Research Center for Biology, LIPI will be Project Co-manager.

(4) JICA Experts

The JICA Experts will give necessary technical assistance, advice and recommendations to BPPT on any matters pertaining to the implementation of the Project.

(5) Joint Coordinating Committee

Joint Coordinating Committee (hereinafter referred to as "JCC") will be established in order to facilitate inter-organizational coordination. JCC will be held at least once a year and whenever deems it necessary. JCC will approve an annual work plan, review overall progress, conduct monitoring and evaluation of the Project, and discuss and take necessary measures to major issues that arise during the Project. Outline and a list of proposed members of JCC are shown in the Annex V.

2. Project Sites and Beneficiaries

(1) Project Sites: Indonesia

(2) Beneficiaries: Indonesian Institutes engaged in the Project

3. Duration

The duration of the Project will be five (5) years starting on April 1, 2015.

4. Reports

Indonesian side and JICA experts will jointly prepare the following reports in English:

(1) Monitoring sheet at every six (6) months until the project completion; and

(2) Project Completion Report at the time of project completion

5. Environmental and Social Considerations

BPPT, AU, LIPI and University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd. agreed to abide by 'JICA Guidelines for Environmental and Social Considerations' in order to ensure that appropriate considerations will be made for the environmental and social impacts of the Project.

III. UNDERTAKINGS OF BPPT

1. BPPT will take necessary measures to:
   (1) ensure that the knowledge and experience acquired by the personnel of the Republic of Indonesia from technical training as well as the equipment provided by JICA will be utilized effectively in the implementation of the Project;
   (2) grant privileges, exemptions and benefits to the JICA experts referred to in ANNEX I and their families, which are no less favorable than those granted to experts of third countries performing similar missions in the Republic of Indonesia under the Colombo Plan Technical Cooperation Scheme;

2. BPPT will take necessary measures to:
   (1) provide security-related information as well as measures to ensure the safety of the JICA experts;
   (2) permit the JICA experts to enter, leave and sojourn in Indonesia for the duration of their assignments therein and exempt them from foreign registration requirements and consular fees;
   (3) exempt the JICA experts from taxes and any other charges on the equipment, machinery and other material necessary for the implementation of the Project; and
   (4) exempt the JICA experts from income tax and charges of any kind imposed on or in connection with any emoluments or allowances paid to them and/or remitted to them from abroad for their services in connection with the implementation of the Project.
   (5) meet taxes and any other charges on the equipment, machinery and other material, referred to in ANNEX III, necessary for the implementation of the Project.

3. BPPT, AU and LIPI will bear claims, if any arises, against the JICA experts resulting from, occurring in the course of, or otherwise connected with, the discharge of their duties in the implementation of the Project, except when such claims arise from gross negligence or willful misconduct on the part of the JICA experts.

IV. MONITORING AND EVALUATION

JICA and the BPPT will jointly and regularly monitor the progress of the Project through the Monitoring Sheets based on PDM and PO. The Monitoring Sheets shall be reviewed every six (6) months.

Also, Project Completion Report shall be drawn up one (1) month before the termination of the Project.

JICA will conduct the following evaluations and surveys to mainly verify sustainability and impact of the Project and draw lessons. The BPPT is required to provide necessary support for them.

1. Ex-post evaluation three (3) years after the project completion, in principle; and

2. Follow-up surveys on necessity basis

V. PROMOTION OF PUBLIC SUPPORT

For the purpose of promoting support for the Project, BPPT will take appropriate measures to make the Project widely known to the people of the Republic of Indonesia.

VI. MISCONDUCT

If JICA receives information related to suspected corrupt or fraudulent practices in the implementation of the Project, BPPT and relevant organizations shall provide JICA with such information as JICA may reasonably request, including information related to any concerned official of the government and/or public organizations of the GOI.
BPPT and relevant organizations shall not, unfairly or unfavorably treat the person and/or company which provided the information related to suspected corrupt or fraudulent practices in the implementation of the Project.

VII. MUTUAL CONSULTATION

JICA and BPPT will consult each other whenever any major issues arise in the course of project implementation.

VIII. AMENDMENTS

The record of discussions may be amended by the minutes of meetings between BPPT and JICA. The minutes of meetings will be signed by authorized persons of each side who may be different from the signers of the record of discussions.

ANNEX I	PDM version 0 (M/M Annex III)
ANNEX II	Tentative PO version 0 (M/M Annex IV)
ANNEX III	List of Equipment (M/M Annex V)
ANNEX IV	Project Implementation Structure (M/M Annex I)
ANNEX V	List of Proposed Members of Joint Coordinating Committee
ANNEX VI	Goods / Services

Center for Pharmaceutical and Medical Technologies of BPPT(PTFM-BPPT)

AMED

Annex I

Project Design Matrix (PDM) (Version 0)

Date: October 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development

Project Duration: 5 years after the date indicated on the Record of

Proposed Project Title for amendment by JICA and ~~JST~~: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Implementing Agencies:

【Indonesia】 ~~Biotech Center of the Agency for Assessment and Application of Technology (BTC-BPPT)~~, Airlangga University (AU), Indonesian Institute of Sciences (LIPI)

【Japan】 University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia

Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)

Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievements	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minities of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			
3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10,000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents			

Activities	Inputs		Pre-conditions
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	Japan	Indonesia	1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project.
1-1 To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.
1-2 To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-3 In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-4 To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-5 To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			↓
1-6 To determine chemical structures of the lead compound candidates.			Issues and Countermeasures
1-7 To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			
1-8 To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			
2-1	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc).			
2-2	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-3	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-4	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.			
2-5	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			
2-6	To determine chemical structures of the lead compound candidates.			
2-7	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.			
2-8	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
3	**Technologies and research system for drug discover y using biological recourses are established at the Indonesian research institutes.**			
3-1	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

[Abbreviations]DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

AMED

Tentative PO version 0

Version 0
Dated Oct. 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development
(Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources)

Inputs	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Monitoring: Issue	Monitoring: Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Expert													
Chief Advisor/Tropical Medicine Researches	Plan												
	Actual												
Project Coordinator	Plan												
	Actual												
Researcher(s) with expertise in malaria	Plan												
	Actual												
Researcher(s) with expertise in amebiasis	Plan												
	Actual												
Researcher(s) with expertise in isolation and purification of chemical compounds	Plan												
	Actual												
Researcher(s) with expertise in structure analysis of chemical compounds	Plan												
	Actual												
Other researcher(s) with necessary expertise for project research activities as necessity arises	Plan												
	Actual												
Equipment													
Instruments and related equipment for protozoal recombinant enzyme	Plan												
	Actual												
Instruments and related equipment for culture of protozoa	Plan												
	Actual												
Instruments and related equipment for chemical compound isolation	Plan												
	Actual												
Instruments and related equipment for mass production system of the lead compound	Plan												
	Actual												
Training in Japan													
Culture techniques of microorganisms and protozoa	Plan												
	Actual												
Screening techniques for inhibitory activity	Plan												
	Actual												
Techniques for Isolation and purification of chemical compounds	Plan												
	Actual												
Techniques for structure analysis of chemical compounds	Plan												
	Actual												
Techniques for mass production of chemical compounds	Plan												
	Actual												
Other training necessary for project research activities as necessity arises	Plan												
	Actual												
In-country/Third country Training	Plan												
	Actual												

Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.			
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	Plan / Actual	KU MBJ	BPPT LIPI
3.2 Establishment of screening systems	Plan / Actual	UT U Tokyo	BPPT AU
3.3 Establishment of culture and evaluation systems	Plan / Actual	UT U Tokyo	BPPT AU
3.4 Introduction of technologies of isolation and purification	Plan / Actual	KU	BPPT
3.5 Introduction of technologies of chemical structure elucidation	Plan / Actual	KU	BPPT
3.6 Establishment and enhancement of a research network in Indonesia	Plan / Actual	ALL	ALL
Duration / Phasing	Plan / Actual		

Monitoring Plan	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Issue	Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Monitoring													
Joint Coordinating Committee	Plan / Actual												
Scientific Meeting	Plan / Actual												
Set-up the Detailed Plan of Operation	Plan / Actual												
Submission of Monitoring Sheet	Plan / Actual												
Monitoring Mission from Japan	Plan / Actual												
Post Monitoring	Plan / Actual												
Reports/Documents													
Project Completion Report	Plan / Actual												
Public Relations													
Establishment and Operation of Web Site	Plan / Actual												
International symposiums are held for drug discovery	Plan / Actual												

Activities / Sub-Activities	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Responsible Organization: Japan	Responsible Organization: GOI	Achievements	Issue & Countermeasures
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV				
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).														
1.1 Primary screening for inhibitory activity of extract to the plasmodium-derived recombinant enzyme	Plan										U Tokyo	BPPT		
	Actual													
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan										U Tokyo	BPPT		
	Actual													
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum* , in parallel with Activity 1-1 and 1-2	Plan										U Tokyo	BPPT		
	Actual													
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan										KU U Tokyo	BPPT		
	Actual													
1.5 Establishment of mass production system of the lead compound candidates	Plan										KU MRI	BPPT		
	Actual													
1.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	Plan										U Tokyo	BPPT		
	Actual													
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)														
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan										UI	AU (BPPT)		
	Actual													
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan										UI	AU (BPPT)		
	Actual													
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica* , in parallel with Activity 2-1 and 2-2	Plan										UI	AU (BPPT)		
	Actual													
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan										KU UI	BPPT (AU)		
	Actual													
2.5 Establishment of mass production system of the lead compound candidates	Plan										KU MRI	BPPT		
	Actual													
2.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan										UI	AU (BPPT)		
	Actual													
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													

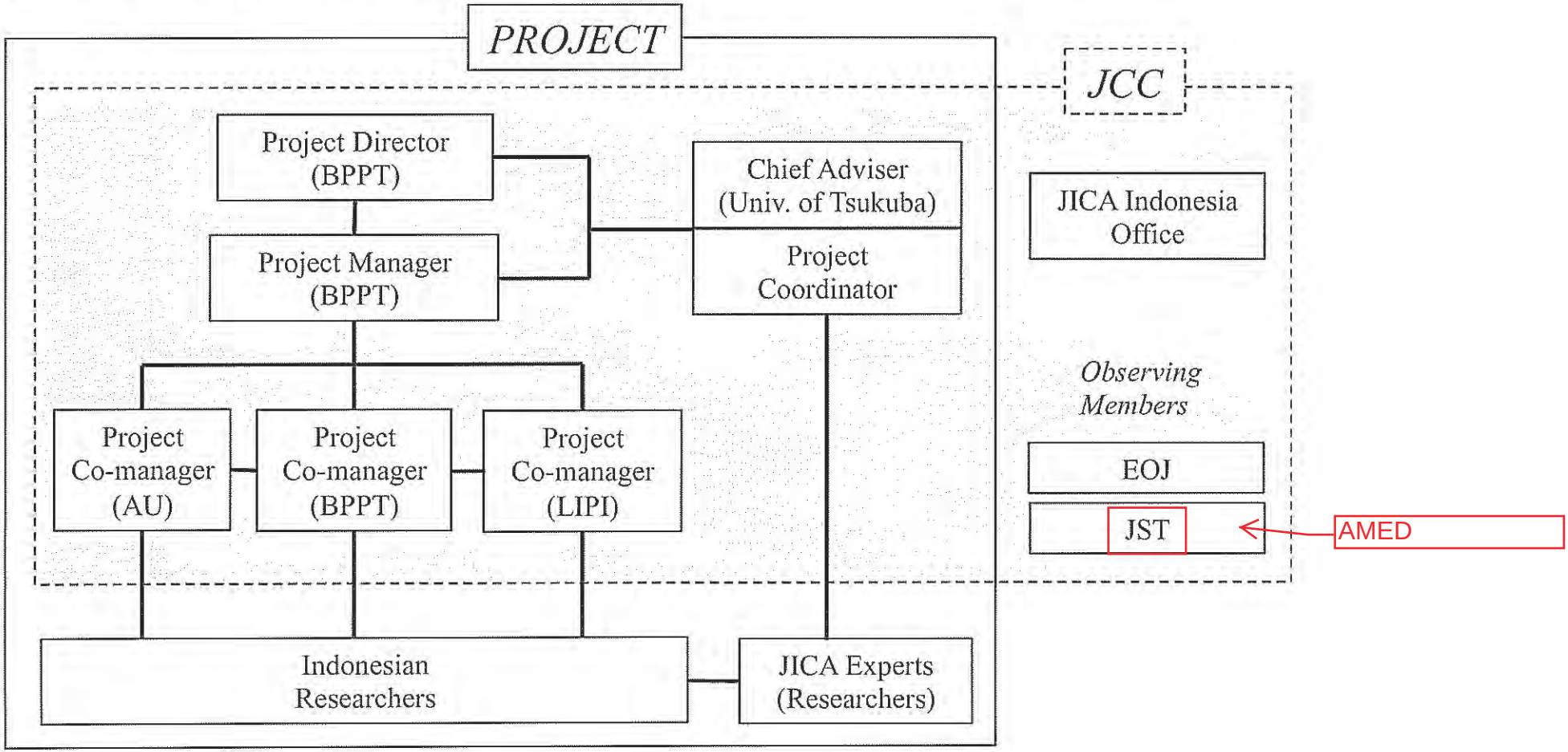
ANNEX III

List of Equipment

Category	Name
Microbial isolation/extract preparation	Freezer -30°C Freezer -30°C 250 ml Flask holder (for large scale shaker incubator)
Microbial storage	Deep freezer -80°C, double compressor
Plant extract	Rotary evaporator/concentrator
Enzyme preparation	UV-vis spectrophotometer Electrophoresis system (for protein)
Enzyme-based screening	96-plate reader
Hit analysis	Analytical HPLC with DAD detector Semi-preparative HPLC (flow rate <20ml/min with UV-vis detector) Photodiode detector for UPLC (waters)
Cell-based screening	Safety cabinet class 2 Autoclave Ultracentrifuge Ultracentrifuge Rotors CO_2/O_2 incubator Incubator Refrigerated centrifuge, table top Centrifuge Rotors, swing and angle Liquid nitrogen tank 30L with canister (box storage) as-one
Scale up production	Mini fermentor (3L (or 5L) x5 jar) Fermentor 30L
Experimental instruments and others	Server and PC Ultrasonic washer Sonicator Fraction collector, UV (for protein purification) Multichannel automatic micropipette 10ml Multichannel automatic micropipette 50ml Multichannel automatic micropipette 200ml Multichannel automatic micropipette 1000ml Micropipette set (2-1000ml) Refrigerator Freezer

ANNEX Ⅳ

Project Implementation Structure



ANNEX V

LIST OF PROPOSED MEMBERS OF JOINT COORDINATING COMMITTEE

1. Functions

The JCC will be held at least once a year and whenever deems it necessary. The functions of JCC are as follows:

(a) To facilitate inter-organizational coordination concerning the Project

(b) To approve an annual work plan of the Project

(c) To review overall progress, conduct monitoring and evaluation of The Project, and

(d) To exchange opinions on major issues that arise during the Project and to take necessary measures.

2. Chairperson: Project Director or person appointed by the Project Director
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

3. Members

(a) The Indonesian side

- Project Manager: ~~Head, the Center for the Assessment of Biotechnology~~; Director,Center for Pharmaceutical and Medical Technologies of BPPT
- Project Co-Managers: ~~Head of Technological Services Division, Biotech Center~~ of BPPT, Director, Institute of Tropical Disease, AU, and Director, Research Center for Biology, LIPI; and
- Other representative(s) from BPPT.

Program Head, Center for Pharmaceutical and Medical Technologies

(b) The Japanese side

- Japanese Chief Advisor;
- JICA Project Coordinator; and
- Representative(s) from the JICA Indonesia Office.

(c) Observers

Stakeholders appointed by the Chairperson such as the Embassy of Japan, AMED and/or other relevant organizations.

ANNEX VI

GOODS / SERVICES

Both sides confirmed that the Project is categorized as "goods / services" stipulated in article 42 (1) c of Government Regulation no. 10/2011. In accordance with regulation of Minister of Finance no. 191/ PMK.05 /2011, BPPT shall submit the handover delivery certificate of goods/services (BAST) to the Ministry of Finance of Indonesia. In order to secure the accuracy of BAST, JICA Indonesia office will provide BPPT with data on semester basis as follows:

1. Goods: name and price (in effective currency) per item of equipment handed over during last six months

2. Services: total expenditure (in effective currency) of last six months for expert, training, and mission

BPPT will make and sign BAST based on the data provided by JICA, and after obtaining JICA's confirmation, submit it to the Ministry of Finance.

APPENDIX 2

MAIN POINTS DISCUSSED

1. Biosafety

   Both sides agree that all laboratory activities should follow the international biosafety regulations.

2. Interectual Property Rights

   Both sides agree that matters related to intellectual property rights should follow the Memorandum of Understanding between University of Tsukuba and BPPT.

MINUTES OF MEETINGS
BETWEEN
THE JAPANESE DETAILED PLANNING SURVEY TEAM
AND
THE AUTHORITIES CONCERNED OF
THE GOVERNMENT OF THE REPUBLIC OF INDONESIA ON JAPANESE TECHNICAL COOPERATION FOR
THE PROJECT FOR UTILIZATION OF INDONESIAN BIORESOURCE FOR ANTI-MALARIAL AND ANTI-AMEBIC DRUG DEVELOPMENT

Japan International Cooperation Agency (hereinafter referred to as "JICA") organized the Detailed Planning Survey Team (hereinafter referred to as "the Team"), headed by Dr. Kaname KANAI, which visited the Republic of Indonesia from 2 October to 10 October, 2014 for the purpose of discussing the framework of the technical cooperation project entitled "Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development" (hereinafter referred to as "the Project").

During their stay in the Republic of Indonesia, the Team had a series of discussions and exchanged views on the Project with the Indonesian authorities.

As a result of the discussions, the Team and the Indonesian authorities concerned agreed on the matters referred to in the document attached hereto.

Jakarta, 10 October 2014

Dr. Kaname KANAI
Team Leader
Detailed Planning Survey Team
Japan International Cooperation Agency
Japan

Dr. Listyani Wijayanti
Deputy Chairperson
Agency for the Assessment and
Application of Technology (BPPT)
Republic of Indonesia

Witnessed by:

Prof. Dr. Kazuro SHIOMI
Professor
Kitasato Institute for Life Sciences,
Kitasato University
Japan

Prof. Dr. Sulaiman Yusuf
Representing Deputy Chairman for Life Sciences
Indonesian Institute of Sciences, LIPI
Republic of Indonesia

Prof. Dr. Soetjipto Vice Rector
Airlangga University
Republic of Indonesia

THE ATTACHED DOCUMENT

I. OBJECTIVES OF THE DETAILED PLANNING SURVEY

The objectives of the survey were to confirm background and contents of the request from the Government of the Republic of Indonesia and to make a cooperation plan (project design) through discussions with the Indonesian authorities concerned. The Team also collected and analyzed necessary information for ex-ante evaluation.

The contents of the survey were as follows:

1. To confirm the contents of the request from the Republic of Indonesia and the research plan of the University of Tsukuba (hereinafter referred to as "UT") and to harmonize the two;
2. To have discussions with the Indonesian authorities concerned on the project design including, Project Design Matrix (hereinafter referred to as "PDM"), a tentative Plan of Operation (hereinafter referred to as "PO"), inputs and implementing structure, and to reach an agreement;
3. To confirm actions and schedule up to the Project's commencement; and
4. To exchange the Minutes of Meetings (hereinafter referred to as "M/M") containing the project design and the draft Record of Discussions (hereinafter referred to as "R/D"), which is to be signed before commencement of the Project as a token of confirmation of result of the discussions.

II. BASIC FRAMEWORK OF THE PROJECT

1. Project Implementation Scheme

Both sides confirmed that the Project should be implemented under the "Science and Technology Research Partnership for Sustainable Development (SATREPS)*" promoted by JICA in collaboration with the ~~Japan Science and Technology Agency~~ Japan Agency for Medical Research and Development (hereinafter referred to as ~~"JST"~~ "AMED").

JICA will take necessary measures for the technical cooperation such as dispatch of experts, provision of equipment and training of personnel, and other supports related to the Project in Indonesia. JST will support UT and other members of the Japanese research team for the project activities implemented in Japan.

The Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT"), as the Indonesian counterpart, will take necessary measures for technical cooperation, such as preparation of research facilities, equipment and materials, personnel, utilities and other support related to the Project.

* "SATREPS" aims to develop new technology and its applications, and also aims at capacity development of researchers and research institutions in both countries.

2. Project Title

It is appropriate to modify the title of the Project from the one indicated in the application entitled "The Project for Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development" to "The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources" so that the agreed contents of the Project are accurately reflected.

Both parties agreed the above change and will propose the title modification to the authorities concerned of each government and, if approved, the title will be changed officially through diplomatic procedure.

3. Term of Cooperation

The duration of the Project will be five (5) years from the date, which will be indicated in the R/D.

4. Implementation Structure of the Project

4-1. Administration

Both sides agreed that the administration of the Project would be organized as shown in Annex I as follows:

There will be:

(1) Project Director (who will bear overall responsibility for the administration and implementation of the Project);
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

(2) Project Manager (who will be responsible for the managerial and technical matters of the Project);
~~Head, the Center for the Assessment of Biotechnology~~ (hereinafter referred to as "PTFM er") of BPPT
Director.Center for Pharmaceutical and Medical Technologies

(3) Project Co-manager (who will be responsible for the managerial and technical matters of the Project in collaboration with the Project Manager);
- Program Head, Pharmaceutical and Medical Technologies of BPPT
- Director, Institute of Tropical Disease, Airlangga University (hereinafter referred to as "AU")
- Director, Research Center for Biology, Indonesian Institute of Sciences (hereinafter referred to as "LIPI")

(4) Indonesian counterpart researchers, as shown in Annex II;

(5) Japanese Chief Advisor (who will provide necessary recommendations and advice to the Project Director and the Project Manager on any matters pertaining to the implementation of the Project): Professor, Graduate School of Life and Environmental Sciences, UT;

(6) JICA Project Coordinator; and

(7) Other JICA Experts (who will give necessary technical guidance and advice to Indonesian counterpart researchers on technical matters pertaining to the implementation of the Project).

4-2. Joint Coordinating Committee

For the effective and successful implementation of technical cooperation for the Project, a Joint Coordinating Committee will be established whose functions and composition are described as follows:

(1) Functions

1) To formulate and authorize the annual activity plan of the Project;
2) To endorse major achievements and products of the Project;
3) To monitor and review overall progress and supervise the Project; and
4) To review and discuss major issues arising from or concerning the Project.

(2) Composition

1) Chairperson: Project Director or person appointed by the Project Director
2) Members
   a. Indonesian side
      - Project Manager
      - Project Co-Managers
      - Other representative(s) from BPPT
   b. Japanese side
      - Japanese Chief Advisor
      - JICA Project Coordinator
      - Representative(s) from the JICA Indonesia Office

AMED

3) Observers
   Stakeholders appointed by the Chairperson such as the Embassy of Japan, JST and/or other relevant organizations

4-3. Scientific Meeting

In order to ensure effective monitoring of the research progress and timely feedback of the technical advice from the experts, researchers and personnel engaged in the Project will have opportunities for exchanging and monitoring research outcomes as well as administrative matters at least once a year. Reports and/or minutes of meetings will be prepared in English and will be shared with the relevant researchers and personnel.

5. Project Design Matrix and Tentative Plan of Operation

The basic framework of the Project is as shown in the PDM in Annex III. The tentative PO is as shown in Annex IV.

6. Inputs

The inputs from each side are as follows:

6-1. Japanese side

(1) Chief Advisor;
(2) Project Coordinator;
(3) Research scientists and staff;
(4) Project local staff, including assistant(s) and driver(s);
(5) Training in Japan for several Indonesian counterpart personnel; and
(6) Necessary equipment for research and development activities, as shown in Annex V.

6-2. Indonesian side

(1) Research scientists and staff;

(2) Office space and laboratory space;

(3) Existing equipment; and

(4) Available data, information and specimens related to the Project.

7. Special Issues

7-1. Memorandum of Understanding between research institutes

Both sides agreed that UT and BPPT should reach an agreement to execute the collaborative research in accordance with the project design immediately after signing R/D. The document (e.g. Memorandum of Understanding) will contain the following items of the collaborative research:

a. Objective and Plan;
b. Implementation;
c. Confidentiality and Intellectual Property Rights;
d. Access to Genetic Resources;
e. Publication of Results;
f. Dispute Resolution;
g. Duration of the Agreement;
h. Compliance with Laws and Regulations; and
i. Other items concerning both sides.

7-2. Intellectual Property Rights

Both sides confirmed that matters related to intellectual property rights should follow the Memorandum of Understanding.

7-3. Research Approvals

Both sides agreed that research approvals from the relevant institutions of Indonesia will be obtained.

7-4. Material Transfer

Both sides agreed that clearance of material transfer from relevant ministry/authority should be obtained. The materials may include pathogens, microorganisms, plants, extracts, substances, etc.

7-5. Compliance of Rules

Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards.

7-6. Biosafety

Both sides agreed that all laboratory activities should follow the international biosafety regulations.

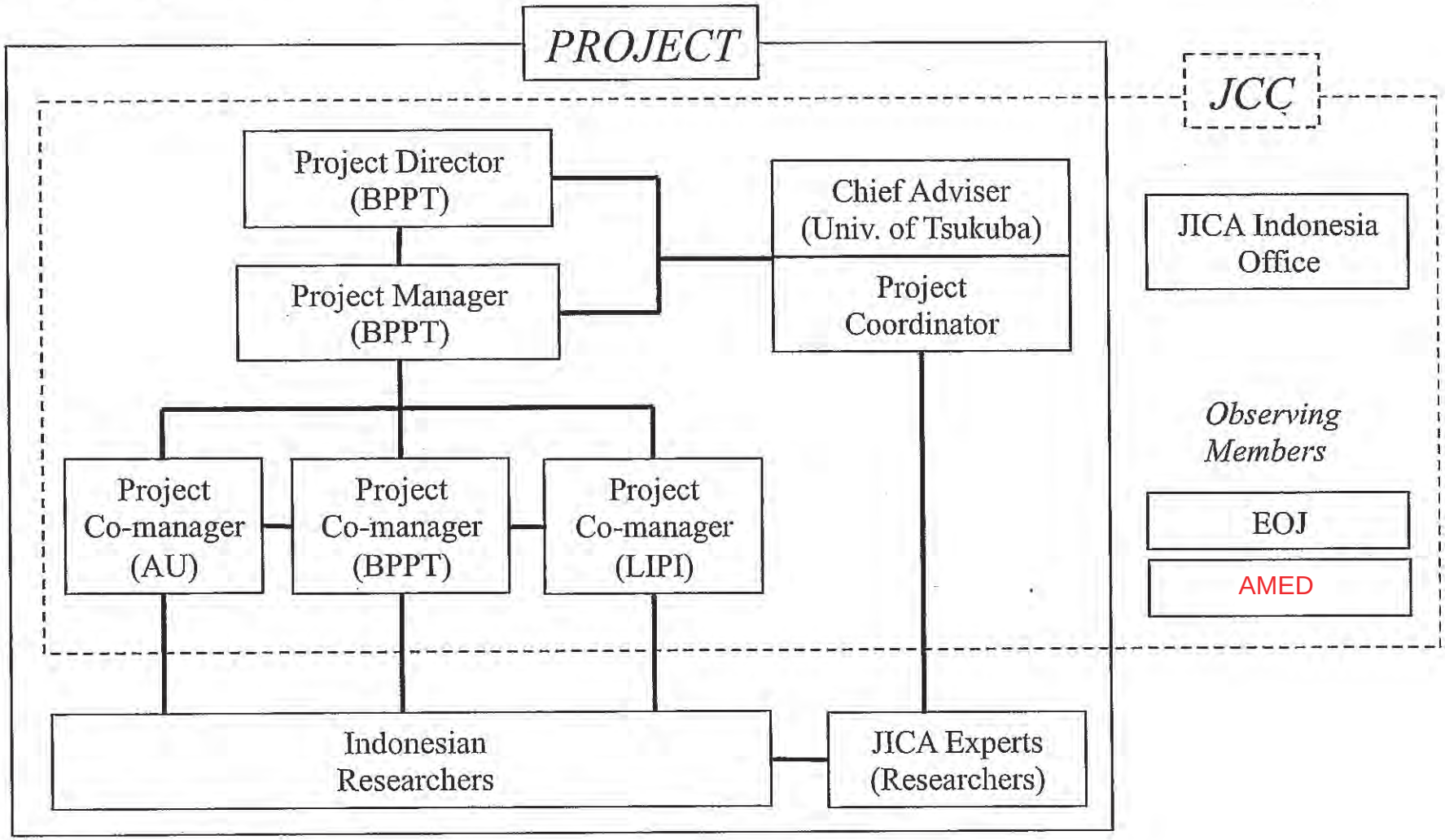
III. WAY FORWARD

1. Based on this M/M and the draft R/D as shown in Annex VI, the Indonesian and the Japanese side will prepare the final version of the R/D.
2. Based on the mutual agreement reached, the R/D should be signed by both sides as soon as possible aiming at the end of January 2015, but no later than the end of February 2015.
3. Memorandum of Understanding between UT and BPPT will be finalized by the end of January 2015.
4. The Project is expected to start in April 2015.
5. The schedule is subject to change in accordance with approval processes of the Project.

LIST OF ANNEXES

Annex I	Project Implementation Structure
Annex II	List of Researchers
Annex III	PDM version 0
Annex IV	Tentative PO Version 0
Annex V	Tentative List of Equipment
Annex VI	Draft R/D

Project Implementation Structure



List of Researchers

Research Subject	The Indonesian Side	The Japanese Side
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).		
1.1 Primary screening for inhibitory activity of extracts to the plasmodium-derived recombinant enzyme	• Erwahyuni E Prabandari (BPPT) • Endah Dwi HartU.Tokyoi (BPPT)	• Daniel Ken Inaoka (U.Tokyo)
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of Plasmodium falciparum	• Astutiati Nurhasanah (BPPT) • Nuralih (BPPT) • Mutia Hardhiyuna (BPPT) • Siska Andrina Kusumastuti (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Keisuke Komatsuya (U. Tokyo)
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1 and 1-2	• Astutiati Nurhasanah (BPPT) • Nuralih (BPPT) • Mutia Hardhiyuna (BPPT) • Siska Andrina Kusumastuti (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Keisuke Komatsuya (U. Tokyo)
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT) • Presetyawan Yunianto (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU)
1.5 Establishment of mass production system of the lead compound candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)
1.6 Determination of chemical structures of the lead compound candidates	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU)

	• Eka Siska (BPPT) • Rudiyono (BPPT) • Presetyawan Yunianto (BPPT)	
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	• Agung Eru Wibowo (BPPT) • Kurnia Agustini (BPPT)	• Daniel Ken Inaoka (U.Tokyo) • Keisuke Komatsuya (U.Tokyo)
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .		
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica*-derived site-specific recombinant enzyme	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	• Achmad Fuad Hafid (AU) • Myrna Adianti (ITD-AU) • Ratna Wahyuni (ITD-AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BTC-BPPT) • Presetyawan Yunianto (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
2.5 Establishment of mass production system of the lead compound candidates	• Diana Dewi (BPPT) • Suyanto (BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)

	• Anna Safarrida (BPPT) • Dyah Noor Hidayati (BPPT)	
2.6 Determination of chemical structures of the lead compound candidates	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT) • Presetyawan Yunianto (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
2.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID)
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT)	• Daniel Ken Inaoka (U. Tokyo) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.		
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	• Achmad Dinoto (LIPI) • Puspita Lisdiyanti (LIPI) • Rifgiyah Nur Umami (LIPI) • Eris Septiana (LIPI) • Muhammad Ilyas (LIPI) • Dyah Noor Hidayati (BPPT)	• AUTko MaUTmoto (KU) • Ken-ichi Nonaka (KU) • Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ) • Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (U. Tokyo)
3.2 Establishment of screening systems	• Erwahyuni E Prabandari (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (U. Tokyo)
3.3 Establishment of culture and evaluation systems	• Astutiati Nurhasanah (BPPT)	• Tomoyoshi Nozaki (UT)

	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU)	• Daniel Ken Inaoka (U. Tokyo)
3.4 Introduction of technologies of isolation and purification	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
3.5 Introduction of technologies of chemical structure elucidation	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Rudiyono (BPPT)	• Kazuro Shiomi (KU) • Miho Mori (KU)
3.6 Establishment and enhancement of a research network in Indonesia	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Ahmad Fuad (AU) • Puspita Lisdyanti (LIPI)	• Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (U. Tokyo) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)

Institution abbreviation:

- BPPT: Agency for the Assessment and Application of Technology
- AU: Institute for Tropical Diseases, Airlangga University
- LIPI: Biotechnology Research Institute, Indonesia Institute of Science
- U.Tokyo: University of Tokyo
- KU: Kitasato University
- MBJ: MicroBiopharm Japan, Co., Ltd.
- UT: University of Tsukuba
- NIID: National Institute of Infectious Diseases of Japan

AMED

Center for Pharmaceutical and Medical Technologies of BPPT (PTFM-BPPT)

Project Design Matrix (PDM) (Version 0)

Date: October 10, 2014
Project Duration: 5 years after the date indicated on the Record of Discussion

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development

Proposed Project Title for amendment by JICA and ~~JST~~: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Implementing Agencies:
【Indonesia】~~Biotech Center of the Agency for Assessment and Application of Technology (BTC~~ BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia
Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievements	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Munities of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			

3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents

Activities	Inputs		Pre-conditions
	Japan	Indonesia	
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).** 1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.). 1-2. To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system. 1-3. In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in ~~BTC-BPPT~~ PTFM-BPPT.

1-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.
1-5.	To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.
1-6.	To determine chemical structures of the lead compound candidates.
1-7.	To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.
1-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.
2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica*-derived recombinant enzymes (SAT, CS, NADK, etc.).
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.
2-6.	To determine chemical structures of the lead compound candidates.
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.

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Issues and Countermeasures

3	**Technologies and research system for drug discover y using biological recourses arc established at the Indonesinn research institutes.**			
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5.	To introducc technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

[Abbreviations] DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

AMED

Tentative PO version 0

Version 0
Dated Oct. 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development
(Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources)

Inputs	Year	1st Year				2nd Year				3rd Year				4th Year				5th Year				6th Year				7th Year				8th Year				9st Year				Remarks	Monitoring: Issue	Monitoring: Solution
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV			
Expert	Plan																																							
Chief Advisor/Tropical Medicine Researches	Actual																																							
Project Coordinator	Plan																																							
	Actual																																							
Researcher(s) with expertise in malaria	Plan																																							
	Actual																																							
Researcher(s) with expertise in amebiasis	Plan																																							
	Actual																																							
Researcher(s) with expertise in isolation and purification of chemical compounds	Plan																																							
	Actual																																							
Researcher(s) with expertise in structure analysis of chemical compounds	Plan																																							
	Actual																																							
Other researcher(s) with necessary expertise for project research activities as necessity arises	Plan																																							
	Actual																																							
Equipment	Plan																																							
Instruments and related equipment for protozoal recombinant enzyme	Actual																																							
Instruments and related equipment for culture of protozoa	Plan																																							
	Actual																																							
Instruments and related equipment for chemical compound isolation	Plan																																							
	Actual																																							
Instruments and related equipment for mass production system of the lead compound	Plan																																							
	Actual																																							
Training in Japan	Plan																																							
Culture techniques of microorganisms and protozoa	Actual																																							
Screening techniques for inhibitory activity	Plan																																							
	Actual																																							
Techniques for Isolation and purification of chemical compounds	Plan																																							
	Actual																																							
Techniques for structure analysis of chemical compounds	Plan																																							
	Actual																																							
Techniques for mass production of chemical compounds	Plan																																							
	Actual																																							
Other training necessary for project research activities as necessity arises	Plan																																							
	Actual																																							
In-country/Third country Training	Plan																																							
	Actual																																							

Activities / Sub-Activities	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Responsible Organization: Japan	Responsible Organization: GOI	Achievements	Issue & Countermeasures
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV				
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).														
1.1 Primary screening for inhibitory activity of extract to the plasmodium-derived recombinant enzyme	Plan										U.Tokyo	BPPT		
	Actual													
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan										U.Tokyo	BPPT		
	Actual													
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1 and 1-2	Plan										U.Tokyo	BPPT		
	Actual													
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan										KU U.Tokyo	BPPT		
	Actual													
1.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBI	BPPT		
	Actual													
1.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	Plan										U.Tokyo	BPPT		
	Actual													
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)														
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan										UT	AU (BPPT)		
	Actual													
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan										UT	AU (BPPT)		
	Actual													
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2	Plan										UT	AU (BPPT)		
	Actual													
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan										KU UT	BPPT (AU)		
	Actual													
2.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBI	BPPT		
	Actual													
2.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan										UT	AU (BPPT)		
	Actual													
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													

Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.						
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	Plan / Actual	KU MBJ	BPPT LIPI			
3.2 Establishment of screening systems	Plan / Actual	UT U.Tokyo	BPPT AU			
3.3 Establishment of culture and evaluation systems	Plan / Actual	UT U.Tokyo	BPPT AU			
3.4 Introduction of technologies of isolation and purification	Plan / Actual	KU	BPPT			
3.5 Introduction of technologies of chemical structure elucidation	Plan / Actual	KU	BPPT			
3.6 Establishment and enhancement of a research network in Indonesia	Plan / Actual	ALL	ALL			
Duration / Phasing	Plan / Actual					

Monitoring Plan	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Issue	Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Monitoring													
Joint Coordinating Committee	Plan	•	•	•	•	•							
	Actual												
Scientific Meeting	Plan	▲	▲	▲	▲	▲	▲						
	Actual												
Set-up the Detailed Plan of Operation	Plan	▲	▲	▲	▲								
	Actual												
Submission of Monitoring Sheet	Plan	■ ■	■ ■	■ ■	■ ■	■ ■	■						
	Actual												
Monitoring Mission from Japan	Plan			■		■							
	Actual												
Post Monitoring	Plan									■			
	Actual												
Reports/Documents													
Project Completion Report	Plan						▲						
	Actual												
Public Relations													
Establishment and Operation of Web Site	Plan	▲											
	Actual												
International symposiums are held for drug discovery	Plan			▲		▲							
	Actual												

Tentative List of Equipment

Category	Name
Microbial isolation/extract preparation	Freezer -30°C
	Freezer -30°C
	250 ml Flask holder (for large scale shaker incubator)
Microbial storage	Deep freezer -80°C, double compressor
Plant extract	Rotary evaporator/concentrator
Enzyme preparation	UV-vis spectrophotometer
	Electrophoresis system (for protein)
Enzyme-based screening	96-plate reader
Hit analysis	Analytical HPLC with DAD detector
	Semi-preparative HPLC (flow rate <20ml/min with UV-vis c
	Photodiode detector for UPLC (waters)
Cell-based screening	Safety cabinet class 2
	Autoclave
	Ultracentrifuge
	Ultracentrifuge Rotors
	CO_2/O_2 incubator
	Incubator
	Refrigerated centrifuge, table top
	Centrifuge Rotors, swing and angle
	Liquid nitrogen tank 30L with canister (box storage) as-one
Scale up production	Mini fermentor (3L (or 5L) x5 jar)
	Fermentor 30L
Experimental instruments and others	Server and PC
	Ultrasonic washer
	Sonicator
	Fraction collector, UV (for protein purification)
	Multichannel automatic micropipette 10ml
	Multichannel automatic micropipette 50ml
	Multichannel automatic micropipette 200ml
	Multichannel automatic micropipette 1000ml
	Micropipette set (2-1000ml)
	Refrigerator
	Freezer

[DRAFT]

RECORD OF DISCUSSIONS
ON
THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES
IN
THE REPUBLIC OF INDONESIA
AGREED UPON BETWEEN
AGENCY FOR THE ASSESSMENT AND APPLICATION OF TECHNOLOGY
AND
JAPAN INTERNATIONAL COOPERATION AGENCY

Jakarta, 〈date〉

Mr. Atsushi Sasaki
Chief Representative
Japan International Cooperation Agency
Indonesia Office

Chairperson
Agency for the Assessment and Application of Technology (BPPT)
The Republic of Indonesia

Based on the minutes of meetings on the detailed planning survey on "the Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources (hereinafter referred to as "the Project") signed on October 10, 2014 between Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT") and the Japan International Cooperation Agency (hereinafter referred to as "JICA"), JICA held a series of discussions with BPPT and relevant organizations to develop a detailed plan of the Project.

Both parties agreed the details of the Project and the main points discussed as described in the Appendix 1 and the Appendix 2 respectively.

Both parties also agreed that BPPT, the counterpart to JICA, will be responsible for the implementation of the Project in cooperation with JICA, coordinate with other relevant organizations and ensure that the self-reliant operation of the Project is sustained during and after the implementation period in order to contribute toward social and economic development of the Republic of Indonesia.

The Project will be implemented within the framework of the Colombo Plan Technical Cooperation Scheme between the Government of Japan (hereinafter referred to as "GOJ") and the Government of the Republic of Indonesia (hereinafter referred to as "GOI").

The effectiveness of the record of discussion is subject to the approval of JICA.

Appendix 1: Project Description
Appendix 2: Main Points Discussed
Appendix 3: Minutes of Meetings on the Detailed Planning Survey
Appendices are integral part of the Record of Discussions

APPENDIX 1

PROJECT DESCRIPTION

Both parties confirmed that there is no change in the project description agreed on in the minutes of meetings on the concerning detailed planning survey on the project signed on October 10, 2014 (appendix 3).

I. BACKGROUND

Indonesia is the second largest biodiversity country in the world. The diversity and uniqueness of bioresources is an important capital for Indonesia to increase wealth and prosperity of the people. GOI declared a strategic directive master plan for Indonesian economic development, through which GOI expects increase its economic competitiveness by transformation from bioresources-based comparative economic activities to innovation-based competitive economic activities. BPPT has been consistently developing innovation for utilization of Indonesian bioresources in order to support industries in Indonesia. One of the prioritized research topics is bio-prospecting for health by utilization of microbial and plant biodiversity. Many achievements have been obtained, however, very little of them had been practically used by industries or people. Thus, capacity building on utilization of bioresource for health, especially drug development, is very urgent.

Malaria and amebiasis are regarded as neglected diseases caused by parasites and a considerable concern in Indonesia which is needed to be overcome. Moreover, resistance of presently available drugs to the parasites is unavoidable, thus drug discovery for anti-malarial and anti-amebic compounds is urgently needed.

This technical cooperation is aimed to strengthen the capacity of Indonesian researchers and institutions in drug discovery against tropical infectious diseases including malaria and amebiasis using Indonesia bioresources.

GOI requested JICA to initiate the Indonesia-Japan collaborative project at BPPT and other associated institutions for conducting research on malaria and amebiasis. The joint research by the Indonesian institutes, BPPT and its collaborating institutes in Indonesia, the Indonesian Institute of Sciences (hereinafter referred to as "LIPI") and the Institute of Tropical Disease, Airlangga University (hereinafter referred to as "AU"), and Japanese institutions, University of Tsukuba, Kitasato University, University of Tokyo, and MicroBiopharm Japan Co. Ltd., aim (i) to strengthen capacity building for Indonesian researchers and institutions, (ii) to reinforce international research collaboration, and (iii) to increase added value of Indonesia bioresources especially for anti-malaria and anti-amebic drug discovery.

II. OUTLINE OF THE PROJECT

Details of the Project are described in the Project Design Matrix (hereinafter referred to as "PDM") (Annex I) and the tentative Plan of Operation (hereinafter referred to as "PO") (Annex II)

1. Project Implementation Structure

The project implementation structure is given in the Annex III. The roles and assignments of relevant organizations are as follows:

(1) BPPT

(a) Project Director will be responsible for overall administration and implementation of the Project. The Project Director will be Deputy Chairperson of Agro-industrial Technology and Biotechnology of BPPT;

(b) Project Manager will be responsible for the managerial and technical matters of the Project. The Project Manager will be Head, Biotechnology Application Center of BPPT; and

(c) Project Co-manager will be responsible for the managerial and technical matters of the Project in collaboration with the Project Manager. The Project Co-managers will be Division Head, Biotechnology Application Center of BPPT.

(2) AU

Director, Institute of Tropical Disease, AU will be Project Co-manager.

(3) LIPI

Director, Research Center for Biotechnology, LIPI will be Project Co-manager.

(4) JICA Experts

The JICA Experts will give necessary technical assistance, advice and recommendations to BPPT on any matters pertaining to the implementation of the Project.

(5) Joint Coordinating Committee

Joint Coordinating Committee (hereinafter referred to as "JCC") will be established in order to facilitate inter-organizational coordination. JCC will be held at least once a year and whenever deems it necessary. JCC will approve an annual work plan, review overall progress, conduct monitoring and evaluation of the Project, and discuss and take necessary measures to major issues that arise during the Project. Outline and a list of proposed members of JCC are shown in the Annex IV.

2. Project Sites and Beneficiaries

(1) Project Sites : Indonesia

(2) Beneficiaries : Indonesian Institutes engaged in the Project

3. Duration

The duration of the Project will be five (5) years starting on April 1, 2015.

4. Reports

Indonesian side and JICA experts will jointly prepare the following reports in English:

(1) Monitoring sheet at every six (6) months until the project completion; and

(2) Project Completion Report at the time of project completion

5. Environmental and Social Considerations

BPPT, AU, LIPI and University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd. agreed to abide by 'JICA Guidelines for Environmental and Social Considerations' in order to ensure that appropriate considerations will be made for the environmental and social impacts of the Project.

III. UNDERTAKINGS OF BPPT

1. BPPT will take necessary measures to:
   (1) ensure that the knowledge and experience acquired by the personnel of the Republic of Indonesia from technical training as well as the equipment provided by JICA will be utilized effectively in the implementation of the Project;
   (2) grant privileges, exemptions and benefits to the JICA experts referred to in ANNEX I and their families, which are no less favorable than those granted to experts of third countries performing similar missions in the Republic of Indonesia under the Colombo Plan Technical Cooperation Scheme;

2. BPPT will take necessary measures to:
   (1) provide security-related information as well as measures to ensure the safety of the JICA experts;
   (2) permit the JICA experts to enter, leave and sojourn in Indonesia for the duration of their assignments therein and exempt them from foreign registration requirements and consular fees;
   (3) exempt the JICA experts from taxes and any other charges on the equipment, machinery and other material necessary for the implementation of the Project; and
   (4) exempt the JICA experts from income tax and charges of any kind imposed on or in connection with any emoluments or allowances paid to them and/or remitted to them from abroad for their services in connection with the implementation of the Project.

3. BPPT, AU and LIPI will bear claims, if any arises, against the JICA experts resulting from, occurring in the course of, or otherwise connected with, the discharge of their duties in the implementation of the Project, except when such claims arise from gross negligence or willful misconduct on the part of the JICA experts.

IV. EVALUATION

JICA and the BPPT will jointly and regularly monitor the progress of the Project through the Monitoring Sheets based on PDM and PO. The Monitoring Sheets shall be reviewed every six (6) months.

Also, Project Completion Report shall be drawn up one (1) month before the

termination of the Project.

V. PROMOTION OF PUBLIC SUPPORT

For the purpose of promoting support for the Project, BPPT will take appropriate measures to make the Project widely known to the people of the Republic of Indonesia.

VI. MISCONDUCT

If JICA receives information related to suspected corrupt or fraudulent practices in the implementation of the Project, BPPT and relevant organizations shall provide JICA with such information as JICA may reasonably request, including information related to any concerned official of the government and/or public organizations of the BPPT and relevant organizations shall not, unfairly or unfavorably treat the person and/or company which provided the information related to suspected corrupt or fraudulent practices in the implementation of the Project.

VII. MUTUAL CONSULTATION

JICA and BPPT will consult each other whenever any major issues arise in the course of project implementation.

VIII. AMENDMENTS

The record of discussions may be amended by the minutes of meetings between BPPT and JICA. The minutes of meetings will be signed by authorized persons of each side who may be different from the signers of the record of discussions.

ANNEX I	PDM version 0 (M/M Annex III)
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ANNEX V	Goods / Services

Note: ANNEX I ,II , and III will be attached when the record of discussions is signed.

ANNEX IV

LIST OF PROPOSED MEMBERS OF JOINT COORDINATING COMMITTEE

1. Functions

The JCC will be held at least once a year and whenever deems it necessary. The functions of JCC are as follows:

(a) To facilitate inter-organizational coordination concerning the Project

(b) To approve an annual work plan of the Project

(c) To review overall progress, conduct monitoring and evaluation of The Project, and

(d) To exchange opinions on major issues that arise during the Project and to take necessary measures.

2. Chairperson: Project Director or person appointed by the Project Director
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

3. Members

(a) The Indonesian side

- Project Manager: Head, the Center for the Assessment of Biotechnology;
- Project Co-Managers: Head of Technological Services Division, Biotech Center of BPPT, Director, Institute of Tropical Disease, AU, and Director, Research Center for Biotechnology, LIPI; and
- Other representative(s) from BPPT.

(b) The Japanese side

- Japanese Chief Advisor;
- JICA Project Coordinator; and
- Representative(s) from the JICA Indonesia Office.

(c) Observers

Stakeholders appointed by the Chairperson such as the Embassy of Japan, JST and/or other relevant organizations.

ANNEX V

GOODS / SERVICES

Both sides confirmed that the Project is categorized as "goods / services" stipulated in article 42 (1) c of Government Regulation no. 10/2011. In accordance with regulation of Minister of Finance no. 191/ PMK.05 /2011, BPPT shall submit the handover delivery certificate of goods/services (BAST) to the Ministry of Finance of Indonesia. In order to secure the accuracy of BAST, JICA Indonesia office will provide BPPT with data on semester basis as follows:

1. Goods: name and price (in effective currency) per item of equipment handed over during last six months

2. Services: total expenditure (in effective currency) of last six months for expert, training, and mission

BPPT will make and sign BAST based on the data provided by JICA, and after obtaining JICA's confirmation, submit it to the Ministry of Finance.

APPENDIX 2

MAIN POINTS DISCUSSED

1. Biosafety

   Both sides agree that all laboratory activities should follow the international biosafety regulations.

2. Interectual Property Rights

   Both sides agree that matters related to intellectual property rights should follow the Memorandum of Understanding between University of Tsukuba and BPPT.

Amendments on 25th January 2017
with the one on 2nd February 2016

RECORD OF DISCUSSIONS
ON
THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES
IN
THE REPUBLIC OF INDONESIA
AGREED UPON BETWEEN
AGENCY FOR THE ASSESSMENT AND APPLICATION OF TECHNOLOGY
AND
JAPAN INTERNATIONAL COOPERATION AGENCY

Jakarta, 17 February 2015

Mr. Atsushi Sasaki
Chief Representative
Japan International Cooperation Agency
Indonesia Office

Dr.Ir. Unggul Priyanto, MSc.
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Program Head,

(2) AU

Director, Institute of Tropical Disease, AU will be Project Co-manager.

(3) LIPI

~~Director, Research Center for Biotechnology~~, LIPI will be Project Co-manager.

Head, Indonesian Culture Collection, Research Center for Biology,

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   (4) exempt the JICA experts from income tax and charges of any kind imposed on or in connection with any emoluments or allowances paid to them and/or remitted to them from abroad for their services in connection with the implementation of the Project.
   (5) meet taxes and any other charges on the equipment, machinery and other material, referred to in ANNEX III, necessary for the implementation of the Project.

3. BPPT, AU and LIPI will bear claims, if any arises, against the JICA experts resulting from, occurring in the course of, or otherwise connected with, the discharge of their duties in the implementation of the Project, except when such claims arise from gross negligence or willful misconduct on the part of the JICA experts.

IV. MONITORING AND EVALUATION

JICA and the BPPT will jointly and regularly monitor the progress of the Project through the Monitoring Sheets based on PDM and PO. The Monitoring Sheets shall be reviewed every six (6) months.

Also, Project Completion Report shall be drawn up one (1) month before the termination of the Project.

JICA will conduct the following evaluations and surveys to mainly verify sustainability and impact of the Project and draw lessons. The BPPT is required to provide necessary support for them.

1. Ex-post evaluation three (3) years after the project completion, in principle; and

2. Follow-up surveys on necessity basis

V. PROMOTION OF PUBLIC SUPPORT

For the purpose of promoting support for the Project, BPPT will take appropriate measures to make the Project widely known to the people of the Republic of Indonesia.

VI. MISCONDUCT

If JICA receives information related to suspected corrupt or fraudulent practices in the implementation of the Project, BPPT and relevant organizations shall provide JICA with such information as JICA may reasonably request, including information related to any concerned official of the government and/or public organizations of the GOI.
BPPT and relevant organizations shall not, unfairly or unfavorably treat the person and/or company which provided the information related to suspected corrupt or fraudulent practices in the implementation of the Project.

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ANNEX VI	Goods / Services

Annex I

AMED

Project Design Matrix (PDM) (Version 0)

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development

Proposed Project Title for amendment by JICA and ~~JST~~: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Date: October 10, 2014
Project Duration: 5 years after the date indicated on the Record of

Implementing Agencies:
【Indonesia】Biotech Center of the Agency for Assessment and Application of Technology (BTC-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia
Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievements	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Minities of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			
3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10,000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents			

Activities	Inputs		Pre-conditions
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	Japan	Indonesia	1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project.
1-1 To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.
1-2 To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-3 In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-4 To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.			
1-5 To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			↓
1-6 To determine chemical structures of the lead compound candidates.			Issues and Countermeasures
1-7 To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.			
1-8 To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			

2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**			
2-1	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica* -derived recombinant enzymes (SAT, CS, NADK, etc).			
2-2	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-3	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.			
2-4	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.			
2-5	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.			
2-6	To determine chemical structures of the lead compound candidates.			
2-7	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.			
2-8	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.			
3	**Technologies and research system for drug discover y using biological recourses are established at the Indonesian research institutes.**			
3-1	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5	To introduce technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

[Abbreviations]DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

AMED

Tentative PO version 0

Version 0
Dated Oct. 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development
(Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources)

Inputs	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Monitoring: Issue	Monitoring: Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Expert													
Chief Advisor/Tropical Medicine Researches	Plan												
	Actual												
Project Coordinator	Plan												
	Actual												
Researcher(s) with expertise in malaria	Plan												
	Actual												
Researcher(s) with expertise in amebiasis	Plan												
	Actual												
Researcher(s) with expertise in isolation and purification of chemical compounds	Plan												
	Actual												
Researcher(s) with expertise in structure analysis of chemical compounds	Plan												
	Actual												
Other researcher(s) with necessary expertise for project research activities as necessity arises	Plan												
	Actual												
Equipment													
Instruments and related equipment for protozoal recombinant enzyme	Plan												
	Actual												
Instruments and related equipment for culture of protozoa	Plan												
	Actual												
Instruments and related equipment for chemical compound isolation	Plan												
	Actual												
Instruments and related equipment for mass production system of the lead compound	Plan												
	Actual												
Training in Japan													
Culture techniques of microorganisms and protozoa	Plan												
	Actual												
Screening techniques for inhibitory activity	Plan												
	Actual												
Techniques for Isolation and purification of chemical compounds	Plan												
	Actual												
Techniques for structure analysis of chemical compounds	Plan												
	Actual												
Techniques for mass production of chemical compounds	Plan												
	Actual												
Other training necessary for project research activities as necessity arises	Plan												
	Actual												
In-country/Third country Training	Plan												
	Actual												

Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.						
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	Plan / Actual	KU MBJ	BPPT LIPI			
3.2 Establishment of screening systems	Plan / Actual	UT U Tokyo	BPPT AU			
3.3 Establishment of culture and evaluation systems	Plan / Actual	UT U Tokyo	BPPT AU			
3.4 Introduction of technologies of isolation and purification	Plan / Actual	KU	BPPT			
3.5 Introduction of technologies of chemical structure elucidation	Plan / Actual	KU	BPPT			
3.6 Establishment and enhancement of a research network in Indonesia	Plan / Actual	ALL	ALL			
Duration / Phasing	Plan / Actual					

Monitoring Plan	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Issue	Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Monitoring													
Joint Coordinating Committee	Plan	III ●	III ●	III ●	III ●	I ●							
	Actual												
Scientific Meeting	Plan	III ▲	III ▲	III ▲	III ▲	I ▲	I ▲						
	Actual												
Set-up the Detailed Plan of Operation	Plan	III ▲	III ▲	III ▲	III ▲								
	Actual												
Submission of Monitoring Sheet	Plan	II ■, IV ■	II ■, IV ■	II ■, IV ■	II ■, IV ■	II ■, IV ■							
	Actual												
Monitoring Mission from Japan	Plan			IV ■		II ■							
	Actual												
Post Monitoring	Plan								IV ■				
	Actual												
Reports/Documents													
Project Completion Report	Plan					IV ▲							
	Actual												
Public Relations													
Establishment and Operation of Web Site	Plan	IV ▲											
	Actual												
International symposiums are held for drug discovery	Plan			I ▲		I ▲							
	Actual												

Activities / Sub-Activities	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Responsible Organization: Japan	Responsible Organization: GOI	Achievements	Issue & Countermeasures
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV				
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).														
1.1 Primary screening for inhibitory activity of extract to the plasmodium-derived recombinant enzyme	Plan										U Tokyo	BPPT		
	Actual													
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan										U Tokyo	BPPT		
	Actual													
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum* , in parallel with Activity 1-1 and 1-2	Plan										U Tokyo	BPPT		
	Actual													
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan										KU U Tokyo	BPPT		
	Actual													
1.5 Establishment of mass production system of the lead compound candidates	Plan										KU MRI	BPPT		
	Actual													
1.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	Plan										U Tokyo	BPPT		
	Actual													
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)														
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan										UI	AU (BPPT)		
	Actual													
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan										UI	AU (BPPT)		
	Actual													
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica* , in parallel with Activity 2-1 and 2-2	Plan										UI	AU (BPPT)		
	Actual													
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan										KU UI	BPPT (AU)		
	Actual													
2.5 Establishment of mass production system of the lead compound candidates	Plan										KU MRI	BPPT		
	Actual													
2.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan										UI	AU (BPPT)		
	Actual													
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													

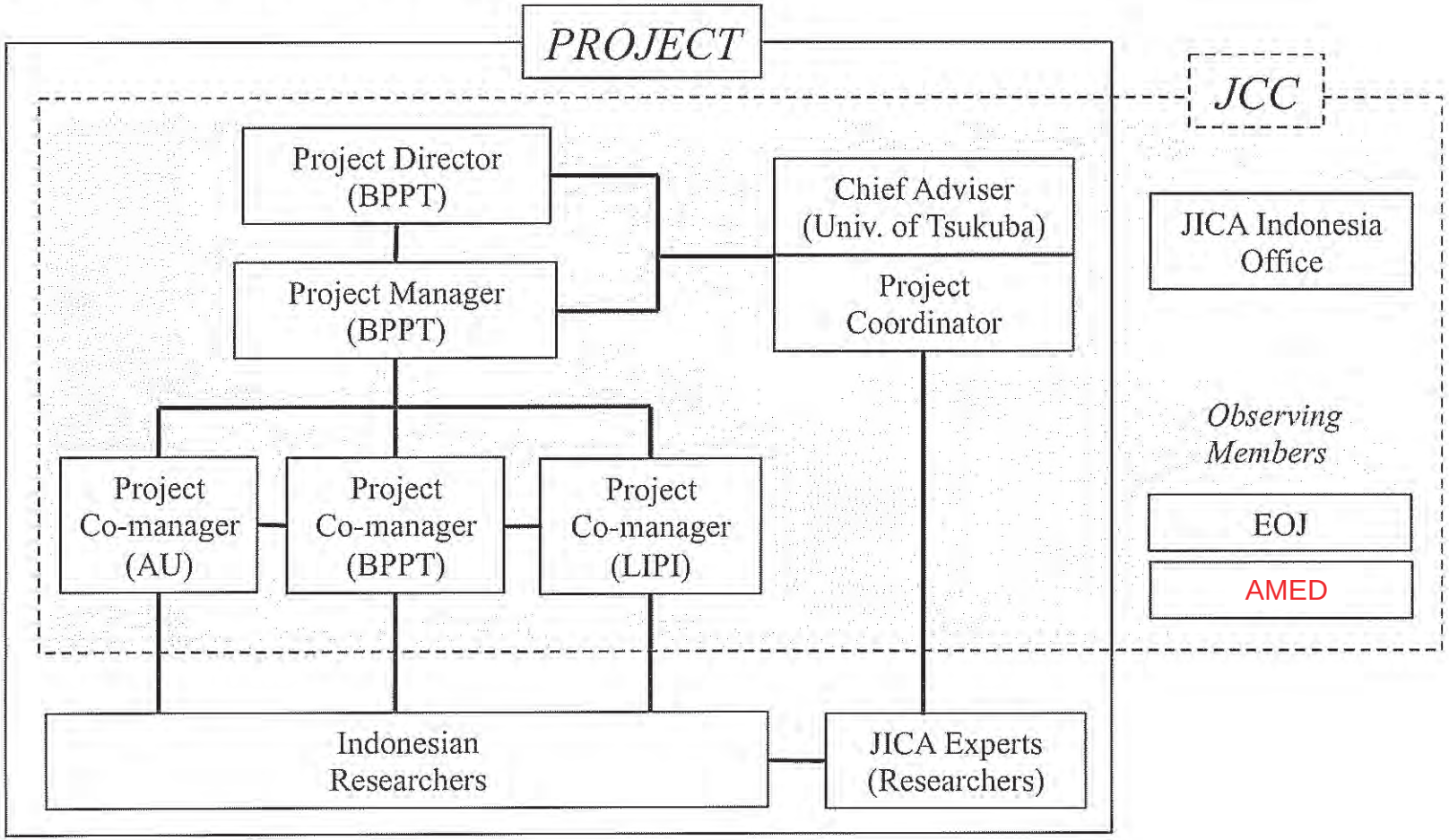
ANNEX III

List of Equipment

Category	Name
Microbial isolation/extract preparation	Freezer -30°C Freezer -30°C 250 ml Flask holder (for large scale shaker incubator)
Microbial storage	Deep freezer -80°C, double compressor
Plant extract	Rotary evaporator/concentrator
Enzyme preparation	UV-vis spectrophotometer Electrophoresis system (for protein)
Enzyme-based screening	96-plate reader
Hit analysis	Analytical HPLC with DAD detector Semi-preparative HPLC (flow rate <20ml/min with UV-vis detector) Photodiode detector for UPLC (waters)
Cell-based screening	Safety cabinet class 2 Autoclave Ultracentrifuge Ultracentrifuge Rotors CO_2/O_2 incubator Incubator Refrigerated centrifuge, table top Centrifuge Rotors, swing and angle Liquid nitrogen tank 30L with canister (box storage) as-one
Scale up production	Mini fermentor (3L (or 5L) x5 jar) Fermentor 30L
Experimental instruments and others	Server and PC Ultrasonic washer Sonicator Fraction collector, UV (for protein purification) Multichannel automatic micropipette 10ml Multichannel automatic micropipette 50ml Multichannel automatic micropipette 200ml Multichannel automatic micropipette 1000ml Micropipette set (2-1000ml) Refrigerator Freezer

ANNEX Ⅳ

Project Implementation Structure



ANNEX V

LIST OF PROPOSED MEMBERS OF JOINT COORDINATING COMMITTEE

1. Functions

The JCC will be held at least once a year and whenever deems it necessary. The functions of JCC are as follows:

(a) To facilitate inter-organizational coordination concerning the Project

(b) To approve an annual work plan of the Project

(c) To review overall progress, conduct monitoring and evaluation of The Project, and

(d) To exchange opinions on major issues that arise during the Project and to take necessary measures.

2. Chairperson: Project Director or person appointed by the Project Director
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

3. Members

(a) The Indonesian side

- Project Manager: Head, the Center for the Assessment of Biotechnology;
- Project Co-Managers: ~~Head of Technological Services Division~~, Biotech Center of BPPT, Director, Institute of Tropical Disease, AU, and ~~Director, Research Center for Biotechnology~~, LIPI; and
- Other representative(s) from BPPT.

Program Head,

Head, Indonesian Culture Collection (InaCC), Research Center for Biology

(b) The Japanese side

- Japanese Chief Advisor;
- JICA Project Coordinator; and
- Representative(s) from the JICA Indonesia Office.

(c) Observers

Stakeholders appointed by the Chairperson such as the Embassy of Japan, AMED and/or other relevant organizations.

ANNEX VI

GOODS / SERVICES

Both sides confirmed that the Project is categorized as "goods / services" stipulated in article 42 (1) c of Government Regulation no. 10/2011. In accordance with regulation of Minister of Finance no. 191/ PMK.05 /2011, BPPT shall submit the handover delivery certificate of goods/services (BAST) to the Ministry of Finance of Indonesia. In order to secure the accuracy of BAST, JICA Indonesia office will provide BPPT with data on semester basis as follows:

1. Goods: name and price (in effective currency) per item of equipment handed over during last six months

2. Services: total expenditure (in effective currency) of last six months for expert, training, and mission

BPPT will make and sign BAST based on the data provided by JICA, and after obtaining JICA's confirmation, submit it to the Ministry of Finance.

APPENDIX 2

MAIN POINTS DISCUSSED

1. Biosafety

   Both sides agree that all laboratory activities should follow the international biosafety regulations.

2. Interectual Property Rights

   Both sides agree that matters related to intellectual property rights should follow the Memorandum of Understanding between University of Tsukuba and BPPT.

MINUTES OF MEETINGS
BETWEEN
THE JAPANESE DETAILED PLANNING SURVEY TEAM
AND
THE AUTHORITIES CONCERNED OF
THE GOVERNMENT OF THE REPUBLIC OF INDONESIA ON JAPANESE TECHNICAL COOPERATION FOR
THE PROJECT FOR UTILIZATION OF INDONESIAN BIORESOURCE FOR ANTI-MALARIAL AND ANTI-AMEBIC DRUG DEVELOPMENT

Japan International Cooperation Agency (hereinafter referred to as "JICA") organized the Detailed Planning Survey Team (hereinafter referred to as "the Team"), headed by Dr. Kaname KANAI, which visited the Republic of Indonesia from 2 October to 10 October, 2014 for the purpose of discussing the framework of the technical cooperation project entitled "Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development" (hereinafter referred to as "the Project").

During their stay in the Republic of Indonesia, the Team had a series of discussions and exchanged views on the Project with the Indonesian authorities.

As a result of the discussions, the Team and the Indonesian authorities concerned agreed on the matters referred to in the document attached hereto.

Jakarta, 10 October 2014

Dr. Kaname KANAI
Team Leader
Detailed Planning Survey Team
Japan International Cooperation Agency
Japan

Dr. Listyani Wijayanti
Deputy Chairperson
Agency for the Assessment and
Application of Technology (BPPT)
Republic of Indonesia

Witnessed by:

Prof. Dr. Kazuro SHIOMI
Professor
Kitasato Institute for Life Sciences,
Kitasato University
Japan

Prof. Dr. Sulaiman Yusuf
Representing Deputy Chairman for Life Sciences
Indonesian Institute of Sciences, LIPI
Republic of Indonesia

Prof. Dr. Soetjipto Vice Rector
Airlangga University
Republic of Indonesia

THE ATTACHED DOCUMENT

I. OBJECTIVES OF THE DETAILED PLANNING SURVEY

The objectives of the survey were to confirm background and contents of the request from the Government of the Republic of Indonesia and to make a cooperation plan (project design) through discussions with the Indonesian authorities concerned. The Team also collected and analyzed necessary information for ex-ante evaluation.

The contents of the survey were as follows:

1. To confirm the contents of the request from the Republic of Indonesia and the research plan of the University of Tsukuba (hereinafter referred to as "UT") and to harmonize the two;
2. To have discussions with the Indonesian authorities concerned on the project design including, Project Design Matrix (hereinafter referred to as "PDM"), a tentative Plan of Operation (hereinafter referred to as "PO"), inputs and implementing structure, and to reach an agreement;
3. To confirm actions and schedule up to the Project's commencement; and
4. To exchange the Minutes of Meetings (hereinafter referred to as "M/M") containing the project design and the draft Record of Discussions (hereinafter referred to as "R/D"), which is to be signed before commencement of the Project as a token of confirmation of result of the discussions.

II. BASIC FRAMEWORK OF THE PROJECT

1. Project Implementation Scheme

Both sides confirmed that the Project should be implemented under the "Science and Technology Research Partnership for Sustainable Development (SATREPS)*" promoted by JICA in collaboration with the ~~Japan Science and Technology Agency~~ (hereinafter referred to as ~~"JST"~~).

Japan Agency for Medical Research and Development "AMED"

JICA will take necessary measures for the technical cooperation such as dispatch of experts, provision of equipment and training of personnel, and other supports related to the Project in Indonesia. JST will support UT and other members of the Japanese research team for the project activities implemented in Japan.

The Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT"), as the Indonesian counterpart, will take necessary measures for technical cooperation, such as preparation of research facilities, equipment and materials, personnel, utilities and other support related to the Project.

* "SATREPS" aims to develop new technology and its applications, and also aims at capacity development of researchers and research institutions in both countries.

2. Project Title

It is appropriate to modify the title of the Project from the one indicated in the application entitled "The Project for Utilization of Indonesian Bioresource for Anti-malarial and Anti-amebic Drug Development" to "The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources" so that the agreed contents of the Project are accurately reflected.

Both parties agreed the above change and will propose the title modification to the authorities concerned of each government and, if approved, the title will be changed officially through diplomatic procedure.

3. Term of Cooperation

The duration of the Project will be five (5) years from the date, which will be indicated in the R/D.

4. Implementation Structure of the Project

4-1. Administration

Both sides agreed that the administration of the Project would be organized as shown in Annex I as follows:

There will be:

(1) Project Director (who will bear overall responsibility for the administration and implementation of the Project);
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

(2) Project Manager (who will be responsible for the managerial and technical matters of the Project);
Head, the Center for the Assessment of Biotechnology (hereinafter referred to as "Biotech Center") of BPPT

(3) Project Co-manager (who will be responsible for the managerial and technical matters of the Project in collaboration with the Project Manager);
- Program Head, Biotech Center of BPPT
- Director, Institute of Tropical Disease, Airlangga University (hereinafter referred to as "AU")
- ~~Director, Research Center for Biotechnology~~, Indonesian Institute of Sciences (hereinafter referred to as "LIPI")

Head, Indonesian Culture Collection, Research Center for Biology,

(4) Indonesian counterpart researchers, as shown in Annex II;

(5) Japanese Chief Advisor (who will provide necessary recommendations and advice to the Project Director and the Project Manager on any matters pertaining to the implementation of the Project): Professor, Graduate School of Life and Environmental Sciences, UT;

(6) JICA Project Coordinator; and

(7) Other JICA Experts (who will give necessary technical guidance and advice to Indonesian counterpart researchers on technical matters pertaining to the implementation of the Project).

4-2. Joint Coordinating Committee

For the effective and successful implementation of technical cooperation for the Project, a Joint Coordinating Committee will be established whose functions and composition are described as follows:

(1) Functions

1) To formulate and authorize the annual activity plan of the Project;
2) To endorse major achievements and products of the Project;
3) To monitor and review overall progress and supervise the Project; and
4) To review and discuss major issues arising from or concerning the Project.

(2) Composition

1) Chairperson: Project Director or person appointed by the Project Director
2) Members
   a. Indonesian side
      - Project Manager
      - Project Co-Managers
      - Other representative(s) from BPPT
   b. Japanese side
      - Japanese Chief Advisor
      - JICA Project Coordinator
      - Representative(s) from the JICA Indonesia Office
3) Observers
   Stakeholders appointed by the Chairperson such as the Embassy of Japan, ~~JST~~ AMED and/or other relevant organizations

4-3. Scientific Meeting

In order to ensure effective monitoring of the research progress and timely feedback of the technical advice from the experts, researchers and personnel engaged in the Project will have opportunities for exchanging and monitoring research outcomes as well as administrative matters at least once a year. Reports and/or minutes of meetings will be prepared in English and will be shared with the relevant researchers and personnel.

5. Project Design Matrix and Tentative Plan of Operation

The basic framework of the Project is as shown in the PDM in Annex III. The tentative PO is as shown in Annex IV.

6. Inputs

The inputs from each side are as follows:

6-1. Japanese side

(1) Chief Advisor;
(2) Project Coordinator;
(3) Research scientists and staff;
(4) Project local staff, including assistant(s) and driver(s);
(5) Training in Japan for several Indonesian counterpart personnel; and
(6) Necessary equipment for research and development activities, as shown in Annex V.

6-2. Indonesian side

(1) Research scientists and staff;

(2) Office space and laboratory space;

(3) Existing equipment; and

(4) Available data, information and specimens related to the Project.

7. Special Issues

7-1. Memorandum of Understanding between research institutes

Both sides agreed that UT and BPPT should reach an agreement to execute the collaborative research in accordance with the project design immediately after signing R/D. The document (e.g. Memorandum of Understanding) will contain the following items of the collaborative research:

a. Objective and Plan;
b. Implementation;
c. Confidentiality and Intellectual Property Rights;
d. Access to Genetic Resources;
e. Publication of Results;
f. Dispute Resolution;
g. Duration of the Agreement;
h. Compliance with Laws and Regulations; and
i. Other items concerning both sides.

7-2. Intellectual Property Rights

Both sides confirmed that matters related to intellectual property rights should follow the Memorandum of Understanding.

7-3. Research Approvals

Both sides agreed that research approvals from the relevant institutions of Indonesia will be obtained.

7-4. Material Transfer

Both sides agreed that clearance of material transfer from relevant ministry/authority should be obtained. The materials may include pathogens, microorganisms, plants, extracts, substances, etc.

7-5. Compliance of Rules

Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards.

7-6. Biosafety

Both sides agreed that all laboratory activities should follow the international biosafety regulations.

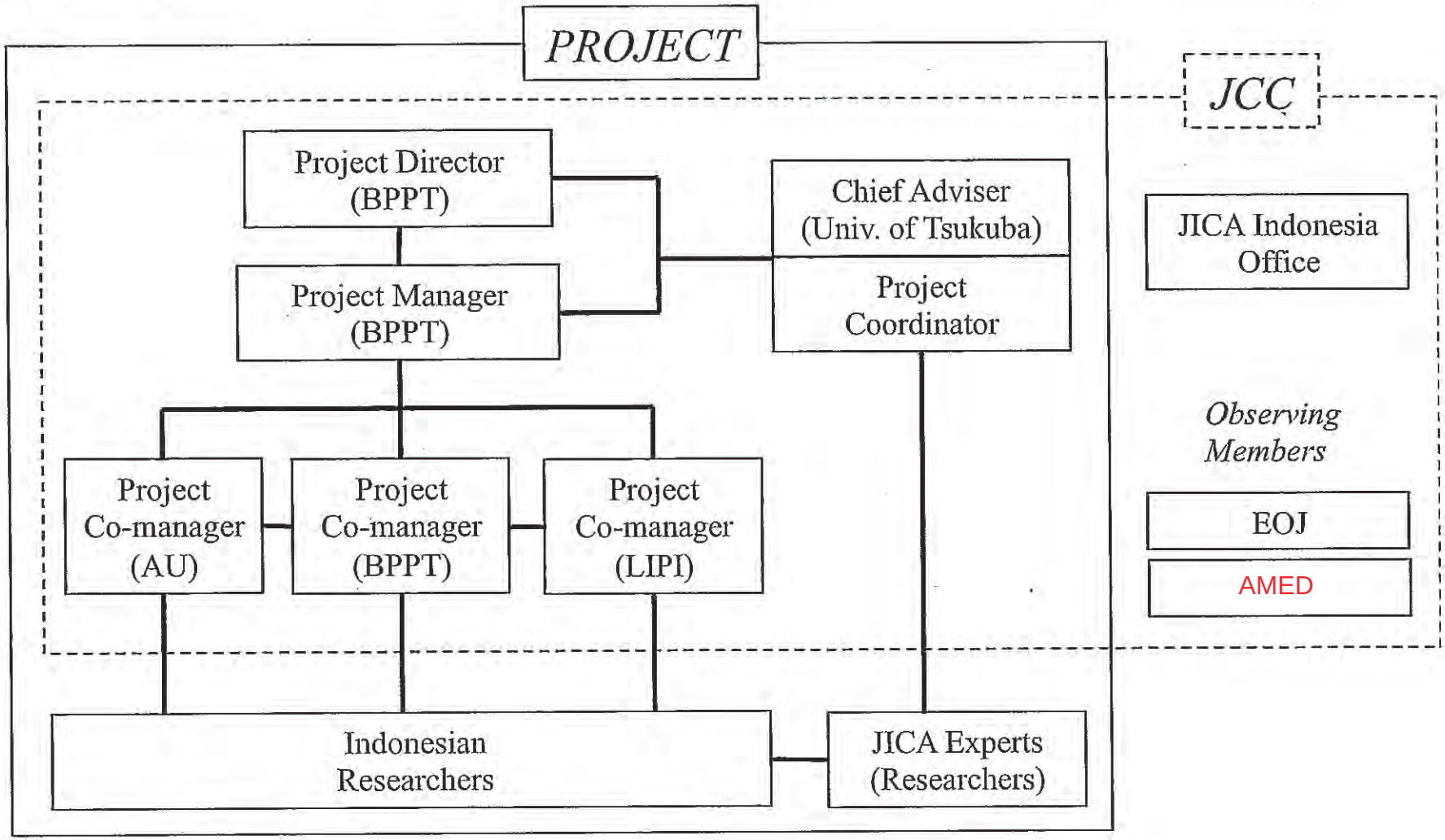
III. WAY FORWARD

1. Based on this M/M and the draft R/D as shown in Annex VI, the Indonesian and the Japanese side will prepare the final version of the R/D.
2. Based on the mutual agreement reached, the R/D should be signed by both sides as soon as possible aiming at the end of January 2015, but no later than the end of February 2015.
3. Memorandum of Understanding between UT and BPPT will be finalized by the end of January 2015.
4. The Project is expected to start in April 2015.
5. The schedule is subject to change in accordance with approval processes of the Project.

LIST OF ANNEXES

Annex I	Project Implementation Structure
Annex II	List of Researchers
Annex III	PDM version 0
Annex IV	Tentative PO Version 0
Annex V	Tentative List of Equipment
Annex VI	Draft R/D

Project Implementation Structure



List of Researchers (As of January 2017)

Reaserch Subject	The Indonesian Side	The Japanese Side
Output 1: Compounds with anti-malarial activity are identified from the extracts on Indonesian biological resources (microorganism, plants, etc.		
1.1. Primary screening for inhibitory activity of extracts to the plasmodium-derived recombinant enzyme	• Erwahyuni E. Prabandari (BPPT) • Endah Dwi Hartuti (BPPT) • Tiara Zovi Putri (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Wan Xinying (U. Tokyo) • Kota Mochizuki (Nagasaki Univ)
1.2. Secondary screening for selective inhibitory activity of the extracts to the proliferation of Plasmodium falciparum	• Danang Waluyo (BPPT) • Dian Japany Puspitasari (BPPT) • Nadia Adipratiwi (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Keisuke Komatsuya (U. Tokyo) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
1.3. Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1- and 1-2	• Danang Waluyo (BPPT) • Dian Japany Puspitasari (BPPT) • Nadia Adipratiwi (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Keusuke Komatsuya (U. Tokyo) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
1.4. Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	• Anis H. Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
1.5. Establishment of mass production system of the lead compounds candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BPPT) • Kristiningrum(BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)

1.6. Determination of chemical structures of the lead compound candidate	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
1.7. Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	• Agung Eru Wibowo (BPPT) • Kurnia Agustini (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Keisuke Komatsuya (U. Tokyo)
1.8. Discussion of future direction of derivatization on the basis of the structure biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT) • Agung Eru Wibowo (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological resources (microorganism, plants, etc)		
2.1. Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica-derived* site-specific recombinant enzyme	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU) •	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)
2.2. Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)

2.3. Screening for selective inhibitory activity of extracts to the extracts of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)
2.4. Isolation and purification of chemical compounds with inhibitory to the proliferation against *Entamoeba histolytica*	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
2.5. Establishment of mass production system of the lead compound candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BBPT) • Kristiningrum(BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)
2.6. Determination of chemical structures of the lead compound candidates	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
2.7. Selection of lead compound(s) through in vitro assessment and subsequent animal testing	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari(AU) • Hikatul Ilmi(AU) • Lidya Tumewu(AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID) •

2.8. Discussion on future direction of derivatization on the basis of the structure biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT) • Agung Eru Wibowo (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 3: Technologies and research system for drug discovery using biological resources are established at the Indonesian research institute		
3.1. Sample collection and additional registration of newly-obtained extracts to the biological resources library	• Puspita Lisdiyanti (LIPI) • Atit Kanti, (LIPI) • Muhammad Ilyas (LIPI) • Ade Lia Putri(LIPI) • Dyah Noor Hidayati (BPPT) • Suryani (BPPT) • Kristiningrum(BPPT)	• Atsuko Matsumoto (KU) • Ken-ichi Nonaka (KU) • Azuma Watanabe (MBJ) • Noriako Sakata (MBJ) • Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (Nagasaki Univ)
3.2. Establishment of screening systems	• Erwahyuni E. Prabandari (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU) • Dwi Peni Kartikasari(AU)	• Tomoyoshi Nozaki (UT) • Daniel Ken Ianoka (Nagasaki Univ) • Wan Xinying (U. Tokyo) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
3.3. Establishment of culture and evaluation system	• Danang Waluyo (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (Nagasaki Univ) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)

3.4. Introduction of technologies of isolation and purification	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
3.5. Introduction of technologies of chemical structure elucidation	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
3.6. Establishment and enhancement of a research network in Indonesia	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Agung Eru Wibowo (BPPT) • Ahmad Fuad Hafid (AU) • Puspita Lisdyanti (LIPI)	• Tomoyoshi Nozaki (UT) • Daniel Ken Ianoka (Nagasaki Univ) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)

Institution Abbreviation:

- BPPT: Agency for the Assessment and Application Technology
- AU: Institute for Tropical Disease, Airlangga University
- LIPI: Biotechnology Research Institute, Indonesia Institute of Science
- U. Tokyo: University of Tokyo
- KU: Kitasato University
- MBJ: MicroBiopharm Japan, Co., Ltd.
- UT: University of Tsukuba
- NIID: National Institute of Infectious Diseases of Japan

AMED

Project Design Matrix (PDM) (Version 0)

Date: October 10, 2014
Project Duration: 5 years after the date indicated on the Record of Discussion

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development

Proposed Project Title for amendment by JICA and ~~JST~~: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Implementing Agencies:
【Indonesia】Biotech Center of the Agency for Assessment and Application of Technology (~~BTC~~-BPPT), Airlangga University (AU), Indonesian Institute of Sciences (LIPI)
【Japan】University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd.

Target Area: the Republic of Indonesia
Project Implementers: Approximately 29 researchers engaged in the Project (21 from BPPT, 3 from AU and 5 from LIPI)
Indirect Beneficiaries: Residents in Indonesia (approx. 250 million)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievements	Remarks
Project Purpose					
Research capacity of the Indonesian research institutes for the development of anti-malarial and anti-amebic agents utilizing biological diversity is enhanced through collaborative research activities with Japanese research institutes.	1. At least one (1) lead compound with anti-malarial activity are determined on the basis of animal experiments for efficacy. 2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy. 3. More than 2 research papers, in which first author is an Indonesian researcher (or comparable responsibility with first author), are published in peer-reviewed journals from Indonesian research institutes.	(1) Experts' project reports (2) Research papers published in scientific journals (3) Munities of the Joint Coordinating Committee (JCC) (4) Handouts and minutes of the Scientific Meetings (5) Other project documents			
Outputs					
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review. 1-2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation. 1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-malarial activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents	1. The Indonesian side properly allocates necessary budget and distribute personnel for the project activities. 2. Trained counterparts do not leave their position so as to affect the outputs of the Project. 3. Necessary cooperation is gained by relevant agencies for the project activities.		1. Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards. 2. JICA experts (researchers) should obtain the foreign research permission from RISTEK in advance of conducting research activities in Indonesia
2 **Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review. 2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation. 2-3. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Other project documents			

3 **Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.**	3-1. More than 10.000 newly-obtained and existing microorganisms, plants and extracts are registered with the biological resource libraries by the end of the 3rd year of the Project. 3-2. Screening systems for inhibitory activity of the extracts from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project. 3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* are established at the Indonesian research institute by the end of the 3rd year of the Project. 3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation. 3-6. International symposiums are held for drug discovery for two(2) times at least.	(1) Experts' project reports (2) Munities of JCC (3) Handouts and minutes of the Scientific Meetings (4) Handouts and minutes of the International Symposium (5) Other project documents		

Activities	Inputs		Pre-conditions
1 **Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).**	Japan	Indonesia	1. The approval is obtained by Indonesian relevant authority for the research subjects conducted in the Project. 2. Institutional review committees and/or boards of biosafety, recombinant DNA experiments, etc. are established in BTC-BPPT.
1-1. To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the plasmodium-derived recombinant enzymes (DHOD, etc.) and plasmodium extracts (DHOD complex etc.).	Experts (1) Chief Advisor/Tropical Medicine Researches (Short-term experts) (2) Project Coordinator (Long-term expert) (3) Researcher(s) with expertise in malaria (Short-term experts) (4) Researcher(s) with expertise in amebiasis (Short-term experts) (5) Researcher(s) with expertise in isolation and purification of chemical compounds (Short-term experts) (6) Researcher(s) with expertise in structure analysis of chemical compounds (Short-term experts) (7) Other researcher(s) with necessary expertise for project research activities (Short-term experts) as necessity arises Training in Japan (1) Culture techniques of microorganisms and protozoa (2) Screening techniques for inhibitory activity (3) Techniques for Isolation and purification of chemical compounds (4) Techniques for structure analysis of chemical compounds (5) Techniques for mass production of chemical compounds (6) Techniques for animal testing (7) Other training necessary for project research activities as necessity arises Equipment and materials Necessary equipment for research activities in the Project Local costs Running expenses necessary for implementation of the project activities other than that are borne by the Indonesian side.	Counterparts (1) Project Director (2) Project Manager (3) Project Co-Managers (4) Researchers with necessary expertise for the project research activities Facilities, equipment and materials (1) Office spaces in BTC-BPPT and AU (2) Laboratory space in BTC-BPPT, AU and LIPI (3) Bioresources possessed in BTC-BPPT, AU and LIPI Local costs Running expenses necessary for implementation of the project activities such as personnel costs of researchers, research activity costs including travel expenses, consumables, and supplies, utility costs such as water, electricity and communication, maintenance costs for research instruments and equipment, etc.	
To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 1-1) to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-2.			
In parallel with the Activity 1-1 and 1-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Plasmodium falciparum* under the condition of *in vitro* culture system.			
1-3.			

1-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against plasmodium from the culture extracts selected at the Activity 1-2 and 1-3.
1-5.	To establish mass production system of the lead compound candidates (Activity 1-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.
1-6.	To determine chemical structures of the lead compound candidates.
1-7.	To select lead compound(s) from the candidates through *in vitro* assessment using malaria clinical strains and animal testing for efficacy assessment.
1-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.
2	**Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.) .**
2-1.	To perform screening for inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to *Entamoeba histolytica*-derived recombinant enzymes (SAT, CS, NADK, etc.).
2-2.	To perform screening for selective inhibitory activity of the extracts with the inhibitory activity against the recombinant enzymes (Activity 2-1) to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.
2-3.	In parallel with the Activity 2-1 and 2-2, to perform screening for selective inhibitory activity of extracts from newly-isolated and/or preserved biological species at BTC-BPPT to the proliferation of *Entamoeba histolytica* under the condition of *in vitro* culture system.
2-4.	To isolate and purify chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica* from the extracts selected at the Activity 2-2 and 2-3.
2-5.	To establish mass production system of the lead compound candidates (Activity 2-4) for determining chemical structure and animal testing by optimizing production system for enhancing productivity of the target compounds.
2-6.	To determine chemical structures of the lead compound candidates.
2-7.	To select lead compound(s) from the candidates through *in vitro* assessment using clinical strains of *Entamoeba histolytica* and animal testing for efficacy assessment.
2-8.	To discuss future direction of derivatization of the lead compound(s) on the basis of the structural biology assessment of the lead compound(s) and the target enzymes.

↓

Issues and Countermeasures

3	**Technologies and research system for drug discover y using biological recourses arc established at the Indonesinn research institutes.**			
3-1.	To conduct sample collection followed by registering newly-obtained and existing microorganisms, plants and extracts additionally to the biological resource libraries for searching compounds with anti-malarial and anti-amebic activities.			
3-2.	To establish screening systems for inhibitory activity of the extracts from biological resources at the Indonesian research institutes.			
3-3.	To establish culture and evaluation systems necessary for each research objective of *Plasmodium falciparum* and *Entamoeba histolytica* at the Indonesian research institutes.			
3-4.	To introduce technologies of isolation and purification of compounds at the Indonesian research institutes.			
3-5.	To introducc technologies of chemical structure elucidation of compounds at the Indonesian research institutes.			
3-6.	To establish and enhance a network for Indonesian research institutes engaged in the drug development for infectious diseases.			

[Abbreviations] DHOD: dihydroorotate dehydrogenase, SAT: serine acetyltransferase, CS: cysteine synthase, NADK: NAD kinase

AMED

Tentative PO version 0

Version 0
Dated Oct. 10, 2014

Project Title: The Project for Utilization of Indonesian Bioresourse for Anti-malarial and Anti-amebic Drug Development
(Proposed Project Title for amendment by JICA and JST: The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources)

Inputs	Year	1st Year				2nd Year				3rd Year				4th Year				5th Year				6th Year				7th Year				8th Year				9st Year				Remarks	Monitoring: Issue	Monitoring: Solution
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV			
Expert	Plan																																							
Chief Advisor/Tropical Medicine Researches	Actual																																							
Project Coordinator	Plan																																							
	Actual																																							
Researcher(s) with expertise in malaria	Plan																																							
	Actual																																							
Researcher(s) with expertise in amebiasis	Plan																																							
	Actual																																							
Researcher(s) with expertise in isolation and purification of chemical compounds	Plan																																							
	Actual																																							
Researcher(s) with expertise in structure analysis of chemical compounds	Plan																																							
	Actual																																							
Other researcher(s) with necessary expertise for project research activities as necessity arises	Plan																																							
	Actual																																							
Equipment	Plan																																							
Instruments and related equipment for protozoal recombinant enzyme	Actual																																							
Instruments and related equipment for culture of protozoa	Plan																																							
	Actual																																							
Instruments and related equipment for chemical compound isolation	Plan																																							
	Actual																																							
Instruments and related equipment for mass production system of the lead compound	Plan																																							
	Actual																																							
Training in Japan	Plan																																							
Culture techniques of microorganisms and protozoa	Actual																																							
Screening techniques for inhibitory activity	Plan																																							
	Actual																																							
Techniques for Isolation and purification of chemical compounds	Plan																																							
	Actual																																							
Techniques for structure analysis of chemical compounds	Plan																																							
	Actual																																							
Techniques for mass production of chemical compounds	Plan																																							
	Actual																																							
Other training necessary for project research activities as necessity arises	Plan																																							
	Actual																																							
In-country/Third country Training	Plan																																							
	Actual																																							

Activities / Sub-Activities	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Responsible Organization: Japan	Responsible Organization: GOI	Achievements	Issue & Countermeasures
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV				
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).														
1.1 Primary screening for inhibitory activity of extract to the plasmodium-derived recombinant enzyme	Plan										U.Tokyo	BPPT		
	Actual													
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan										U.Tokyo	BPPT		
	Actual													
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1 and 1-2	Plan										U.Tokyo	BPPT		
	Actual													
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan										KU U.Tokyo	BPPT		
	Actual													
1.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBI	BPPT		
	Actual													
1.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	Plan										U.Tokyo	BPPT		
	Actual													
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)														
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan										UT	AU (BPPT)		
	Actual													
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan										UT	AU (BPPT)		
	Actual													
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2	Plan										UT	AU (BPPT)		
	Actual													
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan										KU UT	BPPT (AU)		
	Actual													
2.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBI	BPPT		
	Actual													
2.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan										UT	AU (BPPT)		
	Actual													
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													

Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.						
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	Plan / Actual	KU MBJ	BPPT LIPI			
3.2 Establishment of screening systems	Plan / Actual	UT U.Tokyo	BPPT AU			
3.3 Establishment of culture and evaluation systems	Plan / Actual	UT U.Tokyo	BPPT AU			
3.4 Introduction of technologies of isolation and purification	Plan / Actual	KU	BPPT			
3.5 Introduction of technologies of chemical structure elucidation	Plan / Actual	KU	BPPT			
3.6 Establishment and enhancement of a research network in Indonesia	Plan / Actual	ALL	ALL			
Duration / Phasing	Plan / Actual					

Monitoring Plan	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Issue	Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Monitoring													
Joint Coordinating Committee	Plan	•	•	•	•	•							
	Actual												
Scientific Meeting	Plan	▲	▲	▲	▲	▲	▲						
	Actual												
Set-up the Detailed Plan of Operation	Plan	▲	▲	▲	▲								
	Actual												
Submission of Monitoring Sheet	Plan	■ ■	■ ■	■ ■	■ ■	■ ■	■						
	Actual												
Monitoring Mission from Japan	Plan			■		■							
	Actual												
Post Monitoring	Plan									■			
	Actual												
Reports/Documents													
Project Completion Report	Plan						▲						
	Actual												
Public Relations													
Establishment and Operation of Web Site	Plan	▲											
	Actual												
International symposiums are held for drug discovery	Plan			▲		▲							
	Actual												

Tentative List of Equipment

Category	Name
Microbial isolation/extract preparation	Freezer -30°C
	Freezer -30°C
	250 ml Flask holder (for large scale shaker incubator)
Microbial storage	Deep freezer -80°C, double compressor
Plant extract	Rotary evaporator/concentrator
Enzyme preparation	UV-vis spectrophotometer
	Electrophoresis system (for protein)
Enzyme-based screening	96-plate reader
Hit analysis	Analytical HPLC with DAD detector
	Semi-preparative HPLC (flow rate <20ml/min with UV-vis c
	Photodiode detector for UPLC (waters)
Cell-based screening	Safety cabinet class 2
	Autoclave
	Ultracentrifuge
	Ultracentrifuge Rotors
	CO_2/O_2 incubator
	Incubator
	Refrigerated centrifuge, table top
	Centrifuge Rotors, swing and angle
	Liquid nitrogen tank 30L with canister (box storage) as-one
Scale up production	Mini fermentor (3L (or 5L) x5 jar)
	Fermentor 30L
Experimental instruments and others	Server and PC
	Ultrasonic washer
	Sonicator
	Fraction collector, UV (for protein purification)
	Multichannel automatic micropipette 10ml
	Multichannel automatic micropipette 50ml
	Multichannel automatic micropipette 200ml
	Multichannel automatic micropipette 1000ml
	Micropipette set (2-1000ml)
	Refrigerator
	Freezer

[DRAFT]

RECORD OF DISCUSSIONS
ON
THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES
IN
THE REPUBLIC OF INDONESIA
AGREED UPON BETWEEN
AGENCY FOR THE ASSESSMENT AND APPLICATION OF TECHNOLOGY
AND
JAPAN INTERNATIONAL COOPERATION AGENCY

Jakarta, 〈date〉

Mr. Atsushi Sasaki
Chief Representative
Japan International Cooperation Agency
Indonesia Office

Chairperson
Agency for the Assessment and Application of Technology (BPPT)
The Republic of Indonesia

Based on the minutes of meetings on the detailed planning survey on "the Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources (hereinafter referred to as "the Project") signed on October 10, 2014 between Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT") and the Japan International Cooperation Agency (hereinafter referred to as "JICA"), JICA held a series of discussions with BPPT and relevant organizations to develop a detailed plan of the Project.

Both parties agreed the details of the Project and the main points discussed as described in the Appendix 1 and the Appendix 2 respectively.

Both parties also agreed that BPPT, the counterpart to JICA, will be responsible for the implementation of the Project in cooperation with JICA, coordinate with other relevant organizations and ensure that the self-reliant operation of the Project is sustained during and after the implementation period in order to contribute toward social and economic development of the Republic of Indonesia.

The Project will be implemented within the framework of the Colombo Plan Technical Cooperation Scheme between the Government of Japan (hereinafter referred to as "GOJ") and the Government of the Republic of Indonesia (hereinafter referred to as "GOI").

The effectiveness of the record of discussion is subject to the approval of JICA.

Appendix 1: Project Description
Appendix 2: Main Points Discussed
Appendix 3: Minutes of Meetings on the Detailed Planning Survey
Appendices are integral part of the Record of Discussions

APPENDIX 1

PROJECT DESCRIPTION

Both parties confirmed that there is no change in the project description agreed on in the minutes of meetings on the concerning detailed planning survey on the project signed on October 10, 2014 (appendix 3).

I. BACKGROUND

Indonesia is the second largest biodiversity country in the world. The diversity and uniqueness of bioresources is an important capital for Indonesia to increase wealth and prosperity of the people. GOI declared a strategic directive master plan for Indonesian economic development, through which GOI expects increase its economic competitiveness by transformation from bioresources-based comparative economic activities to innovation-based competitive economic activities. BPPT has been consistently developing innovation for utilization of Indonesian bioresources in order to support industries in Indonesia. One of the prioritized research topics is bio-prospecting for health by utilization of microbial and plant biodiversity. Many achievements have been obtained, however, very little of them had been practically used by industries or people. Thus, capacity building on utilization of bioresource for health, especially drug development, is very urgent.

Malaria and amebiasis are regarded as neglected diseases caused by parasites and a considerable concern in Indonesia which is needed to be overcome. Moreover, resistance of presently available drugs to the parasites is unavoidable, thus drug discovery for anti-malarial and anti-amebic compounds is urgently needed.

This technical cooperation is aimed to strengthen the capacity of Indonesian researchers and institutions in drug discovery against tropical infectious diseases including malaria and amebiasis using Indonesia bioresources.

GOI requested JICA to initiate the Indonesia-Japan collaborative project at BPPT and other associated institutions for conducting research on malaria and amebiasis. The joint research by the Indonesian institutes, BPPT and its collaborating institutes in Indonesia, the Indonesian Institute of Sciences (hereinafter referred to as "LIPI") and the Institute of Tropical Disease, Airlangga University (hereinafter referred to as "AU"), and Japanese institutions, University of Tsukuba, Kitasato University, University of Tokyo, and MicroBiopharm Japan Co. Ltd., aim (i) to strengthen capacity building for Indonesian researchers and institutions, (ii) to reinforce international research collaboration, and (iii) to increase added value of Indonesia bioresources especially for anti-malaria and anti-amebic drug discovery.

II. OUTLINE OF THE PROJECT

Details of the Project are described in the Project Design Matrix (hereinafter referred to as "PDM") (Annex I) and the tentative Plan of Operation (hereinafter referred to as "PO") (Annex II)

1. Project Implementation Structure

The project implementation structure is given in the Annex III. The roles and assignments of relevant organizations are as follows:

(1) BPPT

(a) Project Director will be responsible for overall administration and implementation of the Project. The Project Director will be Deputy Chairperson of Agro-industrial Technology and Biotechnology of BPPT;

(b) Project Manager will be responsible for the managerial and technical matters of the Project. The Project Manager will be Head, Biotechnology Application Center of BPPT; and

(c) Project Co-manager will be responsible for the managerial and technical matters of the Project in collaboration with the Project Manager. The Project Co-managers will be Division Head, Biotechnology Application Center of BPPT.

(2) AU

Director, Institute of Tropical Disease, AU will be Project Co-manager.

(3) LIPI

Director, Research Center for Biotechnology, LIPI will be Project Co-manager.

(4) JICA Experts

The JICA Experts will give necessary technical assistance, advice and recommendations to BPPT on any matters pertaining to the implementation of the Project.

(5) Joint Coordinating Committee

Joint Coordinating Committee (hereinafter referred to as "JCC") will be established in order to facilitate inter-organizational coordination. JCC will be held at least once a year and whenever deems it necessary. JCC will approve an annual work plan, review overall progress, conduct monitoring and evaluation of the Project, and discuss and take necessary measures to major issues that arise during the Project. Outline and a list of proposed members of JCC are shown in the Annex IV.

2. Project Sites and Beneficiaries

(1) Project Sites : Indonesia

(2) Beneficiaries : Indonesian Institutes engaged in the Project

3. Duration

The duration of the Project will be five (5) years starting on April 1, 2015.

4. Reports

Indonesian side and JICA experts will jointly prepare the following reports in English:

(1) Monitoring sheet at every six (6) months until the project completion; and

(2) Project Completion Report at the time of project completion

5. Environmental and Social Considerations

BPPT, AU, LIPI and University of Tsukuba, Kitasato University, University of Tokyo, MicroBiopharm Japan Co., Ltd. agreed to abide by 'JICA Guidelines for Environmental and Social Considerations' in order to ensure that appropriate considerations will be made for the environmental and social impacts of the Project.

III. UNDERTAKINGS OF BPPT

1. BPPT will take necessary measures to:
   (1) ensure that the knowledge and experience acquired by the personnel of the Republic of Indonesia from technical training as well as the equipment provided by JICA will be utilized effectively in the implementation of the Project;
   (2) grant privileges, exemptions and benefits to the JICA experts referred to in ANNEX I and their families, which are no less favorable than those granted to experts of third countries performing similar missions in the Republic of Indonesia under the Colombo Plan Technical Cooperation Scheme;

2. BPPT will take necessary measures to:
   (1) provide security-related information as well as measures to ensure the safety of the JICA experts;
   (2) permit the JICA experts to enter, leave and sojourn in Indonesia for the duration of their assignments therein and exempt them from foreign registration requirements and consular fees;
   (3) exempt the JICA experts from taxes and any other charges on the equipment, machinery and other material necessary for the implementation of the Project; and
   (4) exempt the JICA experts from income tax and charges of any kind imposed on or in connection with any emoluments or allowances paid to them and/or remitted to them from abroad for their services in connection with the implementation of the Project.

3. BPPT, AU and LIPI will bear claims, if any arises, against the JICA experts resulting from, occurring in the course of, or otherwise connected with, the discharge of their duties in the implementation of the Project, except when such claims arise from gross negligence or willful misconduct on the part of the JICA experts.

IV. EVALUATION

JICA and the BPPT will jointly and regularly monitor the progress of the Project through the Monitoring Sheets based on PDM and PO. The Monitoring Sheets shall be reviewed every six (6) months.

Also, Project Completion Report shall be drawn up one (1) month before the

termination of the Project.

V. PROMOTION OF PUBLIC SUPPORT

For the purpose of promoting support for the Project, BPPT will take appropriate measures to make the Project widely known to the people of the Republic of Indonesia.

VI. MISCONDUCT

If JICA receives information related to suspected corrupt or fraudulent practices in the implementation of the Project, BPPT and relevant organizations shall provide JICA with such information as JICA may reasonably request, including information related to any concerned official of the government and/or public organizations of the BPPT and relevant organizations shall not, unfairly or unfavorably treat the person and/or company which provided the information related to suspected corrupt or fraudulent practices in the implementation of the Project.

VII. MUTUAL CONSULTATION

JICA and BPPT will consult each other whenever any major issues arise in the course of project implementation.

VIII. AMENDMENTS

The record of discussions may be amended by the minutes of meetings between BPPT and JICA. The minutes of meetings will be signed by authorized persons of each side who may be different from the signers of the record of discussions.

ANNEX I	PDM version 0 (M/M Annex III)
ANNEX II	Tentative PO version 0 (M/M Annex IV)
ANNEX III	Project Implementation Structure (M/M Annex I)
ANNEX IV	List of Proposed Members of Joint Coordinating Committee
ANNEX V	Goods / Services

Note: ANNEX I ,II , and III will be attached when the record of discussions is signed.

ANNEX IV

LIST OF PROPOSED MEMBERS OF JOINT COORDINATING COMMITTEE

1. Functions

The JCC will be held at least once a year and whenever deems it necessary. The functions of JCC are as follows:

(a) To facilitate inter-organizational coordination concerning the Project

(b) To approve an annual work plan of the Project

(c) To review overall progress, conduct monitoring and evaluation of The Project, and

(d) To exchange opinions on major issues that arise during the Project and to take necessary measures.

2. Chairperson: Project Director or person appointed by the Project Director
Deputy Chairperson for Agro-industrial Technology and Biotechnology of BPPT

3. Members

(a) The Indonesian side

- Project Manager: Head, the Center for the Assessment of Biotechnology;
- Project Co-Managers: Head of Technological Services Division, Biotech Center of BPPT, Director, Institute of Tropical Disease, AU, and Director, Research Center for Biotechnology, LIPI; and
- Other representative(s) from BPPT.

(b) The Japanese side

- Japanese Chief Advisor;
- JICA Project Coordinator; and
- Representative(s) from the JICA Indonesia Office.

(c) Observers

Stakeholders appointed by the Chairperson such as the Embassy of Japan, JST and/or other relevant organizations.

ANNEX V

GOODS / SERVICES

Both sides confirmed that the Project is categorized as "goods / services" stipulated in article 42 (1) c of Government Regulation no. 10/2011. In accordance with regulation of Minister of Finance no. 191/ PMK.05 /2011, BPPT shall submit the handover delivery certificate of goods/services (BAST) to the Ministry of Finance of Indonesia. In order to secure the accuracy of BAST, JICA Indonesia office will provide BPPT with data on semester basis as follows:

1. Goods: name and price (in effective currency) per item of equipment handed over during last six months

2. Services: total expenditure (in effective currency) of last six months for expert, training, and mission

BPPT will make and sign BAST based on the data provided by JICA, and after obtaining JICA's confirmation, submit it to the Ministry of Finance.

APPENDIX 2

MAIN POINTS DISCUSSED

1. Biosafety

   Both sides agree that all laboratory activities should follow the international biosafety regulations.

2. Interectual Property Rights

   Both sides agree that matters related to intellectual property rights should follow the Memorandum of Understanding between University of Tsukuba and BPPT.

AMENDMENT
TO
RECORD OF DISCUSSIONS
ON
THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY OF INDONESIAN BIO-RESOURCES IN THE REPUBLIC OF INDONESIA

The Japan International Cooperation Agency (hereinafter referred to as "JICA") and Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT") hereby agree and confirm that the Record of Discussions on the Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources signed on February 17, 2015 with some amendments confirmed by 1st Joint Coordinating (JCC) meeting is amended as follows;

1. Project Implementation Structure confirmed by the minutes of meeting of the 2nd JCC meeting signed on January 25, 2017.

Version confirmed by the 1st JCC meeting on Feb 2 2016	Amended Version
1.Project Implementation Structure (1) BPPT (b) The Project Manager will be Director, Center for Pharmaceutical and Medical Technologies of BPPT; (C)The Project Co-manager will be Program Head, Center for Pharmaceutical and Medical Technologies of BPPT.	1.Project Implementation Structure (1) BPPT (b) The Project Manager will be Head, Laboratory for Biotechnology of BPPT; (c) The Project Co-manager will be Program Head, Laboratory for Biotechnology of BPPT
Reason: Due to BPPT organizational reformation in 2017, implementing institution of this project's activities in BPPT was changed from the Center for Pharmaceutical and Medical Technologies (PTFM)-BPPT to the Laboratory for Biotechnology-BPPT.	

2. ANNEX IV Project Implementation Structure

Before	Amended Version
Chief Advisor (University of Tsukuba)	Chief Advisor (The University of Tokyo)
Reason: Due to the change of the Chief Advisor's affiliation, the Japanese Coordinating Research Institute is changed from University of Tsukuba to The University of Tokyo.	

In witness whereof, the undersigned authorized representatives of JICA and BPPT have signed this amendment. Done in Jakarta on January 15, 2018 and on January 19, 2018 in two original documents in English, both documents are equally authentic.

Annex 1: Record of Discussions (signed on 17 February 2015)
Annex 2: Minutes of Meeting of the 1st Joint Coordinating Committee (signed on 02 February 2016)
Annex 3:Minutes of Meeting of the 2nd Joint Coordinating Committee (signed on 25 January 2017)

Mr.Shunsuke TAKATOI
Senior Representative
Japan International Cooperation Agency
Indonesia Office

Prof.Dr.Eng. Eniya Listiani Dewi, B. Eng.,M.Eng.
Agency for the Assessment and Application of Technology (BPPT)
The Republic of Indonesia

MINUTES OF MEETING
OF
THE 1st JOINT COORDINATING COMMITTEE MEETING
OF THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF
ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY
OF INDONESIAN BIO-RESOURCES (SLeCAMA PROJECT)
IN
THE REPUBLIC OF INDONESIA

The 1st Joint Coordinating Committee Meeting of the Japanese Technical Cooperation for the Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebic Agents by Utilizing Indonesian Bio-resources in the Republic of Indonesia (hereinafter referred to as "SLeCAMA Project") was held at the conference room of Agency of Assessment and Application of Technology, Jakarta, Indonesia on 2nd February, 2016.

As a result of the discussions, both Indonesian side and Japanese side agreed upon the matters in the document attached hereto.

Jakarta, 2nd February 2016

Mr. NAOKI ANDO
Chief Representative
Japan International Cooperation Agency
Indonesia Office

Dr. ENIYA LISTIANI DEWI
Deputy Chairperson for Agricultural
Technology and Biotechnology,
Agency for the Assessment and Application
of Technology (BPPT)
The Republic of Indonesia

Witnessed by

Dr. TOMOYOSHI NOZAKI
Professor,
Graduate School of Life and Environmental
Sciences, University of Tsukuba,
Japan

Prof. MARIA INGE LUSIDA
Chairperson,
Institute of Tropical Disease(ITD),
Airlangga University
The Republic of Indonesia

Dr. ACHMAD DINOTO,
Head, Indonesian Culture Collection (InaCC),
Research Centre for Biology,
Indonesian Institute of Sciences (LIPI),
The Republic of Indonesia

ATTACHED DOCUMENT

I. GENERAL REVIEW

The SLeCAMA Project was commenced on 01 April 2015, for strengthening the capacity of Indonesian researchers and institutions in drug discovery against tropical diseases including malaria and amebiasis using Indonesian bio-resources. through the collaborative joint researches by the Indonesian institutes, BPPT and its collaborating institutes in Indonesia, the Institutes of Sciences (hereinafter referred to as "LIPI") and the Institutes of Tropical Disease, Airlangga University(hereinafter referred to as "AU"), and Japanese institutions, University of Tsukuba (hereinafter referred to as "UT"), Kitasato University (hereinafter referred to as "KU") , University of Tokyo (hereinafter referred to as "U Tokyo") and MicroBiopharm Japan Co. Ltd (hereinafter referred to as "MBJ").

In accordance with the Record of Discussions (hereinafter referred to as the "R/D"), signed on 17th February 2015 by Japan International Cooperation Agency (hereinafter referred to as "JICA") and Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT"), Japanese side has dispatched experts to SLeCAMA Project and has accepted Indonesian counterparts as trainees in KU, U Tokyo and UT. And Japanese side has been providing equipment for the laboratories located in both BPPT and AU to capacitate the drug discovery in the institutes based on requests by the Government of the Republic of Indonesia.

In the preparation phase of the SLeCAMA Project, a coordination meeting between BPPT and Japanese collaborators was organized in February 2014, TU and BPPT exchanged their signatures on the Minutes of Understanding (MoU) to start the project in February 2015. BPPT renovated four (4) laboratory spaces to be BSL 2nd level in March 2015.

The required equipment for the project implementation had listed up and requested by the Government of the Republic of Indonesia to Japanese side in November 2015. There are 43 items of equipment with total 57 units in the list for the laboratories in BPPT and AU. According to the request, UT had procured and has been transferring them from Japan to Indonesia for installing them in the beginning phase of the SLeCAMA Project.

Four working teams according to each stage of process on drug discovery were established, namely A) Microbes and Extract Preparation Team, B) Enzyme-based Screening Team, C) Cell-based Screening Team, and D) Purification Team among BPPT. Each group discussed the activity plan of their own group in the meeting with advises of Japanese members and updated the Tentative of Plan of Operation (hereinafter referred to as "P.O.") , which was designed initially as a part of the R/D.

On 2nd February, 2016, the Kick off Meeting for SLeCAMA Project was organized right before the 1st JCC Meeting in the BPPT in the participation of various stake holders in Indonesia and Japan for proclamation of the project activities.

Both sides reviewed activities in respect to the implementation of the SLeCAMA Project based on the common implementation plan of the Project, which is described in the P.O. and the Tentative Project Design Matrix (hereinafter referred to as the "Tentative PDM") in the R/D and the Minutes of Meeting signed on 10th October, 2014 by related institutions of both sides.

II. SUMMARY OF MEETING

Both sides reviewed and discussed the following issues:

1. Progress of Implementation Activities

1) More than 500 microbes were isolated from the samples collected during the field exploration in July 2015 at Maluku province.

2) Approximately 800 new extracts have been prepared for screening.

3) Approximately 5,000 extracts have been screened for both anti-malarial and anti-amebic activities.

4) Two compounds with anti-malarial activities have been purified and structurally elucidated.

5) Twelve (12) turns of short-term Japanese researchers and a long term JICA expert were dispatched to SLeCAMA Project.

6) Japanese side accepted eleven (11) Indonesian counterparts as trainees in KU, U Tokyo and UT,

7) To introduce technologies on screening, isolation and purification into laboratories in BPPT and AU, tentative plan of training in Japan JFY (Japanese Fiscal Year) 2016 was planned.

8) To implement the activities in 2016, BPPT and AU allocated operational budget for the Project.

9) Coordination Meeting to enhance a network for Indonesian research institutes was established in September 2015 and periodically organized among AU, LIPI and BPPT.

10) Required laboratory equipment which consists of 43 items cost approximate 63 million Japanese yen (excluded transportation cost) were procured in Japan and it is ready to ship for Indonesia. However, due to the complicated process to obtain permission of importation in related several Indonesian administrative offices, the period of the installation have delayed as compared with the P.O.

2. Tentative Plan for the Project Implementation in 2016

1) Microbes and Extract Preparation in 2016
Prepare more than 5,000 extracts, and to isolate more than 500 microbes.

2) Enzyme-based Screening in 2016
Prepare target enzymes and screen more than 5,000 extracts for inhibition activity

3) Cell-based Screening in 2016
Maintain parasite, Plasmodium falciparum 3D7 and Entamoeba histolytica HM-1:IMSS clone 6, and to maintain cell line DLD-1.To screen 5,000 extracts for antiprotozoal activity

4) Purification in 2016
Purify more than 6 extracts (antimalarial: 3, antiamoeba: 3)

5) Regular Managerial Meeting
The project managerial meeting in BPPT will be held every month with representatives of all the working teams of the project in BPPT with JICA experts and chaired by the Project Manager.

6) Coordination Meeting
The coordination meeting will be organized quarterly among AU, LIPI and BPPT.

7) Ten (10) members of working teams of BPPT are planned to participate in the training course organized in Japan, one (1) researcher of AU will participate in post-graduate degree course (Ph.D.) in UT.

8) A field exploration to Biak island is being planned around May 2016.

9) Dispatching Japanese researchers in 2016
Dispatching around thirteen (13) Japanese researchers are planned

10) Laboratory equipment
All equipment procured in Japan in 2015 should be installed in BPPT and AU as soon as possible to catch up the activity plan according to P.O.

11) Implementation Arrangement
To define detail of cooperation scheme between BPPT and UT, both sides should sign on the "Implementation Arrangement "as soon as possible.

3. Others

1) Limited budget for reagents and laboratory-supplies
Operational budget for reagents and laboratory-supplies by Indonesian institutes are essential to implement activities in Indonesia as planned in the Project Design Matrix. However the estimated required cost for those consumables seems to exceed the allocated budgets for 2016. To realize planned outcome, the increment of the budget is necessary

2) Japan Agency for Medical Research and Development
The role of Japan Science and Technology Agency (JST) for the SLECAMA Project was handed over to Japan Agency for Medical Research and Development (AMED) which was newly established in April 2015

3) Alteration of project implementation structure
- Due to re-organization of BPPT in January 2016, the implementing unit of the

SLeCAMA Project of BPPT changed from "Biotech Center of BPPT" to "**Center for Pharmaceutical and Medical Technologies of BPPT**"

- After reviewing the appropriateness as member of the SLeCAMA Project, the main unit of LIPI as SLeCAMA Project changed from "Research Center for Biotechnology, LIPI" to "**Indonesian Culture Collection (InaCC), Research Center for Biology, LIPI**".

4) Alteration of JCC members

In accordance with the alteration of project implementation structure, the members of JCC among BPPT and LIPI changed. The details are shown in the ANNEX 3. "Amendments to the Record of Discussions signed on 17th February 2015 (R/D)"

III. TENTATIVE SCHEDULE OF IMPLEMENTATION

Based on the present status of the Project both sides jointly formulated the tentative Plan of Operation of the SLeCAMA Project. The timelines of the implementation are shown in the attached sheet "Tentative Plan of Operation version 1".

ANNEX

1. Tentative Plan of Operation version 1
2. Project implementation structure (updated)
3. Amendments to the Record of Discussions signed on 17th February 2015(R/D)

ANNEX 1.

Version 1

Dated Feb. 02, 2016

SLeCAMA P.O. (Plan of Operation) version 1

Project Title The Project for Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources (SLeCAMA)

Inputs	JFY	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Monitoring: Issue	Monitoring: Solution
	Year	2015	2016	2017	2018	2019	2020	2021	2022	2023			
	Month	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3	4 5 6 7 8 9 10 11 12 1 2 3			
Expert													
Chief Advisor/Tropical Medicine Researches	Plan												
	Actual												
Project Coordinator	Plan												
	Actual												
Researcher(s) with expertise in malaria	Plan												
	Actual												
Researcher(s) with expertise in amebiasis	Plan												
	Actual												
Researcher(s) with expertise in isolation and purification of chemical compounds	Plan												
	Actual												
Researcher(s) with expertise in structure analysis of chemical compounds	Plan												
	Actual												
Other researcher(s) with necessary expertise for project research activities as necessity arises	Plan												
	Actual												
Equipment													
Instruments and related equipment for protozoal recombinant enzyme	Plan										**Most of provied equipment are under importation process as of Jan. 2016**	Due to complicated importation process, installing schedule have been delayed	To prioritize the follow up of accelerating the process
	Actual												
Instruments and related equipment for culture of protozoa	Plan												
	Actual												
Instruments and related equipment for chemical compound isolation	Plan										**Most of provided equipment are under importation process**	Due to complicated importation process, installing schedule have been delayed	To prioritize the follow up of accelerating the process
	Actual												
Instruments and related equipment for mass production system of the lead compound	Plan										**Detail plan for equipment of mass production is to be discussed**		A-4 Form will be prepared by BPPT
	Actual												
Training in Japan													
Culture techniques of microorganisms and protozoa	Plan												
	Actual												
Screening techniques for inhibitory activity	Plan												
	Actual												
Techniques for Isolation and purification of chemical compounds	Plan												
	Actual												
Techniques for structure analysis of chemical compounds	Plan												
	Actual											Arrangement for trainee acceptance was not smooth in the 1st batch	Administration for acceptance of trainees was rearranged between UT & JICA
Techniques for mass production of chemical compounds	Plan												
	Actual												
Other training necessary for project research activities as necessity arises	Plan												
	Actual												
In-country/Third country Training													
	Plan												
	Actual												

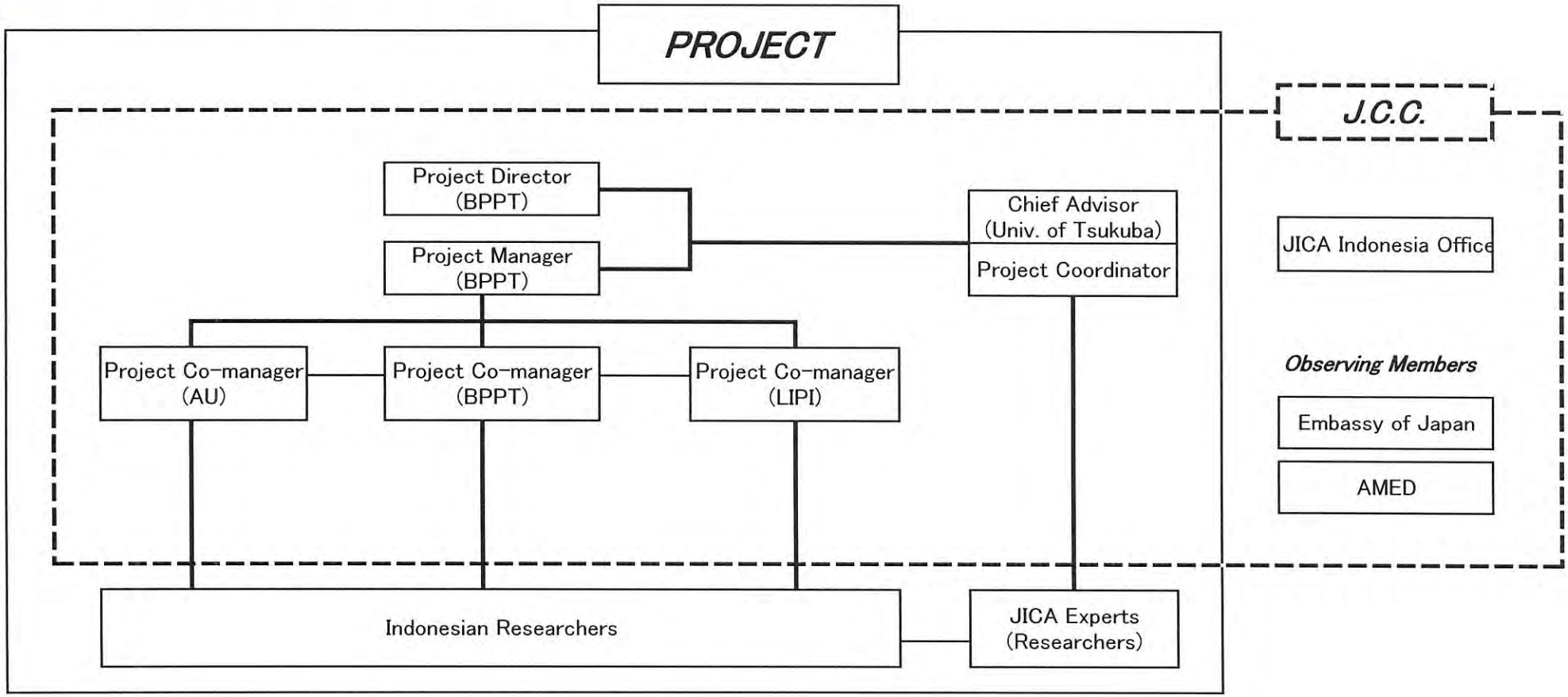
Isolation and Characterization of microbes training in BTC

Activities / Sub-Activities		1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Responsible Organization		Achievements	Issue & Countermeasures
		2015	2016	2017	2018	2019	2020	2021	2022	2023	Japan	GOI		
Output 1: Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).														
1.1 Primary screening for inhibitory activity of extract to the plasmodium-derived recombinant enzyme	Plan										U.Tokyo	BPPT		Equipment are not available in BTC yet
	Actual													
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan										U.Tokyo	BPPT		
	Actual													
1.3 Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum* , in parallel with Activity 1-1 and 1-2	Plan										U.Tokyo	BPPT		
	Actual													
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan										KU U.Tokyo	BPPT		
	Actual													
1.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBJ	BPPT		
	Actual													
1.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
1.7 Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	Plan										U.Tokyo	BPPT		
	Actual													
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)														
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan										UT	AU (BPPT)		
	Actual													
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan										UT	AU (BPPT)		
	Actual													
2.3 Screening for selective inhibitory activity of extracts to the proliferation of *Entamoeba histolytica* , in parallel with Activity 2-1 and 2-2	Plan										UT	AU (BPPT)		
	Actual													
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan										KU UT	BPPT (AU)		
	Actual													
2.5 Establishment of mass production system of the lead compound candidates	Plan										KU MBJ	BPPT		
	Actual													
2.6 Determination of chemical structures of the lead compound candidates	Plan										KU	BPPT		
	Actual													
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan										UT	AU (BPPT)		
	Actual													
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment	Plan										ALL	ALL		
	Actual													

Activities / Sub-Activities	JFY / Year / Month	Responsible Organization: Japan	Responsible Organization: GOI	Achievements	Issue & Countermeasures
Output 3: Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.	1st Year 2015 – 9st Year 2023				
3.1 Sample collection and additional registration of newly-obtained extracts to the biological resource library	Plan / Actual	KU MBJ	BPPT LIPI		
3.2 Establishment of screening systems	Plan / Actual	UT U.Tokyo	BPPT AU		Equipment are not available in BTC/AU yet
3.3 Establishment of culture and evaluation systems	Plan / Actual	UT U.Tokyo	BPPT AU		Equipment are not available in BTC/AU yet
3.4 Introduction of technologies of isolation and purification	Plan / Actual	KU	BPPT		Equipment are not available in BTC/AU yet
3.5 Introduction of technologies of chemical structure elucidation	Plan / Actual	KU	BPPT		
3.6 Establishment and enhancement of a research network in Indonesia	Plan / Actual	ALL	BPPT,AU, LIPI		
Duration / Phasing	Plan / Actual				

Monitoring Plan	Year	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	7th Year	8th Year	9st Year	Remarks	Issue	Solution
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV			
Monitoring													
Joint Coordinating Committee	Plan / Actual	●											
Scientific Meeting	Plan / Actual	● ●											
Set-up the Detailed Plan of Operation	Plan / Actual	●											
Submission of Monitoring Sheet	Plan / Actual												
Monitoring Mission from Japan	Plan / Actual												
Post Monitoring	Plan / Actual												
Reports/Documents													
Project Completion Report	Plan / Actual												
Public Relations													
Establishment and Operation of Web Site	Plan / Actual												
International symposiums are held for drug discovery	Plan / Actual												

Project Implementation Structure (updated)



Amendments to the Record of Discussions signed on 17th February 2015(R/D)

These amendments are made by Japan International Cooperation Agency (JICA) and Agency of Assessment and Application of Technology (BPPT), the parties agree to amend the R/D dated on 17th February, 2015, as below.

1. The first paragraph of the "1. Project Implementation Structure" in the page 4 of the APPENDIX 1 is amended by modifying "ANNEX III" to "**ANNEX IV**".
2. "(b) Project Manager" of the "1. Project Implementation Structure" in the page 4 of the APPENDIX 1 is amended by modifying "Head, Biotechnology Application Center of BPPT" to "**Director, Center for Pharmaceutical and Medical Technologies of BPPT**".
3. "(c) Project Co-manager" of the "1. Project Implementation Structure" in the page 4 of the APPENDIX 1 is amended by modifying "Division Head, Biotechnology Application Center of BPPT" to "**Program Head, Center for Pharmaceutical and Medical Technologies of BPPT** ".
4. "(3) LIPI" of the "1. Project Implementation Structure" in the page 4 of the APPENDIX 1 is amended by modifying "Director, Research Center for Biotechnology, LIPI" to "**Head, Indonesian Culture Collection (InaCC), Research Center for Biology, LIPI** ".
5. The last sentence of the paragraph of "(5) Joint Coordinating Committee" in the page 4 of the APPENDIX 1 is amended by modifying "ANNEX IV" to "**ANNEX V**".
6. The third line on the header of PDM in the ANNEX I of the APPENDIX 1 in the page 7 is amended by modifying "JST" to "**AMED**".
7. The 6th line on the header of PDM in the ANNEX 1 of the APPENDIX 1 in the page 7 is amended by modifying "Biotech Center of the Agency for Assessment and Application of Technology (BTC-BPPT)" to "**Center for Pharmaceutical and Medical Technologies of BPPT (PTFM-BPPT)**".
8. The third line on the header of "Tentative PO version 0" in the ANNEX II of the APPENDIX 1 in the page 10 is amended by modifying "JST" to "**AMED**".
9. The Project Implementation Structure of ANNEX IV of the APPENDIX 1 in the page 14 is amended by modifying "JST" to "**AMED**".
10. "Project Manager" in "(a) The Indonesian Side" in the paragraph of 3. Members in the ANNEX V of the APPENDIX I is amended by modifying "Head, the Center for the Assessment of Biotechnology" to "**Director, Center for Pharmaceutical and Medical Technologies of BPPT** ".
11. "Project Co-Managers" in "(a) The Indonesian Side" in the paragraph of 3. Members in the ANNEX V of the APPENDIX I is amended by modifying "Head of Technological Services Division, Biotech Center of BPPT" to "**Program Head, Center for Pharmaceutical and Medical Technologies of BPPT** " and "-Director,

Research Center for Biotechnology, LIPI" to "**Head, Indonesian Culture Collection (InaCC), Research Center for Biology, LIPI**".

12. The last sentence of the "(c) Observers" in the paragraph of 3. Members in the ANNEX V of the APPENDIX I is amended by modifying "JST" to "**AMED**".
13. The first paragraph of the "1. Project Implementation Scheme" in the page 2 of the APPENDIX 3 (Minutes of Meeting signed on 10 October 2014") is amended by modifying "Japan Science and Technology Agency (hereinafter referred to as "JST") to "**Japan Agency for Medical Research and Development** (hereinafter referred to as **"AMED"**.
14. The second paragraph of the "1. Project Implementation Scheme" in the page 2 of the APPENDIX 3 (Minutes of Meeting signed on 10 October 2014") is amended by modifying "JST" to "**AMED**".
15. "(2) Project Manager" in "4-1. Administration" in the paragraph of "4. Implementation Structure of the Project" in the page 3 of the APPENDIX 3 is amended by modifying "Head, the Center for the Assessment of Biotechnology (hereinafter referred to as "Biotech Center")" to "**Director, Center for Pharmaceutical and Medical Technologies** (hereinafter referred to as "PTFM") ".
16. "(3) Project Co-manager" in "4-1. Administration" in the paragraph of "4. Implementation Structure of the Project" in the page 3 of the APPENDIX 3 is amended by modifying "-Head of Technological Service Division, Biotech Center of BPPT" to "**-Program Head, Center for Pharmaceutical and Medical Technologies of BPPT** " and "-Director, Research Center for Biotechnology, Indonesian Institute of Sciences" to "**-Head, Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute of Sciences**".
17. The paragraph of the "3) Observers" in the page 4 of the APPENDIX 3 is amended by modifying "JST" to "**AMED**".
18. The Project Implementation Structure of ANNEX I of the APPENDIX 3 is amended by modifying "JST" to "**AMED**".
19. The third line on the header of Project Design Matrix (version 0) in the ANNEX III of the APPENDIX 3 is amended by modifying "JST" to "**AMED**".
20. The 6th line on the header of PDM (version 0) in the ANNEX III of the APPENDIX 3 is amended by modifying "Biotech Center of the Agency for Assessment and Application of Technology (BTC-BPPT)" to "**Center for Pharmaceutical and Medical Technologies of BPPT (PTFM-BPPT)**".
21. The third line on the header of "Tentative PO version 0" in the ANNEX IV of the APPENDIX 3 is amended by modifying "JST" to "**AMED**".

MINUTES OF MEETING
OF
THE 2nd JOINT COORDINATING COMMITTEE MEETING
OF THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF
ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY
OF INDONESIAN BIO-RESOURCES (SLeCAMA PROJECT)
IN
THE REPUBLIC OF INDONESIA

The 2nd Joint Coordinating Committee Meeting (hereinafter referred to as "JCC Meeting") of the Japanese Technical Cooperation for the Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebic Agents by Utilizing Indonesian Bio-resources in the Republic of Indonesia (hereinafter referred to as "SLeCAMA Project") was held at the auditorium of the Laboratory of Biotechnology, the Agency of Assessment and Application of Technology, Jakarta, Indonesia on 25th January, 2016.

As a result of the discussions, both Indonesian side and Japanese side agreed upon the matters in the document attached hereto.

Jakarta, 25th January 2017

for **Mr. Mikiya SAITO**
Senior Representative
Japan International Cooperation Agency
Indonesia Office

Prof.Dr.Eng. ENIYA LISTIANI DEWI, B.Eng., M.Eng.
Deputy Chairperson for Agricultural Technology and Biotechnology,
Agency for the Assessment and Application of Technology (BPPT)
The Republic of Indonesia

ATTACHED DOCUMENT

I. GENERAL REVIEW

The SLeCAMA Project was commenced on 01 April 2015, for strengthening the capacity of Indonesian researchers and institutions in drug discovery against tropical diseases including malaria and amebiasis using Indonesian bio-resources, through the collaborative joint researches by the Indonesian institutes, Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT") and its collaborating institutes in Indonesia, the Institutes of Sciences (hereinafter referred to as "LIPI") and the Institutes of Tropical Disease, Airlangga University(hereinafter referred to as "AU"), and Japanese institutions, University of Tsukuba (hereinafter referred to as "UT"), Kitasato University (hereinafter referred to as "KU") , University of Tokyo (hereinafter referred to as "U Tokyo") and MicroBiopharm Japan Co. Ltd (hereinafter referred to as "MBJ"). In April 2016, Nagasaki University came to be another Japanese collaborator.

In accordance with the Record of Discussions (hereinafter referred to as the "R/D"), signed on 17th February 2015 by Japan International Cooperation Agency (hereinafter referred to as "JICA") and BPPT and the Minutes of Meeting of 1st JCC meeting on 2nd February 2016, Japanese side has dispatched experts to SLeCAMA Project and has accepted Indonesian counterparts as trainees in KU, U Tokyo and UT. And Japanese side has been providing equipment for the laboratories located in both BPPT and AU to capacitate the drug discovery in the institutes based on requests by the Government of the Republic of Indonesia, total 80 items of equipment were provided and had been installed in BPPT (65 items) and AU (15 items) so far. There are 14 more items being procured in Indonesia now (5 items for BPPT, 9 items for AU).

Four (4) working teams and Japanese advisory team according to its working-based structure (WBS) of the SLeCAMA were re-established in 2016, namely A) WBS-1: Development of Biological Resources, B) WBS-2: Screening Extract Anti Malaria C) WBS-3: Screening Extract Anti Amebic, and D) WBS-4: Isolation and Purification Active Compounds among BPPT and Japanese advisory team namely E) WBS-5 Technical Support of Active Compounds Development.
Each working team has its leader and the leaders and members participate in the weekly meetings chaired by the Project Co-manager to implement the research activities systematically and effectively.

Both sides reviewed activities in respect to the implementation of the SLeCAMA Project based on the common implementation plan of the Project, which is described in the P.O. and the Tentative Project Design Matrix (hereinafter referred to as the "Tentative PDM") in the R/D and the Minutes of Meeting signed on 2nd February, 2016 by related institutions of both sides.

II. SUMMARY OF MEETING

Both sides reviewed and discussed the following issues:

1. Progress of Project Implementation in 2016

1) Microbes and Extract Preparation in 2016
As many as 883 microbes from the samples collected during the field exploration in May 2016 at Biak Island were isolated. More then 8400 extracts for first screening were prepared from the microbes that were registered in the culture collection.

2) Enzyme-based Screening in 2016
Enzyme-based screening was done over 6000 and 4000 extracts against DHODH/MQO and SAT1/CS3 enzymes for searching anti-malarial and anti-amebic activities, respectively.

3) Cell-based Screening in 2016
Cell-based screenings were done over 400 and 1200 extracts against *Plasmodium falciparum* 3D7 and *Entamoeba histolytica* HM-1:IMSS clone 6 for searching anti-malarial and anti-amebic activities, respectively. Both parasites, as well as 5 lines of mammalian cell for toxicity test, are maintained. Cell toxicity assay system against mammalian cells were also established.

4) Purification in 2016
21 extracts are still on the process of purification (antimalarial: 14, antiamoeba: 7)

5) Thirteen (13) turns of short-term Japanese researchers and a long term JICA Coordinator have been dispatched to SLeCAMA Project.

6) Japanese side accepted nine (9) Indonesian counterparts as short term trainees in KU, U Tokyo and UT

7) One researcher of AU was accepted as a long term trainee to join Ph.D. course in U.T.

8) The disbursement of budget for the SLeCAMA 2016 by BPPT was approximate Rp.345,000,000-.

9) Coordination Meeting to enhance a network for Indonesian research institutes was organized between AU and BPPT in August 2016.

10) Regular Meeting
The project technical meetings in BPPT have been organized weekly with all the working teams of the project in BPPT chaired by the Project Co-manager.

11) Laboratory equipment
All equipment procured since 2015 in Japan had been installed successfully in BPPT (54 items) and AU (9 items) in 2016

Total 17 items of locally procured equipment have been installed as well (BPPT:11 items, AU:6 items) in 2016

2. Tentative Plan for the Project Implementation in 2017

1) Field Exploration for collecting samples in 2017
The Togean island of Central Sulawesi would be the area to collect samples.

2) Microbial isolation and identification
More than 1,000 identified isolates are expected from the newly collected samples in 2017

3) Extracts preparation
More than 5,000 extracts are expected to be prepared for screening using microbial isolates from the collection, as well as from newly isolated microbes as mentioned in 2) above.

4) Screening of active extracts
More than 5,000 extracts expected to be screened in both fields of antimalarial and antiamoeba

5) Purification in 2017
The target in 2017 is to get 4 purified and structure-elucidated compounds

6) International Symposium
An international symposium is planned to organize around August 2017 to strengthen networks for drug discovery, the detail should be determined urgently.

7) Ten (10) members of working teams of BPPT are planned to participate in the training course organized in Japan, one (1) researcher of AU will participate in post-graduate degree course (Ph.D.) in UT.

8) Dispatching Japanese researchers in 2017
Around twenty six (26) turns dispatching Japanese researchers are planned tentatively

9) Laboratory equipment
Now 14 items of equipment (BPPT: 5, AU: 9) are being procured locally and the project is proposing budget for 8 more items (BPPT:6, AU 2) of equipment to JICA in 2017

3. Others

1) Implementation Arrangement including MTA
To define detail of cooperation scheme between BPPT and Japanese side, both sides should sign on the "Implementation Arrangement " as soon as possible.

2) Requirements of material transfer for microbial isolates
Due to low number of active extracts that were produced from recultured microbes and to accelerate the progress, especially in purification stage, deep analysis on microbial properties during storaging, handling, and reculturing is very urgent. Translocation of the interested microbial strains from Indonesia to Japan for this purpose, as well as preparation of related MTA (material transfer agreement), should be prepared as soon as possible.

3) Japanese Implementing Agency
Nagasaki University became member of implementing agency for SLeCAMA from Japan side since April 2016.

4) Handing over of equipment
Property right of provided equipment should be handed over to Indonesian side from JICA. After confirmation of the required official transaction by BPPT, JICA will hand over to BPPT.

5) Alteration of project implementation structure in 2017
Due to decision of BPPT in January 2017, the implementing unit of the SLeCAMA Project of BPPT re-changed from "Center for Pharmaceutical and Medical Technologies of BPPT" to "**Biotech Center of BPPT**"

6) Alteration of JCC members
In accordance with the alteration of project implementation structure, the members of JCC among Indonesian side have changed as follows;

(a) The Indonesian side
- Project Manager : Head, Biotech Center of BPPT
- Project Co-Managers: Program Head, Biotech Center-BPPT, Director, Institute of Tropical Disease, AU, and Head, Indonesian Culture Collection (InaCC) Research Center for Biology-LIPI

7) Update on research members
The List of Researchers was updated as the attached version #1.

ANNEX
1. List of Researchers as of Jan 2017
2. Progress 2016 and Planning 2017 (Biotech Center-BPPT)
3. Report activities of ITD-AU
4. 2016 ACCOMPLISHMENT / 2017 PLAN "Issues to be solved" (Chief Advisor)

List of Researchers (As of January 2017)

Reaserch Subject	The Indonesian Side	The Japanese Side
Output 1: Compounds with anti-malarial activity are identified from the extracts on Indonesian biological resources (microorganism, plants, etc.		
1.1. Primary screening for inhibitory activity of extracts to the plasmodium-derived recombinant enzyme	• Erwahyuni E. Prabandari (BPPT) • Endah Dwi Hartuti (BPPT) • Tiara Zovi Putri (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Wan Xinying (U. Tokyo) • Kota Mochizuki (Nagasaki Univ)
1.2. Secondary screening for selective inhibitory activity of the extracts to the proliferation of Plasmodium falciparum	• Danang Waluyo (BPPT) • Dian Japany Puspitasari (BPPT) • Nadia Adipratiwi (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Keisuke Komatsuya (U. Tokyo) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
1.3. Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1- and 1-2	• Danang Waluyo (BPPT) • Dian Japany Puspitasari (BPPT) • Nadia Adipratiwi (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Keusuke Komatsuya (U. Tokyo) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
1.4. Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	• Anis H. Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
1.5. Establishment of mass production system of the lead compounds candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BPPT) • Kristiningrum(BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)

1.6. Determination of chemical structures of the lead compound candidate	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
1.7. Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	• Agung Eru Wibowo (BPPT) • Kurnia Agustini (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Keisuke Komatsuya (U. Tokyo)
1.8. Discussion of future direction of derivatization on the basis of the structure biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT) • Agung Eru Wibowo (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological resources (microorganism, plants, etc)		
2.1. Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica-derived* site-specific recombinant enzyme	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU) •	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)
2.2. Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)

2.3. Screening for selective inhibitory activity of extracts to the extracts of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)
2.4. Isolation and purification of chemical compounds with inhibitory to the proliferation against *Entamoeba histolytica*	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
2.5. Establishment of mass production system of the lead compound candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BBPT) • Kristiningrum(BPPT)	• Azuma Watanabe (MBJ) • Noriaki Sakata (MBJ)
2.6. Determination of chemical structures of the lead compound candidates	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
2.7. Selection of lead compound(s) through in vitro assessment and subsequent animal testing	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari(AU) • Hikatul Ilmi(AU) • Lidya Tumewu(AU)	• Tomoyoshi Nozaki (UT) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID) •

2.8. Discussion on future direction of derivatization on the basis of the structure biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT) • Agung Eru Wibowo (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Tomoyoshi Nozaki (UT) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 3: Technologies and research system for drug discovery using biological resources are established at the Indonesian research institute		
3.1. Sample collection and additional registration of newly-obtained extracts to the biological resources library	• Puspita Lisdiyanti (LIPI) • Atit Kanti, (LIPI) • Muhammad Ilyas (LIPI) • Ade Lia Putri(LIPI) • Dyah Noor Hidayati (BPPT) • Suryani (BPPT) • Kristiningrum(BPPT)	• Atsuko Matsumoto (KU) • Ken-ichi Nonaka (KU) • Azuma Watanabe (MBJ) • Noriako Sakata (MBJ) • Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (Nagasaki Univ)
3.2. Establishment of screening systems	• Erwahyuni E. Prabandari (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU) • Dwi Peni Kartikasari(AU)	• Tomoyoshi Nozaki (UT) • Daniel Ken Ianoka (Nagasaki Univ) • Wan Xinying (U. Tokyo) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
3.3. Establishment of culture and evaluation system	• Danang Waluyo (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (UT) • Daniel Ken Inaoka (Nagasaki Univ) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)

3.4. Introduction of technologies of isolation and purification	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
3.5. Introduction of technologies of chemical structure elucidation	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita
3.6. Establishment and enhancement of a research network in Indonesia	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Agung Eru Wibowo (BPPT) • Ahmad Fuad Hafid (AU) • Puspita Lisdyanti (LIPI)	• Tomoyoshi Nozaki (UT) • Daniel Ken Ianoka (Nagasaki Univ) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)

Institution Abbreviation:

- BPPT: Agency for the Assessment and Application Technology
- AU: Institute for Tropical Disease, Airlangga University
- LIPI: Biotechnology Research Institute, Indonesia Institute of Science
- U. Tokyo: University of Tokyo
- KU: Kitasato University
- MBJ: MicroBiopharm Japan, Co., Ltd.
- UT: University of Tsukuba
- NIID: National Institute of Infectious Diseases of Japan

BPPT
Japan International Cooperation Agency
LIPI Indonesian Institue of Science
Airlangga University
筑波大学 University of Tsukuba
北里大学 KITASATO UNIVERSITY
東京大学 The University of Tokyo
MicroBiopharm Japan
AMED Agency for Medical Research and Development

The 2nd Joint Coordinating Committee Meeting

The Project for Searching Lead Compound of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

SATREPS SLeCAMA Project

Progress 2016 and Planning 2017

Danang Waluyo
Program Head

Laboratory for Biotechnology, BPPT, Serpong
January 25th, 2017

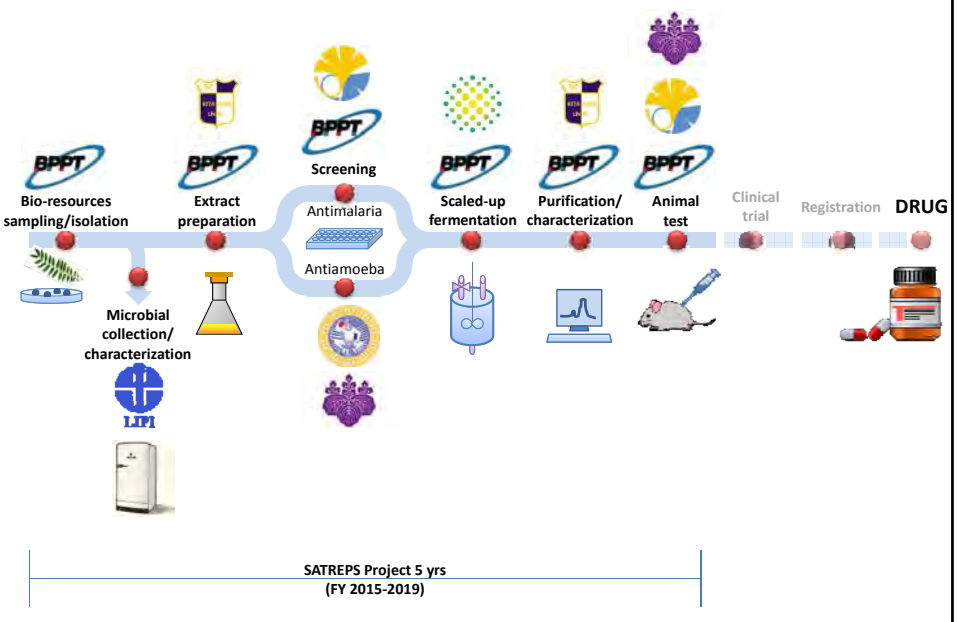
Content

Target Review

Project purpose/Outputs	Indicator	Time achievement (est. time)
0. Research capacity is enhanced	0-1. 1< lead compound (antimalaria) 0-2. 1< lead compound (antiamoeba) 0.3. 2< papers	0-1. 5th year (Mar 2020) 0-2. 5th year (Mar 2020) 0-3. 5th year (Mar 2020)
1. Compounds with anti-malarial activity are identified	1-1. 1< isolated and purified compound 1-2. 1< structure elucidated compound 1-3. 1< efficacy tested compound	1-1. Mid-term review (Jan 2018) 1-2. Terminal evaluation (Oct 2019) 1-3. 5th year (Mar 2020)
2. Compounds with anti-amebic activity are identified	2-1. 1< isolated and purified compound 2-2. 1< structure elucidated compound 2-3. 1< efficacy tested compound	1-1. Mid-term review (Jan 2018) 1-2. Terminal evaluation (Oct 2019) 1-3. 5th year (Mar 2020)
3. Technologies and research system for drug discovery using biological resources are established	3-1. 10,000< microbes, plants, extracts are registered 3-2. Enzyme-based screening system are established 3-3. Cell-based screening system are established 3-4. Technologies of Isolation and purification are introduced 3-5. Technologies of chemical structure analysis are introduced 3-6. 2< international symposium are held	3-1. 3rd year (Mar 2018) 3-2. 2nd year (Mar 2017) 3-3. 3rd year (Mar 2018) 3-4. Terminal evaluation (Oct 2019) 3-5. Terminal evaluation (Oct 2019) 3-6. 3rd and 5th year (Aug 2017 and Aug 2019)

Ref: Record of Discussion

Research Flowchart



Progress 2016 Overview

	2015	2016	Total
Newly Isolated microbes	901	883	1784 (Total collection 25,435)
Total prepared extracts for screening	800	8,480	9,280
Enzyme based screening: DHODH	1440	6039	7479
Enzyme based screening: MQO	480	3319	3,799
Enzyme based screening: CS3	5200	2240	7,440
Enzyme based screening: SAT1	0	2240	2,240
Cell-based screening: *P.falciparum*	320	480	800
Cell-based screening: *E.histolytica*	320	1240	1560
Purification (finished/undergone)	DHODH: 3	DHODH: 0/7 CS3: 0/4 MQO: 0/7 *E.histolytica*: 0/3	3/21
Structure elucidation (finished/undergone)	DHODH: 2	DHODH: 0/1	2/1

Progress 2016 Field Exploration

Sampling

Sampling location	Biak island
Sampling date	June 23-27, 2016
Number of sample	127 (soils)
Number of sampling point	24

Isolation

Method

Type of Microbes	Isolation method
Actinomycetes	High Heating, Wet Heating, Matsumoto's method
Fungi	Serial dilution method with 6 medium (LCA, OGA, SEA, MEA, LiClA, MRBA)

Result

Type of Microbes	Number of soil sample	Number of isolate
Actinomycetes	30	405
Fungi	30	478

Data as per Dec 30th, 2016

Progress 2016

Microbes Isolation and Extract Preparation

Identification Result

Type of Microbes	Method	Number of Identified isolate
Actinomycetes	Morphological observation	359
Fungi	Morphological observation	701

Data as per Dec 30th, 2016

Current Status of Microbial Collection

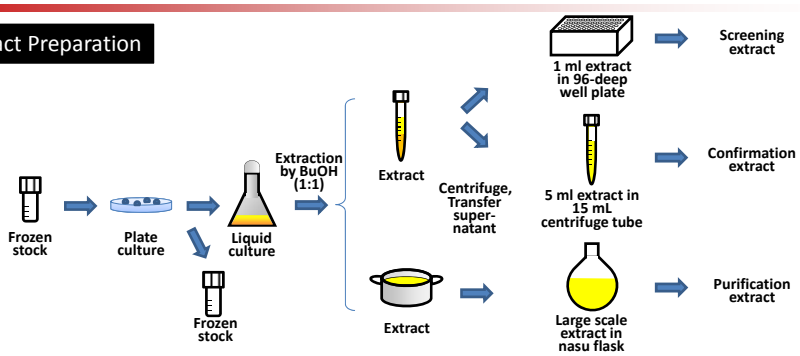
Type of Microbes	Old collection (<2015)	Ambon collection (2015)	Biak collection (2016)	Total
Actinomycetes	11,266	500	405	12,221
Fungi	12,335	401	478	13,214
Total	23,601	901	883	25,435

Data as per Dec 30th, 2016

Progress 2016

Microbes Isolation and Extract Preparation

Extract Preparation



Type of Extract	Scale	Actinomycetes	Fungi	Total
Screening extract	30 ml culture 1 ml extract	4,480	4,000	8,480
Confirmation extract	30 ml culture 5 ml extract	87	254	341
Purification extract	5 L or 100 mL culture, all were extracted	-	19	19

Data as per Dec 30th, 2016

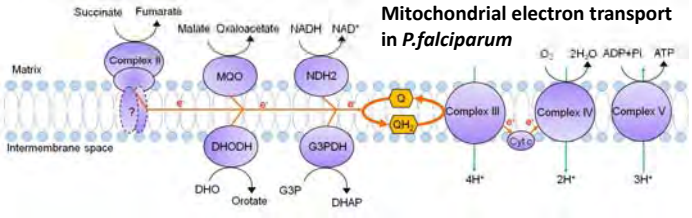
Progress 2016 | Screening of Active Extract | Anti-malaria

Enzyme-based screening

Screening target: extracts with inhibitory activity for DHODH and MQO

DHODH : Dihidroorotate dehydrogenase
MQO : Malate-quinone oxidoreductase

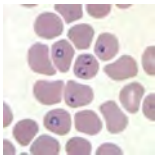
Mitochondrial electron transport in *P.falciparum*



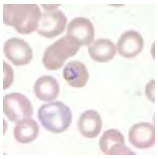
Cell-based screening

Screening target: extracts with inhibitory activity for proliferation of *Plasmodium falciparum*

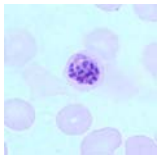
Life-stage of *Plasmodium falciparum*



Ring-form trophozoites



Trophozoites

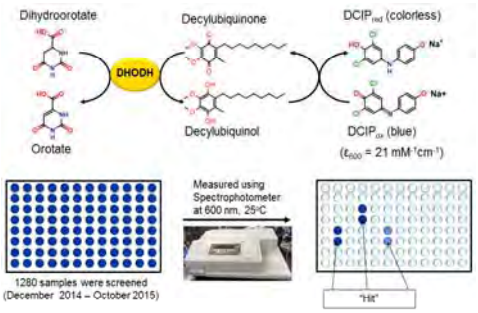


Schizonts

Progress 2016 | Screening of Active Extract | Anti-malaria

Screening (pfDHODH)

Method



Transfered **2 µl** of microbial extract to 96 well plate

↓

Added 192 **µl** of assay mix
[100 mM HEPES (pH 8.0), 150 mM NaCl, 10% (v/v) glycerol, 0.05% (w/v) Triton X-100, 20 nM, PfDHODH 18 µM decylubiquinone, 120 µM DCIP]

↓

Added 8 µl of 5mM (final 200 uM)

↓

Followed reduction of DCIP every 1 min for 1200 seconds
25°C at 600 nm

↓

Calculated % remaining activity to control

↓

Define "hit" as: < 50% of remaining activity

Remark :

- Amount of microbial extract (x µl) will be vary depending on the sample preparation. Amount of assay mix (y µl) was added up to final volume 200 µl.
- Sample preparation :
  1 ml of extract (exact concentration in mg/ml was unknown) was evaporate to dry. Dissolved the dry extract in 100 µl of DMSO (1st screening) or 40 µl of DMSO (2nd and 3rd screening)

Progress 2016 — Screening of Active Extract — Anti-malaria

Screening (pfDHODH)

Result

Number of extracts	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
5200 (prepared <2013)	Takemoto	50	50	9
1280 (including extracts prepared in 2015)	Nuni, Endah, Ery	6	6	1 isolate *)
6039 (including 119 plant extracts)	Nuni, Tiara	117	47	21**)

Data as per Dec 30th, 2016

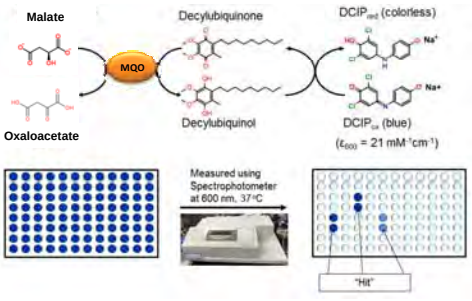
Total number screened extract = **12,519 extracts**

*) in solid state fermentation
**) 2 of those are being purified

Progress 2016 — Screening of Active Extract — Anti-malaria

Screening (pfMQO)

Method



Transfered **x µl** of microbial extract of to 96 well plate

↓

Added **y µl** of assay mix
[50 mM HEPES (pH 7.0), 1 mM KCN, 60 µM decylubiquinone, 120 µM DCIP, 1.51 µl/ml PfMQO membrane]

↓ 37°C at 600 nm

Measured the background for 180 seconds

↓

Added 10 mM of Sodium malate

↓

Followed reduction of DCIP every 1 min for 480 seconds

↓ 37 °C at 600 nm

Calculated % remaining activity relative to control

↓

Define "hit" as: < 20% of remaining activity

Remark :

- Amount of microbial extract (x µl) will be vary depending on the sample preparation. Amount of assay mix (y µl) was added up to final volume 200 µl.
- Sample preparation :
  1 ml of extract (exact concentration in mg/ml was unknown) was evaporate to dry. Dissolved the dry extract in 100 µl of DMSO (1st screening) or 40 µl of DMSO (2nd and 3rd screening)

Progress 2016 — Screening of Active Extract — Anti-malaria

Screening (pfMQO)

Result

Extract	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
480 (including extract prepared in 2015)	Nuni, Ery	74	74 (only 56 was revived)	29
1399	Nuni, Tiara	89	*)	

Data as per Dec 30th, 2016

Total number screened extract = **1,879 extracts**

*) To be recultured soon

Progress 2016 — Screening of Active Extract — Anti-amoeba

Enzyme preparation ⇨ Enzyme-based screening ⇨ Hit confirmation

Enzyme preparation

Method

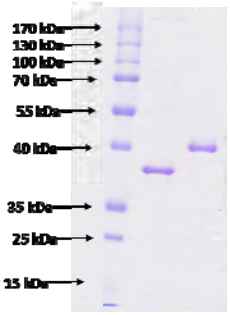



Enzyme	Producer	Cultivation method	Lysis	Purification
CS3	*E.Coli* BL21 (DE3) pET 15b	500 ml 2xYT (in 2L flask), 37°C, 200 rpm, induced by IPTG 200 uM at OD_{600}=0.6. Continue at 20^{0}C, 200 rpm, overnight	Sonication	Ni-NTA column
SAT1	*E.coli* BL21 (DE3) pET 15b	500 ml 2xYT (in 2L flask), 37°C, 200 rpm, induced by IPTG 200 uM at OD_{600}=0.6. Continue at 20^{0}C, 200 rpm, overnight	Sonication	Ni-NTA column

Progress 2016 Screening of Active Extract Anti-amoeba

Result

Enzyme	Specific activity	Yield/stock concentration	Storage
EhCS3	ND	1.7ml/34.86 mg/ml	-80°C
EhSAT1	ND	Precipitated	-



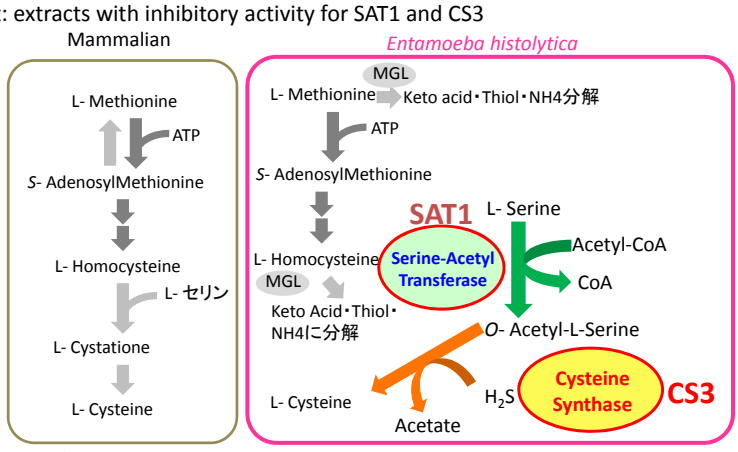
Remarks:
1 : Marker
2 : CS3
3 : SAT1

Progress 2016 Screening of Active Extract Anti-amoeba

Enzyme-based screening

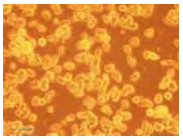
Screening target: extracts with inhibitory activity for SAT1 and CS3

Cysteine biosynthesis pathway



Cell-based screening

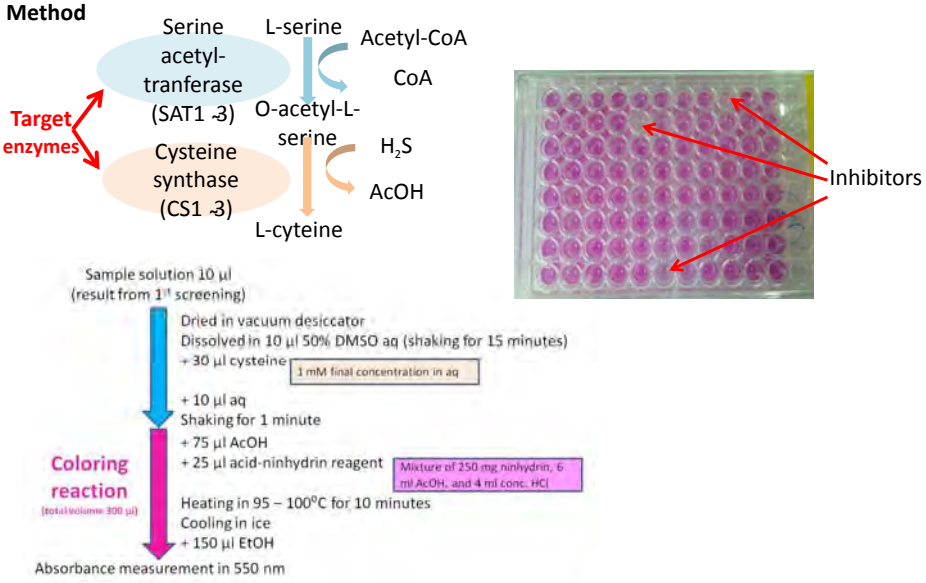
Screening target: extracts with inhibitory activity for proliferation of *Entamoeba histolytica*



Progress 2016 — Screening of Active Extract — Anti-amoeba

Screening **(EhCS3 and EhSAT1)**

Method



Progress 2016 — Screening of Active Extract — Anti-amoeba

Screening **(EhCS3 and EhSAT1)**

Result

Enzyme	Extract	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
CS1/CS3	5200 (extracts prepared <2013)	Amila	33	15	4*)
	2240	Myrna, Ratna, Peny	21	**)	
SAT1	2240	Myrna, Ratna, Peny	28	28 (only 17 were revived)	***)

Data as per Dec 30th, 2016

Total number screened extract = **6,720 extracts**

*) in progress for purification
**) Being revived from frozen stock
***) To be assayed

Progress 2016

Screening of Active Extract

Anti-amoeba

Screening **(*E.histolytica* cell-based screening)**

Method

- Samples dilute in 1ml 50% DMSO
- Concentration DMSO in culture medium 1% :
  - Prepare sample mix = 245µl media BIS + 5µl sample in DMSO 50% → mix pipetting
- *E. histolytica* clone-6 subculture in 96 well plate; 200µl/well; 6000cell/well; incubation 1hr 35.5°C
- After 1hr, discard medium BIS; add 200µl sample mix in to each well; incubate 24hr 35.5°C
- After 24hr, discard medium BIS;
- add 10x WST-1 in Opti MEM1x, 100µl/well
  - Prepare 10x WST-1 : 1plate 96well (1ml WST-1 + 9ml Opti MEM1x)
- Incubate 20 min 37°C, read absorbance 450nm

Progress 2016

Screening of Active Extract

Anti-amoeba

Screening **(*E.histolytica* cell-based screening)**

Result

Extract	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
1,240	Myrna, Ratna, Peny	49	4	4*)

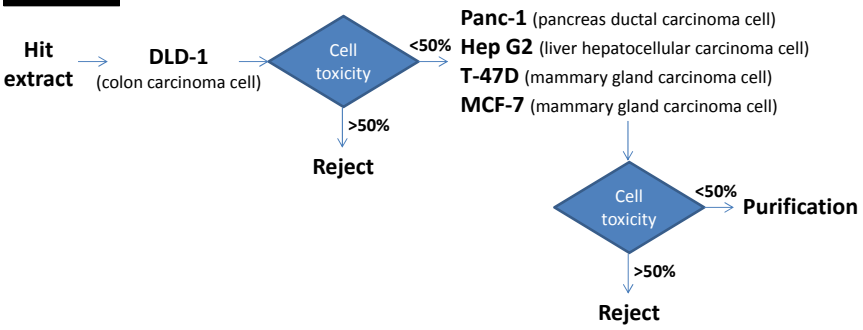
Data as per Dec 30th, 2016

Total number screened extract = **1,240 extracts**

*) Recultured microbes were determined after dose-dependent test and toxicity test against MRC-5

Progress 2016 — Screening of Active Extract — Toxicity Assay

Screening **(Cell toxicity test)**



Activity	Number of extracts	Non-toxic agaist DLD-1	Non-toxic against others
Inhibitor of pfDHODH	38	28	24
Inhibitor of pfMQO	55	49	20
Inhibitor of proliferation of *E.histolytica*	32	-*)	4*)

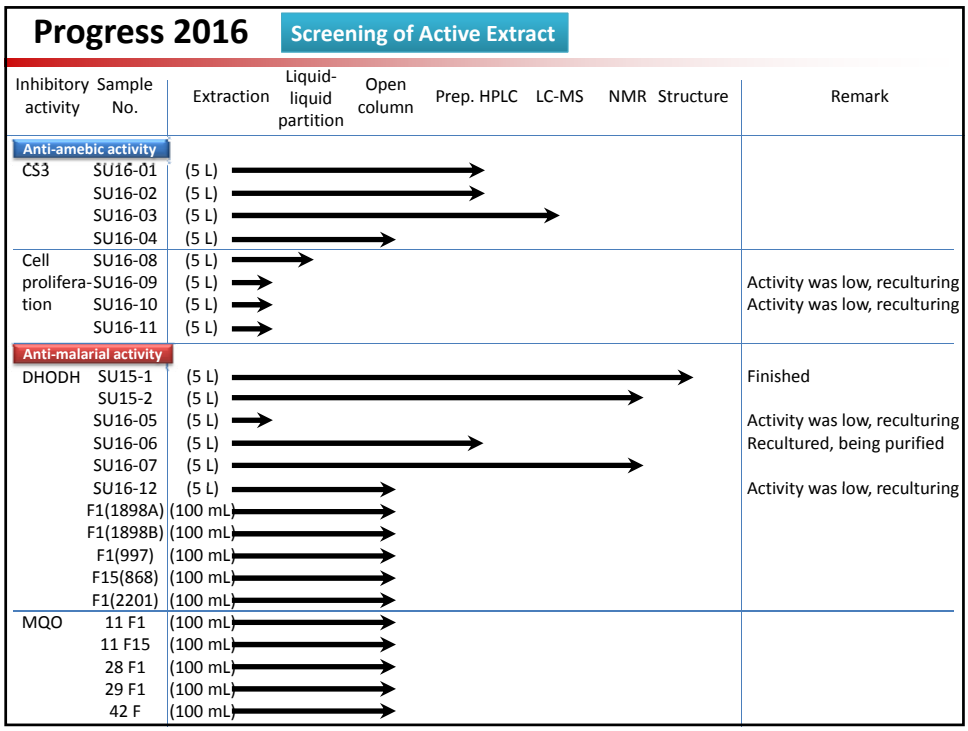
Data as per Dec 30th, 2016

*) Toxicity test were done against MRC-5 cell (fetal lung fibroblast cell)

Progress 2016 — Screening of Active Extract

Cultivation
↓
Extraction – – – – – Assay
↓
Liquid-liquid partition – – – – – TLC and assay
↓
Column chromatograpy I, II ... – – – – – TLC, LC-UV and assay
↓
Preparative HPLC I, II – – – – – TLC, LC-UV and assay
↓
Structure elucidation – – – – – LC-MS and NMR

Progress 2016 — Screening of Active Extract



Progress 2016 — Technical Support

Training in Japan

No	Name	Home Institution	Title of Training	Duration of Training			Days	Training Venue
1	Ms. Ratna Wahyuni Zainuri	Airlangga University	Cultivation of Entamoeba Histolytica and Production, Purification and Assays of Amebic Enzymes	18-Jan-2016	~	17-Mar-2016	60	National Institute of Infectious Diseases
2	Mr. Dwi Peni Kartikasari	Airlangga University	Cultviation and screening of microorganisms and enzymes for the development of anti amebic compounds	9-May-2016	~	20-Jun-2016	43	National Institute of Infectious Diseases
3	Ms. Eka Siska	BPPT	Isolation and Purification of active compounds	2-Oct-2016	~	29-Oct-2016	28	Kitasato University
4	Ms. Diana Dewi	BPPT	Microbial isolation and extract production	2-Oct-2016	~	29-Oct-2016	28	Kitasato University
5	Ms. Amila Pramisandi	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Purification)	23-Oct-2016	~	5-Nov-2016	14	Kitasato University
6	Mr. Danang Waluyo	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Screening)	6-Nov-2016	~	17-Dec-2016	42	National Institute of Infectious Diseases
7	Dr. Erwahyuni E. Prabandari	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Screening)	6-Nov-2016	~	17-Dec-2016	42	Kitasato University
8	Dr. Anis H. Mahsunah	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Purification)	7-Nov-2016	~	3-Dec-2016	27	Kitasato University
9	Ms. Nurlaila	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Purification)	7-Nov-2016	~	3-Dec-2016	27	Kitasato University
10	Ms. Ratna Wahyuni Zainuri	Airlangga University	(Long-term training)	1-Apr-2016	~	31-Mar-2019	(3 yrs)	University of Tsukuba

Progress 2016 Technical Support

Training in Indonesia

No	Name of Expert	University	Expertise	Duration of Visit		days
1	Dr. Ken Daniel INAOKA	University of Tokyo	Malaria (Investigation and Analysis)	25/Jan/16	4/Mar/16	40
2	Prof. Tomoyoshi NOZAKI	University of Tsukuba	Chief Advisor/Tropical Medicine Research	31/Jan/16	2/Feb/16	3
3	Dr. Atsuko MATSUMOTO	Kitasato University	Collection and Isolation of Microbial Reserources	31/Jan/16	18/Feb/16	19
4	Dr. Azuma WATANABE	MicroBiopharma Japan	Isolation, Purification and Structure Analysis of Chemical Compounds	31/Jan/16	4/Feb/16	5
5	Dr. Kazuro SHIOMI	Kitasato University	Isolation, Purification, and Structure Analysis of Chemical Compounds	31/Jan/16	3/Feb/16	4
6	Dr. Daisuke TAKEMOTO	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	18/Apr/16	16/Jun/16	60
7	Prof. Tomoyoshi NOZAKI	University of Tsukuba	Chief Advisor/Tropical Medicine Research	22/May/16	25/May/16	4
8	Dr. Ken Daniel INAOKA	University of Tokyo	Malaria (Investigation and Analysis)	7/Aug/16	9/Sep/16	34
9	Dr. Yukiko MIYAZAKI	University of Tokyo	Malaria (Investigation and Analysis)	7/Aug/16	9/Sep/16	34
10	Dr. Mihoko MORI	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	5/Sep/16	25/Sep/16	21
11	Prof. Tomoyoshi NOZAKI	University of Tsukuba	Chief Advisor/Tropical Medicine Research	14/Nov/16	22/Nov/16	9

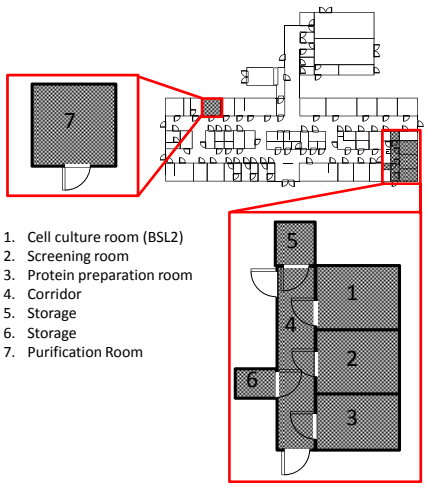
Progress 2016 Technical Support Equipment Installation

Name	Maker	Location
Biosafty Cabinet IIA	AIRTECH	ITD AU
Microscope	CKX41	ITD AU
High Speed Refriegerated Micro Centrifuge	MX-107	ITD AU
Bio Freezer	GS-5210HC	ITD AU
Bench-top Centrifuge	LC-230, Roter TS-40LB, Adaptor	ITD AU
Bio Medical showcase	BMS-501F3(500L)	ITD AU
Incubator	IS401	ITD AU
Biosafty Cabinet IIA (2)	AIRTECH	BPPT
UV-Vis Spectrophotometer	JASCO	BPPT
Ultrasonic Crusher(DIGITAL)	Branson	BPPT
96-well Microtiter Plate Reader	Molecular Device	BPPT
Ultracentrifuge	HITACHI	BPPT
Rotor for Ultracentrifuge	HITACHI	BPPT
HPLC (PDA Detector) (2)	Shimadzu	BPPT
Incubator	ASTEC	BPPT
HPLC-Column (2 sets)	SHISEIDO	BPPT
Incubator	ASTEC	BPPT
Flask Plate for Rotary Shaker	IWASHIYA BIO SCIENCE	BPPT
High Speed Refrigerated Centrifuge	TOMY	BPPT
Rotor	TOMY	BPPT
High Speed Refrigerated Centrifuge	TOMY	BPPT
Resin and Gel for Chromatography		BPPT
Electric Pipette 12 channel (4 sets)	Mettler Toledo	BPPT
Multichannel Pipette (8)	Nichiryo	BPPT
Ergonomic pipette (10)	Nichiryo	BPPT
Glass column		BPPT
Ultrasonic Cleaner	AS ONE	BPPT
Liquid Nitrogen Tank 30L	CEBELL	BPPT
Biomedical Freezer (513Lt)	Nihon Freezer	BPPT
Glasswares		BPPT
Analytical Balances	Shimadzu	BPPT
Agarose Gel Electrophoresis	Atto	BPPT
Fraction Collector	BIO RAD	BPPT
EGP Combo	BIO RAD	BPPT

Progress 2016

Technical Support | Equipment Installation

Laboratory layout



1. Cell culture room (BSL2)
2. Screening room
3. Protein preparation room
4. Corridor
5. Storage
6. Storage
7. Purification Room

Arrival


Installation


After installation


Progress 2016

Budget Arrangement

- Initial budget = Rp. 450.000.000
- 1st Budget optimization = Rp. 426.370.000
- 2nd Budget optimization = Rp. 390.050.000

Description	Budget (Rp.)	Realization (Rp.)	Note
Reagents and consumables	185.000.000	184.452.400	
Salaries	160.000.000	128.000.000	Budget optimization (the remained budget could not be used)
Stationaries	4.630.000	4.629.900	
Travels	40.420.000	27.873.900	Budget optimization (the remained budget could not be used)
TOTAL	**390.050.000**	**344.956.200**	

Planning 2017

1. **Field expedition**
   - Location: Togean Island, Central Sulawesi

2. **Microbial isolation and identification**
   - Target: 1000 identified isolates
3. **Extract preparation**
   - Target: 5000 extracts for screening
4. **Screening of active extract**
   - Target:
     a. Anti-malaria : 5000 extracts
     b. Anti-ameba : 5000 extracts
5. **Purification of active compound**
   - Target: 4 purified and structure-elucidated compounds
6. **International symposium**
   - Time and venue: (to be determined)
7. **Publication**
   - Target: submission of 2 international peer-reviewed papers

Planning 2017

Training and Technical Support

Training in Japan

No	Name	Home Institution	Title of Training	Duration of Training		Days	Training Venue
1	Mr. Danang Waluyo	BPPT	Cell toxicity test of active compounds/in vivo assay of active compounds	3-Feb-2018	31-Mar-2018	28	University of Tokyo
2	Dr. Erwahyuni E. Prabandari	BPPT	Production of enzyme for screening of antiparasitic active compounds	23-Apr-2017	20-May-2017	28	University of Tokyo
3	Dr. Anis H. Mahsunah	BPPT	Structure elucidation of active compound	4-Feb-2018	3-Mar-2018	28	Kitasato University
4	Ms. Diana Dewi	BPPT	Optimization of large scale cultivation for active compound production	4-Feb-2018	3-Mar-2018	56	Kitasato University
5	Ms. Eka Siska	BPPT	Structure elucidation of active compound	17-Sep-2017	11-Nov-2017	56	Kitasato University
6	Ms. Nurlaila	BPPT	Purification of active compound	17-Sep-2017	11-Nov-2017	29	Kitasato University
7	Sasmito	BPPT	Purification of active compound	9-Jul-2017	6-Aug-2017	28	Kitasato University
8	Nuki Bambang Nugroho	BPPT	Purification of active compound	9-Jul-2017	5-Aug-2017	28	Kitasato University
9	Ms. Endah Dwi Hartuti	BPPT	(Long-term training)	(TBD)		(3 yrs)	Nagasaki University
10	Ms. Amila Pramisandi	BPPT	(Long-term training)	1-Apr-2017	~ 31-Mar-2020	(3 yrs)	Kitasato University
11	Ms. Dian Japany Puspitasari	BPPT	(Long-term training)	(TBD)		(3 yrs)	(TBD)
12	Dr. Myrna Adianti	Airlangga University	Cell toxicity assay and new enzyme assays for antiamebic compound discovery	23-Apr-2017	23-Jun-2017	62	U Tokyo (April 23-June 20)
13	Mr. Dwi Peni Kartikasari	Airlangga University	(Long-term training)	(TBD)		(3 yrs)	(TBD)
14	Rini Riffiani	LIPI	Drug discovery of antimalarials	(TBD)			(TBD)
15	A'liyatur Rosyidah	LIPI	Drug discovery of antiamebics	(TBD)			(TBD)

Planning 2017

Budget Arrangement

- BPPT allocated budget for FY 2017 as much as Rp. 500.000.000
- BPPT is currently applying some proposals to several funding agency, including Ministry of Research, Technology and Higher Education, and DIPI (The Indonesian Science Fund), with total of proposed budget is as much as Rp. 3.245.000.000

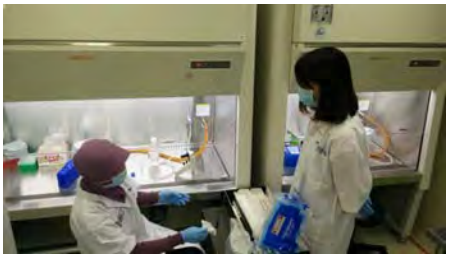
Description	Budget (Rp.)	Note
Salaries	196.000.000	7 persons
Meeting	46.530.000	JCC meeting, international symposium, internal meeting
Reagents and consumables	207.360.000	Microbial isolation, extract preparation, screening, purification
Travels	50.110.000	Field exploration, meeting
TOTAL	**500.000.000**	

Planning 2017

Project Management

Implementing unit	**Laboratory for Biotechnology-BPPT (Biotech Center)**
Project Director	**Prof. Dr. Eng. Eniya Listyani Dewi, B.Eng., M.Eng.** (Deputy Chairperson of Technology for Agroindustry and Biotechnology, BPPT)
Project Manager	**Dr. Agung Eru Wibowo, Apt.** (Head of Laboratory for Biotechnology, BPPT)
Project Co-manager	**Danang Waluyo, M.Eng.** (Program Head, BPPT)
Project Co-manager	**Prof. Maria Inge Lusida, M.Kes., Sp.MK(K), Ph.D.** (Head of Institute of Tropical Disease, Airlangga University)
Project Co-manager	**Dr. Atit Kanti, M.Sc.** (Head of InaCC, LIPI)

THANK YOU



Report activities of ITD-UNAIR

"Project for Searching Lead Compounds of anti-Malarial and Anti-Amebic Agent by Utilizing Diversity of Indonesian Bio-Resources"

BPPT-Biotech Center,
25 January 2017

Second Year activities

- Training in Japan:
  - Mrs. Peni : 2 x training (May-June 2016 & January-February 2017)
  - Mrs. Ratna (scholarship for doctoral program start from April 2016)
- Amoeba laboratorium set up
- Consumables (reagents and plasticware)
- Training from Japanese researcher to ITD-UNAIR for enzyme production
- Screened dried extract from BPPT (Cell culture based and enzymatic based screening)

Lab. set up

- Laboratorium set up for Entamoeba cell culture system.

ITEM NO.	EQUIPMENT NAME	Mfr	MODEL	Q'TY
1	BIO FREEZER	NIHON FREEZER CO.,LTD.	GS-5210HC	1 set
2	BIO MEDICAL SHOWCASE	NIHON FREEZER CO.,LTD.	BMS-501F3	1 set
3	INCUBATOR	Yamato Scientific Co., Ltd.	IS401	4 sets
4	Stacking Support	Yamato Scientific Co., Ltd.	OD40	2 sets
5	BIOSAFETY CABINET Ⅱ A	AIRTECH JAPAN.LTD	BHC-1007 Ⅱ A2	1 set
6	HIGH SPEED REFRIGERATED MICRO CENTRIFUGE	TOMY KOGYO CO., LTD.	MX-107	1 unit
7	angle rotor for MX-107	TOMY KOGYO CO., LTD.	TMP-24	1 pc
8	LOW SPEED BENCH-TOP CENTRIFUGE	TOMY KOGYO CO., LTD.	LC-230	1 unit
9	INVERTED MICROSCOPE	Olympus Corporation	CKX41+DP22	2 sets
10	MONITOR (for Microscope)	Olympus Corporation		2 sets
11	swing-out rotor for LC-230	TOMY KOGYO CO., LTD.	TS-40LB, B240-96D, AS40-96D	1 set

- Additional equipment
  - PCR machine
  - Gel documentation system
  - Incubator shaker
  - Pipettes
  - Sonicator
  - Autoclave
- Laboratory assistance
  (2 persons start from November 2016)

Lab Equipments

- First batch of extracts (December 2015):
  - Fungi – 400 extracts
  - *Actinomycetes* – 400 extracts
- Second batch of extracts (March 2016):
  - Fungi – 640 extracts
  - *Actinomycetes* – 800 extracts
- Third Batch of extracts (Oktober 2016):
  - Fungi – extracts
  - *Actinomycetes* - extracts

Results

- Cell based screening
  - 48 hit extracts
- Enzymatic based screening (CS3 & SAT1)
  - 21 & 28 hit extracts
- Cell based with serial dilution concentration
  - 32 selected hit extracts
- Toxicity assay with MRC5 (*done in NIID by Ratna*)

Future Plan

- Toxicity assay training for ITD-UNAIR
- Primary screening and **secondary** screening of BPPT samples

JCC SECOND YEAR

The Project for Searching Lead Compound of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

2016 ACCOMPLISHMENT / 2017 PLAN

Issues to be solved

TOMO NOZAKI
CHIEF ADVISOR

Laboratory for Biotechnology, BPPT, Serpong
January 25th, 2017

Content

By Danang WALUYO

Progress 2016 Overview

	2015	2016	Total
Newly Isolated microbes	901	883	1,784 (Total collection 25,435)
Total prepared extracts for screening	800	8,480	9,280
Enzyme based screening: DHODH	1440	6039	7,479
Enzyme based screening: MQO	480	3319	3,799
Enzyme based screening: CS3	5200	2240	7,440
Enzyme based screening: SAT1	0	2240	2,240
Cell-based screening: *P.falciparum*	320	480	800
Cell-based screening: *E.histolytica*	320	1240	1,560
Purification (finished/undergone)	DHODH: 3	DHODH: 0/7 CS3: 0/4 MQO: 0/7 *E.histolytica*: 0/3	3/21
Structure elucidation (finished/undergone)	DHODH: 2	DHODH: 0/1	2/1

By Danang WALUYO

Progress 2016 Field Exploration

Sampling

Sampling location	Biak island
Sampling date	June 23-27, 2016
Number of sample	127 (soils)
Number of sampling point	24

Isolation

Method

Type of Microbes	Isolation method
Actinomycetes	High Heating, Wet Heating, Matsumoto's method
Fungi	Serial dilution method with 6 medium (LCA, OGA, SEA, MEA, LiClA, MRBA)

Result

Type of Microbes	Number of soil sample	Number of isolate
Actinomycetes	30	405
Fungi	30	478

Data as per Dec 30th, 2016

By Danang WALUYO

Progress 2016

Microbes Isolation and Extract Preparation

Identification Result

Type of Microbes	Method	Number of Identified isolate
Actinomycetes	Morphological observation	359
Fungi	Morphological observation	701

Data as per Dec 30th, 2016

Current Status of Microbial Collection

Type of Microbes	Old collection (<2015)	Ambon collection (2015)	Biak collection (2016)	Total
Actinomycetes	11,266	500	405	12,221
Fungi	12,335	401	478	13,214
Total	23,601	901	883	25,435

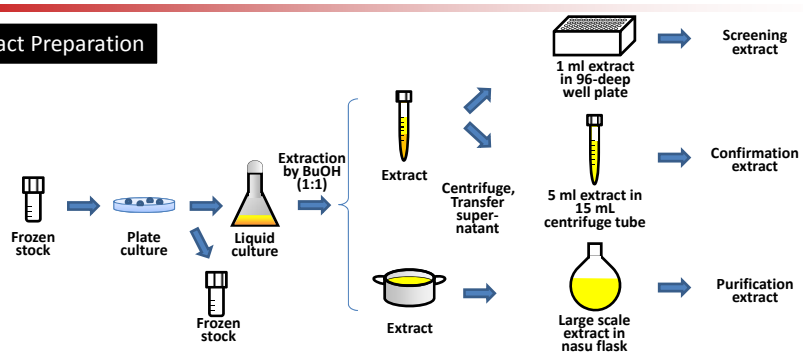
Data as per Dec 30th, 2016

By Danang WALUYO

Progress 2016

Microbes Isolation and Extract Preparation

Extract Preparation



Type of Extract	Scale	Actinomycetes	Fungi	Total
Screening extract	30 ml culture 1 ml extract	4,480	4,000	8,480
Confirmation extract	30 ml culture 5 ml extract	87	254	341
Purification extract	5 L or 100 mL culture, all were extracted	-	17	17

Data as per Dec 30th, 2016

By Danang WALUYO

ISSUES TO BE SOLVED

1. Characterization/archiving of Actinomycetes/fungal strains…..Publication
2. Cordination between BC/Airlangga U/InaCC……Sample transfer/record…..suggestion: every three months
3. Delay in cell-based screening
4. Loss of activities after reculture/confirmation
5. Exploration of new targets
6. Selection of primary and secondary

Progress 2016

Screening of Active Extract | **Anti-malaria**

Screening **(PfDHODH)**

Result

Number of extracts	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
5200 (prepared <2013)	Takemoto	50	50	9
1280 (including extracts prepared in 2015)	Nuni, Endah, Ery	6	6	1 isolate *)
6039 (including 119 plant extracts)	Nuni, Tiara	117	47	21**)

Data as per Dec 30th, 2016

Total number screened extract = **12,519 extracts**

*) in solid state fermentation
**) 2 of those are being purified

By Danang WALUYO

Progress 2016 — Screening of Active Extract — Anti-malaria

Screening (PfMQO)

Result

Extract	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
480 (including extract prepared in 2015)	Nuni, Ery	74	74 (only 56 was revived)	29
1399	Nuni, Tiara	89	*)	

Data as per Dec 30th, 2016

Total number screened extract = **1,879 extracts**

*) To be recultured soon

By Danang WALUYO

Progress 2016 — Screening of Active Extract — Anti-amoeba

Screening (EhCS3 and EhSAT1)

Result

Enzyme	Extract	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
CS1/CS3	5200 (extracts prepared <2013)	Amila	33	15	4*)
	2240	Myrna, Ratna, Peny	21	**)	
SAT1	2240	Myrna, Ratna, Peny	28	28 (only 17 were revived)	***)

Data as per Dec 30th, 2016

Total number screened extract = **6,720 extracts**

*) in progress for purification
**) Being revived from frozen stock
***) To be assayed

By Danang WALUYO

ISSUES TO BE SOLVED

1. Characterization/archiving of Actinomycetes/fungal strains…..Publication
2. Cordination between BC/Airlangga U/InaCC……Sample transfer/record…..suggestion: every three months
3. Exploration of new targets
4. Delay in cell-based screening
5. Loss of activities after reculture/confirmation
6. Selection of primary and secondary

Progress 2016

Screening of Active Extract

Anti-amoeba

Screening (*E.histolytica* cell-based screening)

Result

Extract	Screened by	No. of 1st screening hit	Re-culture status	No. of proposed hit
1,240	Myrna, Ratna, Peny	49	4	4*)

Data as per Dec 30th, 2016

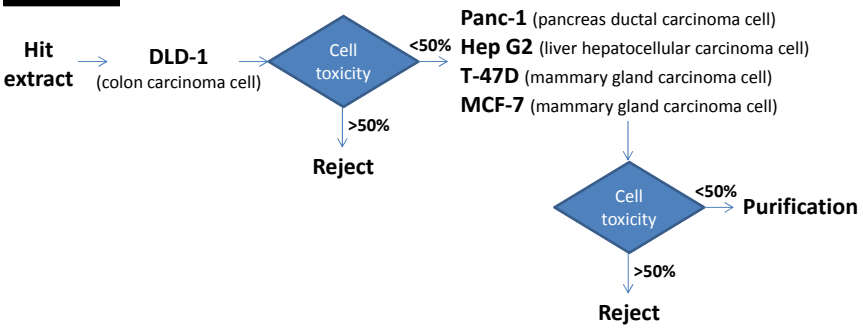
Total number screened extract = **1,240 extracts**

*) Recultured microbes were determined after dose-dependent test and toxicity test against MRC-5

Progress 2016

Screening of Active Extract | **Toxicity Assay**

Screening **(Cell toxicity test)**



Activity	Number of extracts	Non-toxic agaist DLD-1	Non-toxic against others
Inhibitor of pfDHODH	38	28	24
Inhibitor of pfMQO	55	49	20
Inhibitor of proliferation of *E.histolytica*	32	-*)	4*)

Data as per Dec 30th, 2016

*) Toxicity test were done against MRC-5 cell (fetal lung fibroblast cell)

By Danang WALUYO

ISSUES TO BE SOLVED

1. Characterization/archiving of Actinomycetes/fungal strains…..Publication
2. Cordination between BC/Airlangga U/InaCC……Sample transfer/record…..suggestion: every three months
3. Exploration of new targets
4. Delay in cell-based screening
5. Selection of primary and secondary mammalian cell lines for toxicity (counter) assay

Progress 2016 — Screening of Active Extract

Cultivation

↓

Extraction — Assay

↓

Liquid-liquid partition — TLC and assay

↓

Column chromatograpy I, II ... — TLC, LC-UV and assay

↓

Preparative HPLC I, II — TLC, LC-UV and assay

↓

Structure elucidation — LC-MS and NMR

By Danang WALUYO

Progress 2016 — Screening of Active Extract

Currently Undergone Active Compound Purification

Activity	Producer	Purified by	Current Status
Inhibitor of CS3	*Aspergillus fumigatus*	Nurlaila	Preparative HPLC
Inhibitor of CS3	(Not identified yet)	Eka	Preparative HPLC
Inhibitor of CS3	(Not identified yet)	Nuki	Liquid-liquid partition
Inhibitor of CS3	(Not identified yet)	Sasmito, Anis	Preparative HPLC
Inhibitor of pfDHODH	*Acremonium cellulolyticus*	Amila	Structure elucidation
Inhibitor of pfDHODH	(Not identified yet)	Amila	Structure elucidation

Data as per Dec 30th, 2016

Structure-elucidated compound

Activity	Producer	Purified by	Structure name
Inhibitor of DHODH	*Penicillium chrysogenum*	Anis, Amila	4-quinolone

By Danang WALUYO

ISSUES TO BE SOLVED

1. Characterization/archiving of Actinomycetes/fungal strains.....Publication
2. Cordination between BC/Airlangga U/InaCC.....Sample transfer/record.....suggestion: every three months
3. Delay in cell-based screening
4. Loss of activities after reculture/confirmation
5. Exploration of new targets
6. Selection of primary and secondary mammalian cell lines for counter assay
7. Prioritization of hits
8. Broadening of the bottleneck process(es) (purification/structure)

Progress 2016

Technical Support

Training in Japan

9 short term trainees: ~11 months
1 long term trainee: full year

No	Name	Home Institution	Title of Training	Duration of Training			Days	Training Venue
1	Ms. Ratna Wahyuni Zainuri	Airlangga University	Cultivation of Entamoeba Histolytica and Production, Purification and Assays of Amebic Enzymes	18-Jan-2016	~	17-Mar-2016	60	National Institute of Infectious Diseases
2	Mr. Dwi Peni Kartikasari	Airlangga University	Cultviation and screening of microorganisms and enzymes for the development of anti amebic compounds	9-May-2016	~	20-Jun-2016	43	National Institute of Infectious Diseases
3	Ms. Eka Siska	BPPT	Isolation and Purification of active compounds	2-Oct-2016	~	29-Oct-2016	28	Kitasato University
4	Ms. Diana Dewi	BPPT	Microbial isolation and extract production	2-Oct-2016	~	29-Oct-2016	28	Kitasato University
5	Ms. Amila Pramisandi	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Purification)	23-Oct-2016	~	5-Nov-2016	14	Kitasato University
6	Mr. Danang Waluyo	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Screening)	6-Nov-2016	~	17-Dec-2016	42	National Institute of Infectious Diseases
7	Dr. Erwahyuni E. Prabandari	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Screening)	6-Nov-2016	~	17-Dec-2016	42	Kitasato University
8	Dr. Anis H. Mahsunah	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Purification)	7-Nov-2016	~	3-Dec-2016	27	Kitasato University
9	Ms. Nurlaila	BPPT	Cultivation of entamoeba histolytica and production, purification, and assays of amebic enzyme (Purification)	7-Nov-2016	~	3-Dec-2016	27	Kitasato University
10	Ms. Ratna Wahyuni Zainuri	Airlangga University	(Long-term training)	1-Apr-2016	~	31-Mar-2019	(3 yrs)	University of Tsukuba

By Mitsuhiko IWASHITA/Danang WALUYO

Progress 2016 Technical Support

Expert dispatch to Indonesia

9 short term dispatch: 232 days

No	Name of Expert	University	Expertise	Duration of Visit		days
1	Dr. Ken Daniel INAOKA	University of Tokyo	Malaria (Investigation and Analysis)	25/Jan/16	4/Mar/16	40
2	Prof. Tomoyoshi NOZAKI	University of Tsukuba	Chief Advisor/Tropical Medicine Research	31/Jan/16	2/Feb/16	3
3	Dr. Atsuko MATSUMOTO	Kitasato University	Collection and Isolation of Microbial Reserources	31/Jan/16	18/Feb/16	19
4	Dr. Azuma WATANABE	MicroBiopharma Japan	Isolation, Purification and Structure Analysis of Chemical Compounds	31/Jan/16	4/Feb/16	5
5	Dr. Kazuro SHIOMI	Kitasato University	Isolation, Purification, and Structure Analysis of Chemical Compounds	31/Jan/16	3/Feb/16	4
6	Dr. Daisuke TAKEMOTO	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	18/Apr/16	16/Jun/16	60
7	Prof. Tomoyoshi NOZAKI	University of Tsukuba	Chief Advisor/Tropical Medicine Research	22/May/16	25/May/16	4
8	Dr. Ken Daniel INAOKA	University of Tokyo	Malaria (Investigation and Analysis)	7/Aug/16	9/Sep/16	34
9	Dr. Yukiko MIYAZAKI	University of Tokyo	Malaria (Investigation and Analysis)	7/Aug/16	9/Sep/16	34
10	Dr. Mihoko MORI	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	5/Sep/16	25/Sep/16	21
11	Prof. Tomoyoshi NOZAKI	University of Tsukuba	Chief Advisor/Tropical Medicine Research	14/Nov/16	22/Nov/16	9

By Mitsuhiko IWASHITA/Danang WALUYO

Progress 2016 Technical Support Equipment Installation

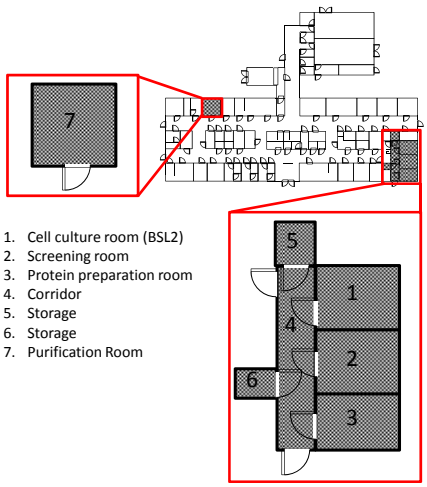
Name	Maker	Location
Biosafty Cabinet IIA	AIRTECH	ITD AU
Microscope	CKX41	ITD AU
High Speed Refriegerated Micro Centrifuge	MX-107	ITD AU
Bio Freezer	GS-5210HC	ITD AU
Bench-top Centrifuge	LC-230, Roter TS-40LB, Adaptor	ITD AU
Bio Medical showcase	BMS-501F3(500L)	ITD AU
Incubator	IS401	ITD AU
Biosafty Cabinet IIA (2)	AIRTECH	BPPT
UV-Vis Spectrophotometer	JASCO	BPPT
Ultrasonic Crusher(DIGITAL)	Branson	BPPT
96-well Microtiter Plate Reader	Molecular Device	BPPT
Ultracentrifuge	HITACHI	BPPT
Rotor for Ultracentrifuge	HITACHI	BPPT
HPLC (PDA Detector) (2)	Shimadzu	BPPT
Incubator	ASTEC	BPPT
HPLC-Column (2 sets)	SHISEIDO	BPPT
Incubator	ASTEC	BPPT
Flask Plate for Rotary Shaker	IWASHIYA BIO SCIENCE	BPPT
High Speed Refrigerated Centrifuge	TOMY	BPPT
Rotor	TOMY	BPPT
High Speed Refrigerated Centrifuge	TOMY	BPPT
Resin and Gel for Chromatography		BPPT
Electric Pipette 12 channel (4 sets)	Mettler Toledo	BPPT
Multichannel Pipette (8)	Nichiryo	BPPT
Ergonomic pipette (10)	Nichiryo	BPPT
Glass column		BPPT
Ultrasonic Cleaner	AS ONE	BPPT
Liquid Nitrogen Tank 30L	CEBELL	BPPT
Biomedical Freezer (513Lt)	Nihon Freezer	BPPT
Glasswares		BPPT
Analytical Balances	Shimadzu	BPPT
Agarose Gel Electrophoresis	Atto	BPPT
Fraction Collector	BIO RAD	BPPT
EGP Combo	BIO RAD	BPPT

By Mitsuhiko IWASHITA/Danang WALUYO

Progress 2016

Technical Support | Equipment Installation

Laboratory layout



1. Cell culture room (BSL2)
2. Screening room
3. Protein preparation room
4. Corridor
5. Storage
6. Storage
7. Purification Room

Arrival

Installation

After installation

By Mitsuhiko IWASHITA/Danang WALUYO

Planning 2017

1. **Field expedition**
   - Location: Togean Island, Central Sulawesi
2. **Microbial isolation and identification**
   - Target: 1000 identified isolates
3. **Extract preparation**
   - Target: 5000 extracts for screening
4. **Screening of active extract**
   - Target:
     a. Anti-malaria : 5000 extracts
     b. Anti-amoeba : 5000 extracts
5. **Purification of active compound**
   - Target: 4 purified and structure-elucidated compounds
6. **International symposium**
   - Time and venue: (to be determined)
7. **Publication**
   - Target: 2 international peer-reviewed papers

By Danang WALUYO

Planning 2017

Training and Technical Support

8 short term trainees: ~9 months
4 long term trainees: full year

Training in Japan

No	Name	Home Institution	Title of Training	Duration of Training		Days	Training Venue
1	Mr. Danang Waluyo	BPPT	Cell toxicity test of active compounds/in vivo assay of active compounds	3-Feb-2018	31-Mar-2018	28	University of Tokyo
2	Dr. Erwahyuni E. Prabandari	BPPT	Production of enzyme for screening of antiparasitic active compounds	23-Apr-2017	20-May-2017	28	University of Tokyo
3	Dr. Anis H. Mahsunah	BPPT	Structure elucidation of active compound	4-Feb-2018	3-Mar-2018	28	Kitasato University
4	Ms. Diana Dewi	BPPT	Optimization of large scale cultivation for active compound production	4-Feb-2018	3-Mar-2018	56	Kitasato University
5	Ms. Eka Siska	BPPT	Structure elucidation of active compound	17-Sep-2017	11-Nov-2017	56	Kitasato University
6	Ms. Nurlaila	BPPT	Purification of active compound	17-Sep-2017	11-Nov-2017	29	Kitasato University
7	Sasmito	BPPT	Purification of active compound	9-Jul-2017	6-Aug-2017	28	Kitasato University
8	Nuki Bambang Nugroho	BPPT	Purification of active compound	9-Jul-2017	5-Aug-2017	28	Kitasato University
9	Ms. Endah Dwi Hartuti	BPPT	(Long-term training)	(TBD)		(3 yrs)	Nagasaki University
10	Ms. Amila Pramisandi	BPPT	(Long-term training)	1-Apr-2017	~ 31-Mar-2020	(3 yrs)	Kitasato University
11	Ms. Dian Japany Puspitasari	BPPT	(Long-term training)	(TBD)		(3 yrs)	(TBD)
12	Dr. Myrna Adianti	Airlangga University	Cell toxicity assay and new enzyme assays for antiamebic compound discovery	23-Apr-2017	23-Jun-2017	62	U Tokyo (April 23-June 20)
13	Mr. Dwi Peni Kartikasari	Airlangga University	(Long-term training)	(TBD)		(3 yrs)	(TBD)
14	Rini Riffiani	LIPI	Drug discovery of antimalarials	(TBD)			(TBD)
15	[illegible]	[illegible]	[illegible]				

By Mitsuhiko IWASHITA/Danang WALUYO

Dispatching Japanese Researchers (short term)

	2015JFY	2016JFY	2017JFY(plan)
Univ Tokyo	Twice		6 times
Univ of Tsukuba	3 times	4 times	
Kitasato Univ	5 times	4 times	8 times
MBJ	once	once	twice
Ngasaki Univ		4 times	6 times
Symposium Speakers			4 times
Total	11 turns of dispatching	13 turns of dispatching	26 turns of dispatching

By Mitsuhiko IWASHITA/Danang WALUYO

Provided Equipment

Number of provided equipment (as of Jan 2017)		
	BPPT	ITD-AU
Installed	65 items	15 items
Now Procuring	5 items	9 items
Total	70 items	24 items

By Mitsuhiko IWASHITA/Danang WALUYO

Plan of Equipment Provision in 2017 JFY

Equipment plan 2017 JFY		Place	Quant
1	Thermostatic incubator	BTC	1
2	Vacuum pump	BTC	2
3	Water purification system	BTC	1
4	Mini centrifuge	BTC	2
5	Photodiode detector (for UPLC)	BTC	1
6	Mini fermenter	BTC	3
7	Micropipets sets	ITD-AU	1
8	Biosafety Cabinet	ITD-AU	1

By Mitsuhiko IWASHITA/Danang WALUYO

Tentative Budget Plan

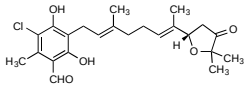
	Tentative Budget Allocation Design (Japanese Side supported by JICA)					
	Approximate data in Japanese Yen					
		2015-2016	2017	2018	2019	total
1	Dispatching Japanese Researchers	19,000,000	16,200,000	15,200,000	13,150,000	63,550,000
2	Acceptance of Indonesian Trainees	25,000,000	15,350,000	15,060,000	12,000,000	67,410,000
3	Equipment & Implements	100,000,000	15,000,000	8,500,000	7,000,000	130,500,000
4	Miscellaneous	2,300,000	1,200,000	1,200,000	1,200,000	5,900,000
	Total	146,300,000	47,750,000	39,960,000	33,350,000	267,360,000

Accomplishment: Discovery of Malaria DHODH inhibitors

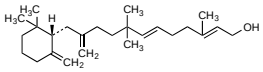
(1) Screening of Kitasato Natural Products Library

(215 compounds, final 50 μg/ml)

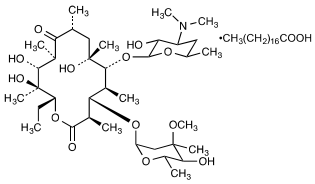
MQO inhibitors: 8 compounds



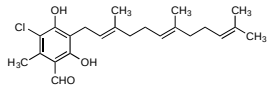
Ascofuranone



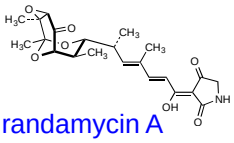
Diumycinol



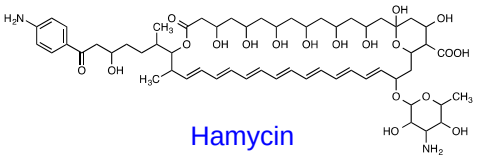
Erythromycin stearate



LLZ-1272a



Tirandamycin A



Hamycin

BA-17039-A (peptide, structure unknown)

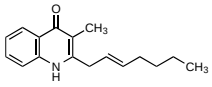
Virantmycin B (structure unknown)

By Mihoko MORI

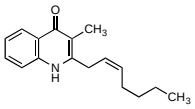
Accomplishment: Discovery of Malaria DHODH inhibitors

(2) Isolating DHODH inhibitors from microbial broths

(2-1) 2-(2-Heptenyl)-3-methyl-4-quinolones



trans compound



cis compound

Both compounds showed 60% inhibition against DHODH at 10 μM.

(2-2) One terpene compound isolated from Indonesian fungal extract (Dr. Anis)

The structure is under determination.

(2-3) Three compounds isolated from Indonesian fungal extract (Ms. Amila)

MS measurement revealed further purification needed for these compounds.

By Mihoko MORI

Searching for Malaria MQO inhibitors

(2) Screening of Kitasato microbial broths (2,640 samples)

Samples showed >60% inhibition against malaria MQO:
111 broths

Actinomycetes: 68 / 1,600 samples

Fungi : 43 / 1,040 samples

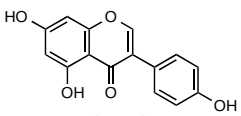
By Mihoko MORI

Problem during screening of Malaria MQO inhibitors

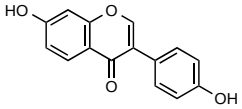
We found the medium contain soy bean meal showed strong PfMQO inhibitory activity.

Methanol extract of soy bean meal has an inhibitory activity.

Well-known bioactive component of soy bean meal is isoflavones



genistein
(no MQO inhibition)

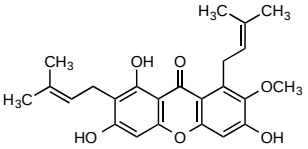


daidzein
(MQO inhibition is unknown)

Now under isolating the inhibitors from methanol extract of soy bean meal.

Search for Malaria MQO inhibitors

fruits of mangosteen
(*Garcinia mangostana*)



α-Mangostin
and analogs

α-Mangostin and related xanthone compounds has antimalarial activity.

Malaria MQO inhibitory activity of α-mangostin was confirmed.

Searching more potent inhibitors has been started with Indonesian *Garcinia* plants.

Screening of Active Compound for Anti-malarial Agent

Enzyme preparation ⇨ Enzyme-based screening ⇨ Hit confirmation

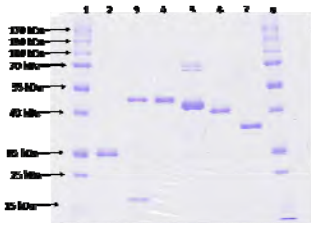
Enzyme preparation

Method

Enzyme	Producer	Cultivation method	Lysis	Purification
PfDHODH	*E. coli* BL21Star (DE3)pETSUMO/PfDHODH	500 ml TB (in 2L flask), 37°C, 200 rpm, induced by IPTG 250 uM at OD_{600}=0.6. Continue at 20^{0}C, 200 rpm, overnight	Sonication	Ni-NTA column
HsDHODH	*E. coli* BL21(DE3)*PyrD*$^-$ pET19b/HsDHODH	500 ml 2YT (in 2L flask), 37°C, 200 rpm, induced by IPTG 25 uM at OD_{600}=0.6. Continue at 20^{0}C, 200 rpm, overnight	Sonication	Ni-NTA column
PfMQO	*E. coli* BL21Star(DE3)pETSUMO/PfMQO	500 ml TB (in 2L flask), 37°C, 200 rpm, induced by IPTG 20 uM at OD_{600}=0.6. Continue at 20^{0}C, 200 rpm, overnight	Sonication	Ultracentrifuge 104.000 ×g
SUMO protease	*E. coli* BL21(DE3)pET28a/SUMO protease	500 ml LB (in 2L flask), 37°C, 200 rpm, induced by IPTG 100 uM at OD_{600}=0.6. Continue at 20^{0}C, 200 rpm, overnight	Sonication	Ni-NTA column

Enzyme preparation for screening

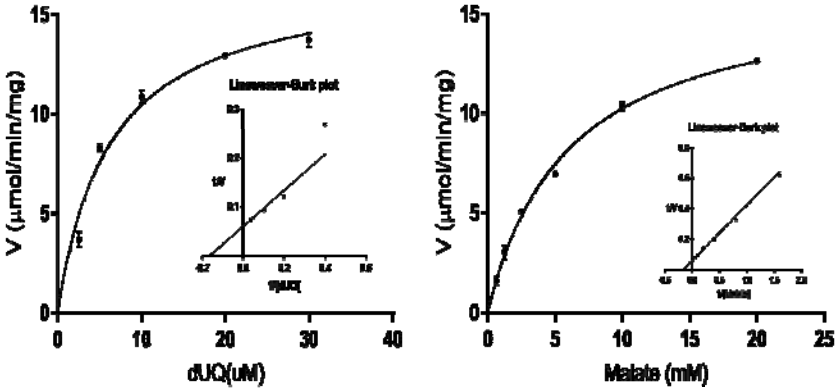
Enzyme	Specific activity	Yield/stock concentration	Storage
PfDHODH	45.2 µmol/min/mg	8.5 ml, 11.3 mg/ml	-30°C
HsDHODH	39.9 µmol/min/mg	1.9 ml, 12.3 mg/ml	-30°C
PfMQO	11.0 µmol/min/mg	16.4 ml, 17.1 mg/ml	-30°C
SUMO protease	ND	25.0 ml, 26.3 mg/ml	-30°C



Remarks:
1,8 : Marker
2 : SUMO Protease
3 : PfDHODH + protease
4 : Cleaved PfDHODH
5 : HSDHODH
6 : CS3
7 : SAT1

By Daniel INAOKA

Biochemical characterization of PfMQO-overexpressed bacterial membrane fraction



K_m (µM)	6.209 ± 0.649	5,996 ± 344
V_{max} (µmol/min/mg)	16.96 ± 0.587	16.40 ± 0.384

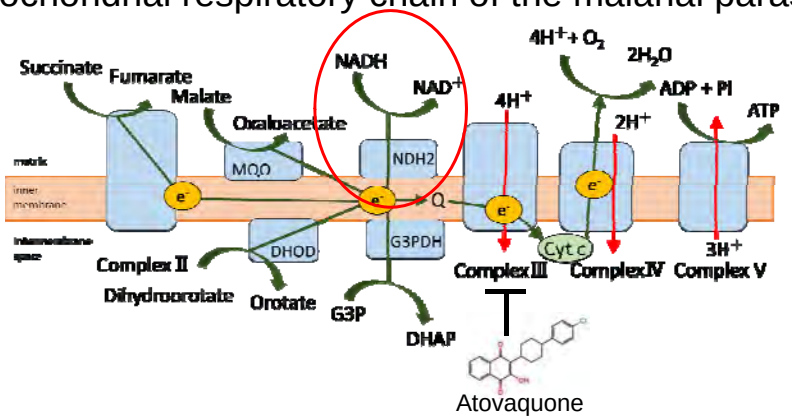
Data obtained from Malate-dependent dUQ reduction activity (dUQ e_{278} = 15 $mM^{-1}cm^{-1}$) and analyzed by GraphPad Prism 7. Assay buffer contained 1.0% ethanol.

By Daniel INAOKA

Biochemical characterization and discovery of novel inhibitors against mitochondrial type II NADH dehydrogenase from *Plasmodium falciparum*

37

Mitochondrial respiratory chain of the malarial parasite



Atovaquone

- NDH2-deficient *P. berghei* ookinetes failed to develop into mature oocysts in the mosquito midgut. (Katja *et al.*, *J. Biol. Chem.*, 2011)

- NDH2 inhibitor showed synergistic growth inhibition of *P. falciparum* with complex III inhibitor, atovaquone.

(Biagini *et al.*, *Antimicrob. Agents Chemother.*, 2006)

38

Preparation of crude membrane

E. coli Complex I and NDH2 knockout strain ANN0221
transformed with pTrcHis2/PfNDH2

Incubate (37 ℃ until A_{600} reached 0.6)
Express His_{10}-PfNDH2 (37 ℃ for 18 h)

Homogenate

10,000 x g, 4℃, 10 min

Precipitate

Supernatant (lysate)

250,000 x g, 4℃, 60 min

Supernatant

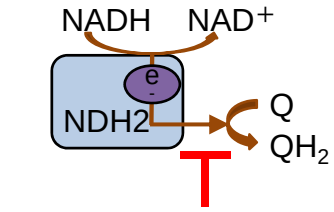
Precipitate (membrane)
(Used for assay and development of screening system)

Suspension buffer
50 mM potassium phosphate, 2 mM EDTA

(Adapted from Fisher *et al.*, *Methods in Enzymology*, 2009)

39

Search for new inhibitors



Respiratory chain inhibitors

Q-site inhibitor library (300 compounds)

1st Screening → Inhibition at 10 μM > 50%

Determination of IC_{50} values

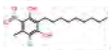
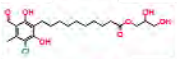
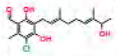
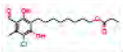
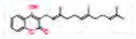
IC_{50} < 1 μM

Biochemical analysis

Co-crystallization of NDH2 with inhibitor

40

Inhibitory effects of top 10 hit compounds on PfNDH2

Compound	Structure	Inhibition at 10 μM (%)
Lauryl gallate		92.2
K5-9		88.9 (IC_{50} = 63.3 nM)
500-15-G		84.7
215-11-O-Piv		81.8
215-11-COOEt		76.6
277-9-OH		76.3
250		75.3
140-1		73.2
273-12		72.9
Ferulenol		70.3

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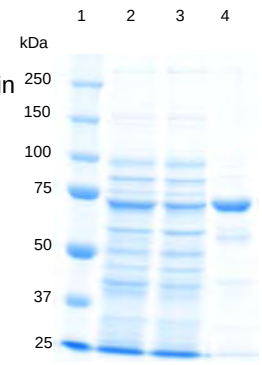
His6-SUMO-PfNDH2 purification

4.0 mg/ml membrane
0.1% Triton X-100
1 mg/ml Asolectin
200,000 x g, 4℃, 60 min
Supernatant (soluble fraction)
Ni-NTA column
Wash
Elution
Concentration



Lane1: marker
Lane2: membrane
Lane3: soluble fraction
Lane4: His_6-SUMO-PfNDH2, 4 μg

←72 kDa
His_6-SUMO-PfNDH2

	Total protein (mg)	Total activity (μmol/min)	Specific activity (μmol/min/mg)	Purification (x-fold)	Yield (%)
Membrane	99.0	537	5.46	1.00	100
Soluble fraction	35.2	452	11.6	2.12	75.8
Purified	2.36	229	97.0	17.8	42.6

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Stability of purified His6-SUMO-PfNDH2

2.0 mg/ml membrane
0.1% Triton X-100
1 mg/ml Asolectin

200,000 x g, 4℃, 60 min

Supernatant (soluble fraction)

Ni-NTA column

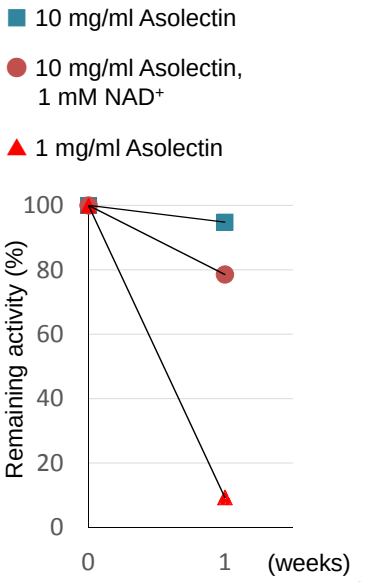
Wash

Elution 10 mg/ml Asolectin

Concentration (500 ×g, overnight)

1 mM NAD^+

Stored at -80℃



43

Conclusion and Future plans

- 42 hits from chemical library (>50% inhibition at 10 μM)
- Purification of PfNDH2
- Next step
  → Establishment of PfNDH2 screening system in Indonesia
  → Screening of Indonesian extracts against PfNDH2

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Searching for *Entamoeba histolytica* cysteine synthase inhibitors

(1) Screening of microbial broth extracts

(1-1) Indonesian microbial broths: total 5,200 samples

33 samples have a potent inhibitory activity against *Entamoeba histolytica* cysteine synthase (EhCS).

Selected 5 microbial broth extracts have been purified by purification team of BC.

Searching for antiamoebic compounds

Screening in Airlangga Univ. using Indonesian microbial broths (1,280 samples)

→ 32 samples had antiamebic activity

→ 4 samples had selective activity compared to mammalian cell lines.

In BC

→ This 4 samples were prepared in large scale.

In Kitasato

→ Antiamoebic activity was confirmed in only 1 sample. (Another 3 samples were cultured again in BC.)

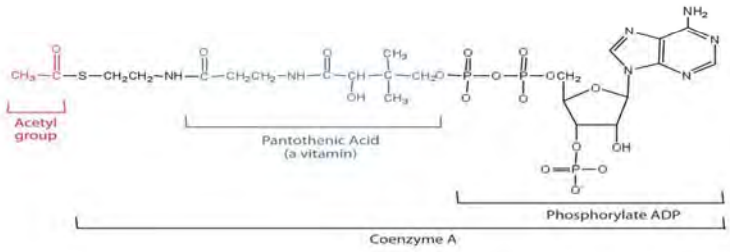
Now purification is progress in Kitasato Univ.

Coenzyme A biosynthesis as new drug target for anti-malaria and anti-amebiasis drugs

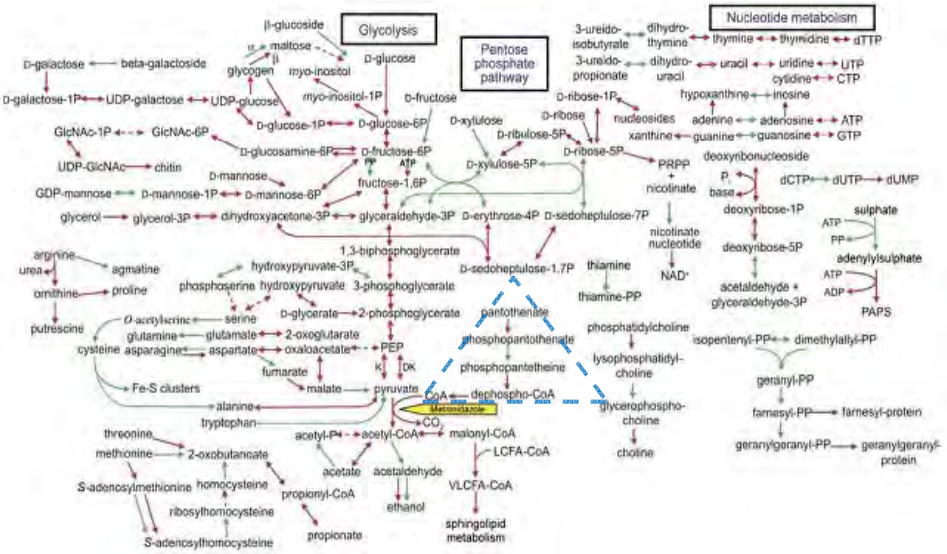
INTRODUCTION

The importance of CoA

- **Numerous reaction central to cellular metabolism**
- **CoA is an indispensable cofactor in all living organism: functional as an acyl group carrier, Acetyl-CoA is the most important.**
- **CoA is a carrier of acyl groups for more than 100 cellular reactions, with estimated as cofactor for 9% of identified enzymatic reactions (Strauss, 2010).**
- **Some enzymes in CoA biosynthesis have low homology with human (26-40%) → potential drug target**

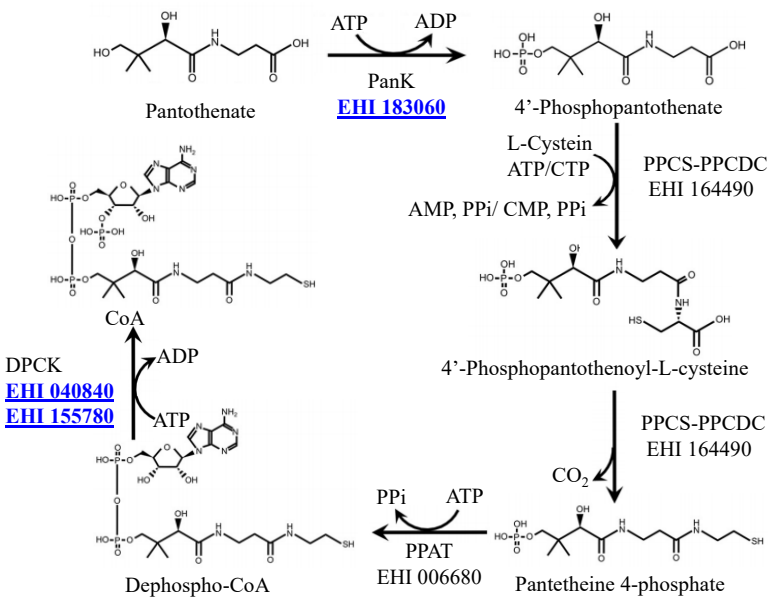


Predicted Metabolism in *E. histolytica*

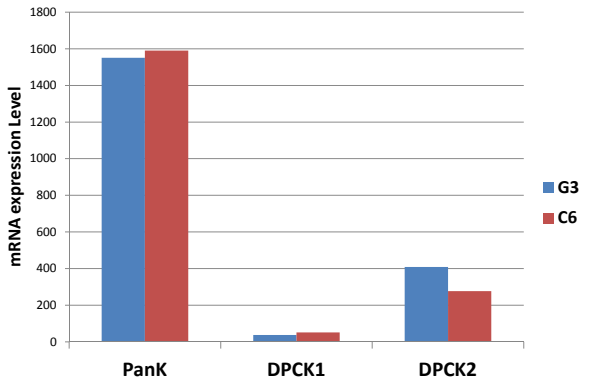


(Lotfus *et al*; 2005 in *Letter of Nature*)

Prediction of CoA biosynthesis *E. histolytica*



mRNA expression level of PanK, DPCK1 and DPCK2 from E. histolytica (Clone 6 and G3 strain)

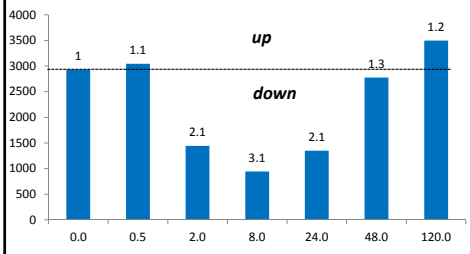


Comparative Individual gene Transcriptomic microarray in *E. Invadens*

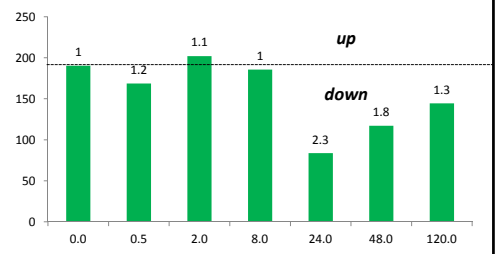
Up and down regulation in *E. invadens*

0 h = trophozoite stage
0.5 – 120 h = encystation stage

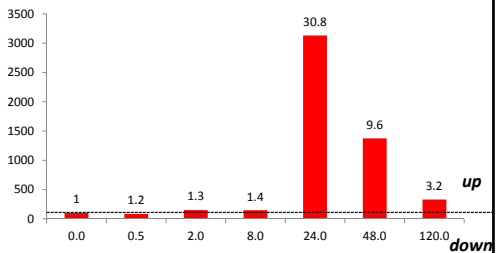
Pantothenate kinase (EIN_016770)



Dephospho CoA kinase 2 (EIN_256160)

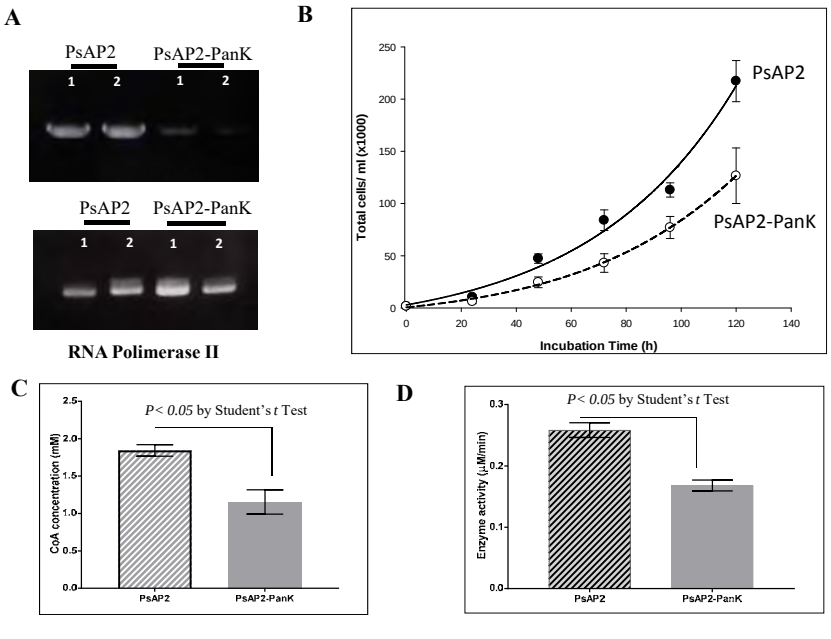


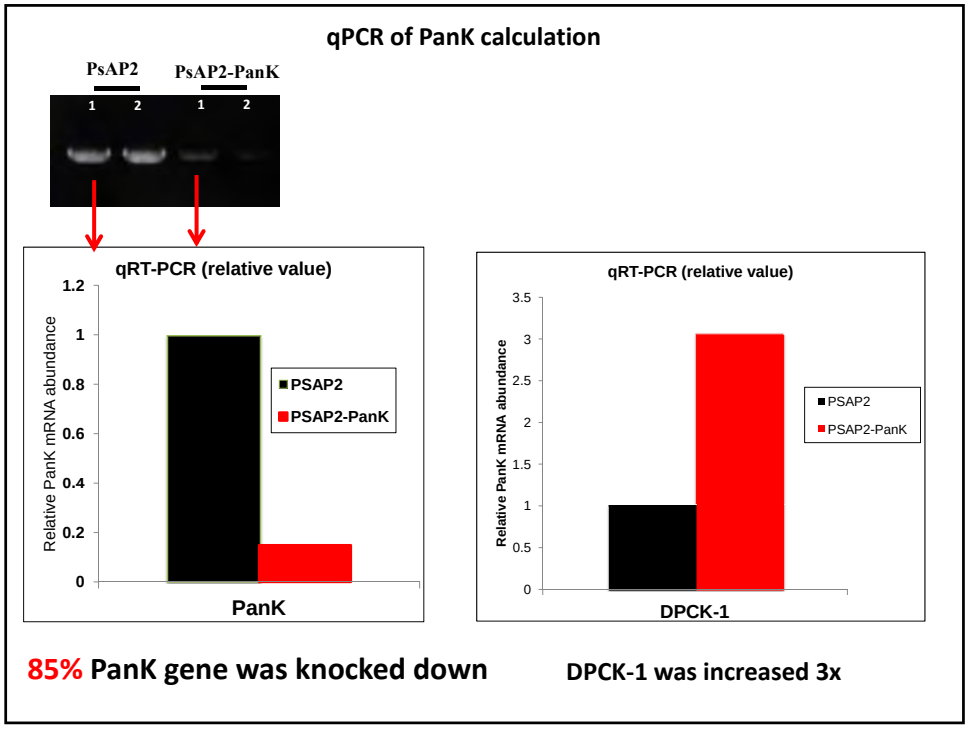
Dephospho CoA kinase 1 (EIN_147090)

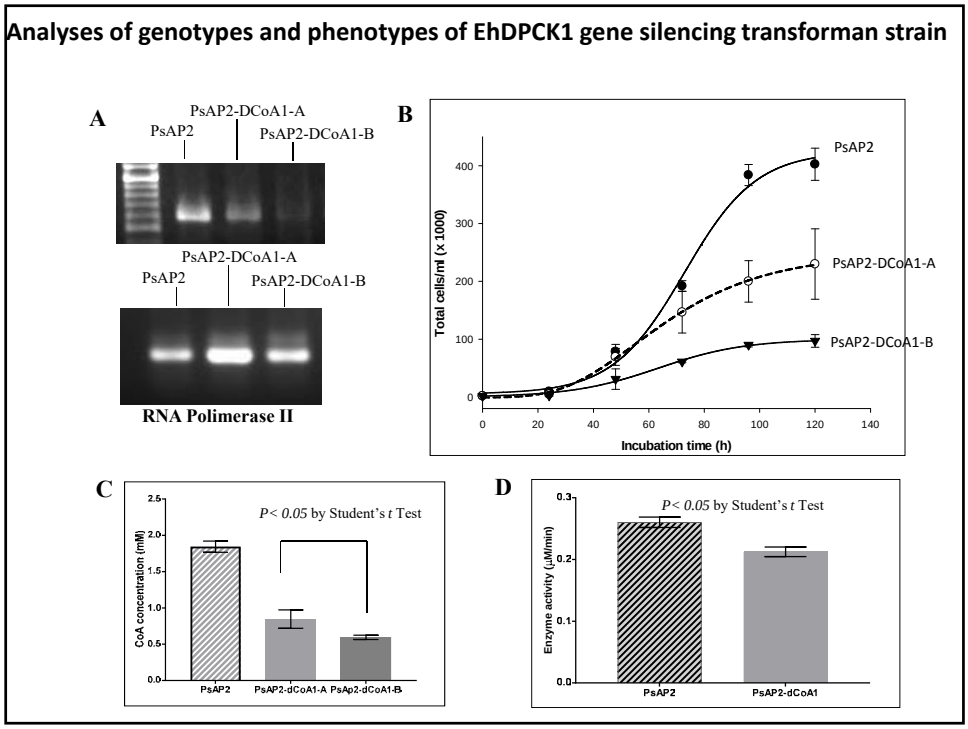


(Raw data sources : De Cadiz *et al*, 2013)

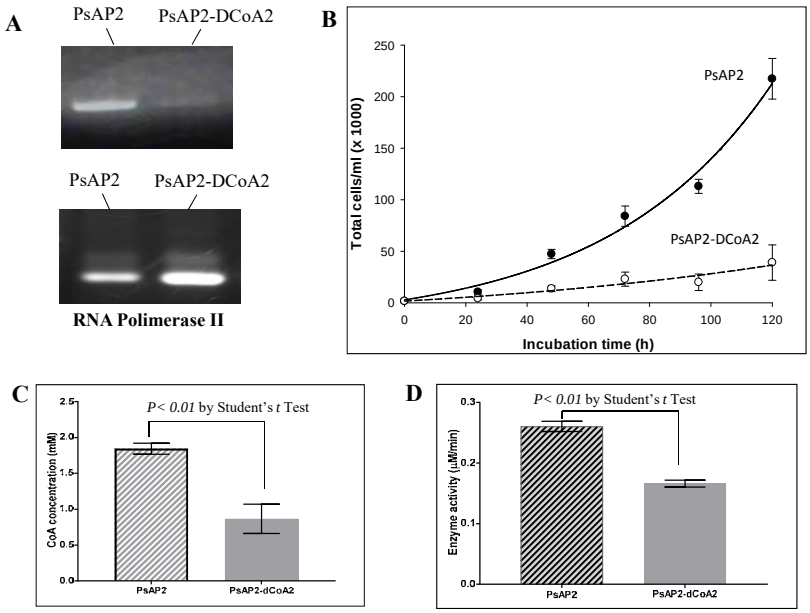
Analyses of genotypes and phenotypes of EhPanK gene silencing transforman strain



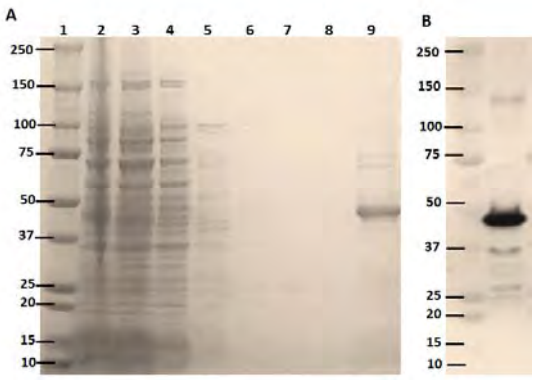

qPCR of PanK calculation
PsAP2
PsAP2-PanK
1 2 1 2
qRT-PCR (relative value)
Relative PanK mRNA abundance
1.2
1
0.8
0.6
0.4
0.2
0
PSAP2
PSAP2-PanK
PanK
qRT-PCR (relative value)
Relative PanK mRNA abundance
3.5
3
2.5
2
1.5
1
0.5
0
PSAP2
PSAP2-PanK
DPCK-1
85% PanK gene was knocked down
DPCK-1 was increased 3x


Analyses of genotypes and phenotypes of EhDPCK1 gene silencing transforman strain
A
PsAP2-DCoA1-A
PsAP2
PsAP2-DCoA1-B
PsAP2-DCoA1-A
PsAP2
PsAP2-DCoA1-B
RNA Polimerase II
B
PsAP2
PsAP2-DCoA1-A
PsAP2-DCoA1-B
Total cells/ml (x 1000)
400
300
200
100
0
0 20 40 60 80 100 120 140
Incubation time (h)
C
P< 0.05 by Student's t Test
CoA concentration (mM)
2.5
2.0
1.5
1.0
0.5
0.0
PsAP2
PsAP2-dCoA1-A
PsAp2-dCoA1-B
D
P< 0.05 by Student's t Test
Enzyme activity (μM/min)
0.3
0.2
0.1
0.0
PsAP2
PsAP2-dCoA1

Analyses of genotipes and phenotypes of EhDPCK2 gene silencing transforman strain

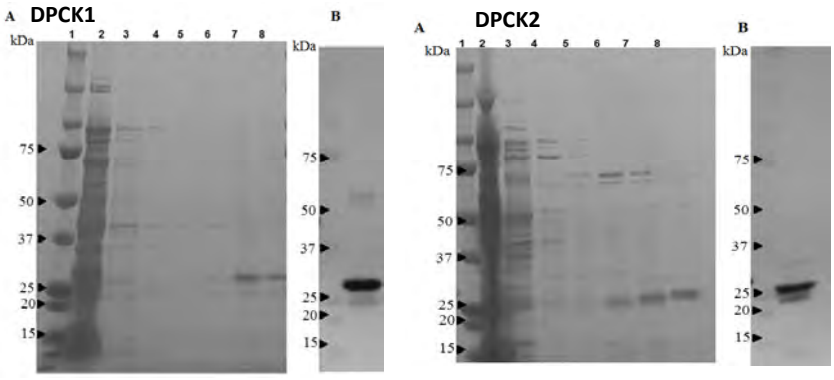


Expression and purification of recombinant EhPanK



A, Protein samples at each step of purification.
Lane 1, molecular weight markers;
lane 2, whole cells;
lane 3, the total lysate;
lane 4, unbound fraction;
lanes 5-8, fractions eluted with 10, 30, 50, and 100 mM imidazole, respectively;
lane 9, purified recombinant EhPanK, eluted with 300 mM imidazole.
B, Immunoblot analysis of purified recombinant PanK using anti-His antibody.

Expression and purification of recombinant DPCK1 and DPCK2



A, Protein samples at each step of purification.
Lane 1, molecular weight markers;
lane 2, whole cells;
lane 3, the total lysate;
lane 4, unbound fraction;
lanes 5-6, fractions eluted with 30 and 50 mM imidazole, respectively;
lane 7, purified recombinant EhPanK, eluted with 300 mM imidazole.
Lane 8, final purified with dialysis
B, Immunoblot analysis of purified recombinant PanK using anti-His antibody.

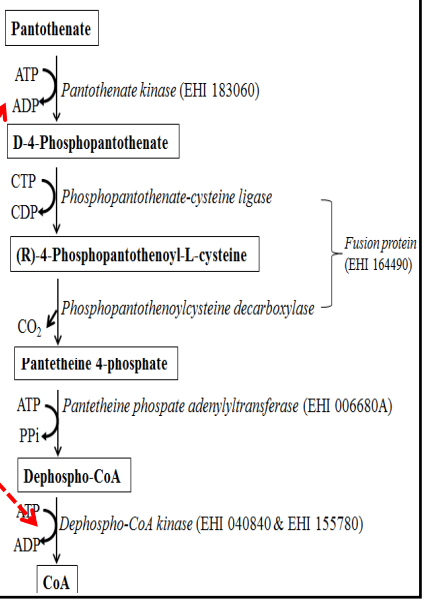
Enzymatic assay of natural compounds againts *E. histolytica*

Extract sources	Total
Fungi	560
Actinomycetes	840
Total	1,400

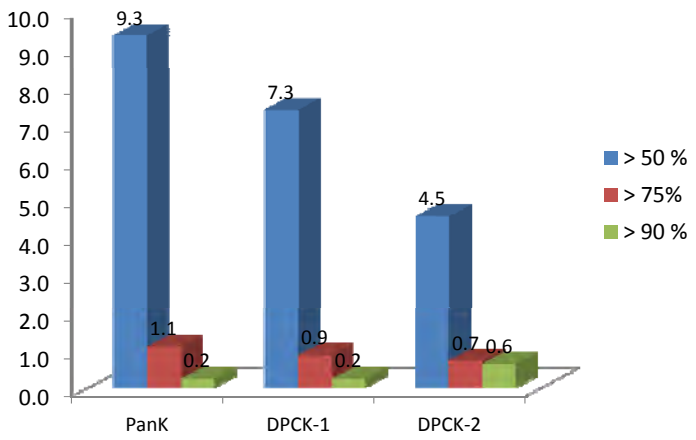
1. Pantothenate kinase (PanK)

2. Depospho-CoA Kinase 1 (DPCK1)

3. Dephospho-CoA Kinase 2 (DPCK2)



Profile of microbial extracts that inhibit PanK, DPCK-1 and DPCK-2



Hit candidate compounds for next study

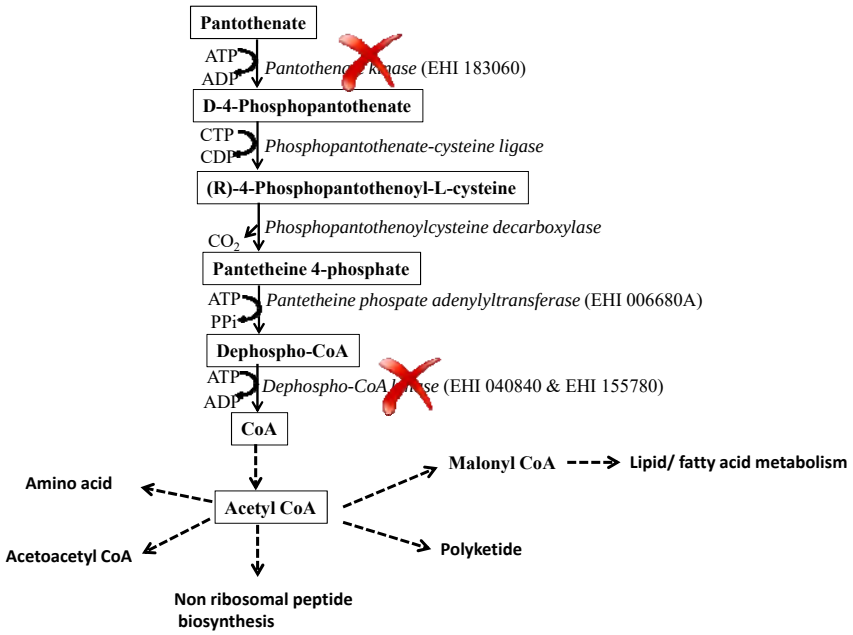
Criteria to determine :

- ✓ **Inhibitor specific to enzyme target, not inhibited other enzymes used for coupled assay**
- ✓ **Stable activity**
- ✓ **Extract-dependent for inhibiting**

	Extract ID	Sources	Enzymes inhibit	Cell based inhibit (*E. his*)*	Human cell inhibit (MRC5)*
1	C-155	Actinomycetes	PanK : 95% DPCK1 : 22 % DPCK2 : 33 %	100 %	> 1 %
2	F15.0511	Fungi	PanK : 57% DPCK1 : 21% DPCK2 : 98 %	24 %	Not checked

**Data provided by Ratna*

Metabolomic analyses on genes silencing



The importance of metabolomic analyses in our research :

- Figure out metabolism profile from knocked down PanK and DPCK strain of *E. his*
- Predict mode of action of EhPanK and/or EhDPCK specific inhibitor from natural compounds.

MINUTES OF MEETING
OF
THE 3rd JOINT COORDINATING COMMITTEE MEETING
OF THE PROJECT FOR SEARCHING LEAD COMPOUNDS OF
ANTI-MALARIAL AND ANTI-AMEBIC AGENTS BY UTILIZING DIVERSITY
OF INDONESIAN BIO-RESOURCES (SLeCAMA PROJECT)
IN
THE REPUBLIC OF INDONESIA

The 3rd Joint Coordinating Committee Meeting (hereinafter referred to as "JCC Meeting") of the Japanese Technical Cooperation for the Project for Searching Lead Compounds of Anti-Malarial and Anti-Amebic Agents by Utilizing Indonesian Bio-resources in the Republic of Indonesia (hereinafter referred to as "SLeCAMA Project") was held at the conference room of Agency for the Assessment and Application of Technology, Jakarta, Indonesia on 31st January, 2018.

As a result of the discussions, both Indonesian side and Japanese side agreed upon the matters in the document attached hereto.

Jakarta, 31st January 2018

Mr. Shunsuke TAKATOI
Senior Representative
Japan International Cooperation Agency
Indonesia Office

Prof.Dr.Eng. ENIYA LISTIANI DEWI, B.Eng., M.Eng.
Deputy Chairperson for Agricultural Technology and Biotechnology,
Agency for the Assessment and Application of Technology (BPPT)
The Republic of Indonesia

ATTACHED DOCUMENT

I. GENERAL REVIEW

The SLeCAMA Project was commenced on 01 April 2015, for strengthening the capacity of Indonesian researchers and institutions in drug discovery against tropical diseases including malaria and amebiasis using Indonesian bio-resources. through the collaborative joint researches by the Indonesian institutes, BPPT and its collaborating institutes in Indonesia, the Institutes of Sciences (hereinafter referred to as "LIPI") and the Institutes of Tropical Disease, Airlangga University(hereinafter referred to as "AU"), and Japanese institutions, University of Tsukuba (hereinafter referred to as "UT"), Kitasato University (hereinafter referred to as "KU") , the University of Tokyo (hereinafter referred to as "U. Tokyo") and MicroBiopharm Japan Co. Ltd (hereinafter referred to as "MBJ").
In April 2016, Nagasaki University (hereinafter referred to as "Nagasaki Univ.") came to be another Japanese collaborator. Then in April 2017, U. Tokyo became to the Japanese coordinating institute instead of UT.

In accordance with the Record of Discussions (hereinafter referred to as the "R/D"), signed on 17th February 2015 by Japan International Cooperation Agency (hereinafter referred to as "JICA") and Agency for the Assessment and Application of Technology (hereinafter referred to as "BPPT") and the Minutes of Meeting of 2nd JCC meeting on 25th January 2017, Japanese side has dispatched experts to SLeCAMA Project and has accepted Indonesian counterparts as trainees in KU, Nagasaki Univ. and U.Tokyo. And Japanese side has been providing equipment for the laboratories located in both BPPT and AU to capacitate the drug discovery in the institutes based on requests by the Government of the Republic of Indonesia. According to the requests, total 102 items of equipment were provided and had been installed in BPPT (77 items) and AU (25 items) since the start of the SLeCAMA Project. There are 2 more items are being procured in Indonesia now (1 item for BPPT, 1 item for AU).

In this year, the project was conducted in BPPT according to work-breakdown system (WBS) composed by four (4) working teams: A) Microbial and extract preparation, B) Enzyme-based screening of active extract, and C) Cell-based screening of active extract, and D) Purification of active compound.
Each working team has its leader, and the leaders with other members participate in the periodical meeting weekly chaired by the Project Co-manager at the Laboratory for Biotechnology-BPPT to implement the research activities systematic and effectively.

Both sides reviewed activities in respect to the implementation of the SLeCAMA Project based on the common implementation plan of the project, which is described in the P.O. with the Tentative Project Design Matrix (hereinafter referred to as the "Tentative PDM") in the R/D and the Minutes of Meeting of Joint Coordinating Committee signed on 25th January, 2017 by both sides.

II. SUMMARY OF MEETING

Both sides reviewed and discussed the following issues:

1. Progress of Project Implementation in 2017

1) Microbes and Extract Preparation at BPPT in 2017
As of December 2017, 485 microbes from the samples collected during the field exploration in May 2017 at Togean Island were isolated. More than 4,380 extracts for first screening were prepared from the microbes that were registered in the culture collection.

2) Enzyme-based Screening at BPPT in 2017
Enzyme-based screenings were done more than 10,616 extracts against PfDHODH/PfMQO enzymes for searching anti-malarial activities.

3) Cell-based Screening at BPPT in 2017
Cell-based screenings were done more than 5,720 extracts against *Plasmodium falciparum* 3D7 for searching anti-malarial activities, respectively.
Malaria and amebic parasites, as well as 5 lines of mammalian cell for toxicity test, are maintained. Toxicity assay of active extracts inhibit proliferation of malaria parasite against mammalian cells were also done with 451 extracts.

4) Purification at BPPT in 2017
6 extracts are still on the process of purification for anti-malarial agents

5) Enzyme-based Screening at AU in 2017
At AU, enzyme-based screenings were done with 3,840 extracts against CS3 enzyme and 1,460 extracts against SAT1 enzyme for searching anti-amebic activities.

6) Cell-based Screening at AU in 2017
At AU, cell-based screening were done with 5,120 extracts against *Entamoeba histolytica*

7) Purification at AU in 2017
1 extract is still on the process of purification for anti-amebic agents

8) Twenty three (23) turns of short-term Japanese researchers and a long term JICA Coordinator have been dispatched to SLeCAMA Project.

9) Japanese side accepted nine (9) Indonesian counterparts as short term trainees in KU, U Tokyo and Nagasaki Univ.

10) Three Indonesian researchers have been participating in Ph.D. degree courses at U.Tokyo and KU as a long term trainee since April 2017.

11) The disbursement of budget for the SLeCAMA Project in 2017 by BPPT was Rp.822,595,576-.

12) "International Symposium on Natural Resources-based Drug Development"

To build a consortium on drug development in Indonesia, the project organised an international symposium on 21-22 August 2017 at BPPT headquarter. During the sessions, some of key persons from related ministries and industries shared strategies and policies that are needed for promoting innovation on drug development in Indonesia. And prominent leading researchers in drug development from Japan and Indonesia also delivered recent update on advanced technology in drug development. Total 297 participants attended this 2-days symposium.

13) Publication in 2017
A scientific paper written by Indonesian researcher as the first author was published by an international peer-reviewed journal ("BBA Bioenergetics").
BPPT also participated in The 9th International Seminar of Indonesian Society for Microbiology held at Palembang, 14-15 November 2017, by presenting 4 topics related to this project.

14) Coordination Meeting to enhance a network for Indonesian research institutes was organized between LIPI and BPPT in Sep 2017.

15) Regular Meeting
The project technical meeting in BPPT have been organizing weekly with all the working teams of the SLeCAMA Project in BPPT chaired by the Project Co-manager.

16) Laboratory equipment

In 2017, total 22 items of procured equipment have been installed (BPPT:12 items, AU:10 items),.
The cumulative numbers of items had installed are 102 (BPPT 77 items, AU 25 items) as of December 2017.
In addition to that two(2) more items are being procured (BPPT:1 item, AU:1 item)

2. Tentative Plan for the Project Implementation in 2018

1) Field Exploration for collecting samples at BPPT in 2018
The area to collect samples in 2018 would be the "Puspiptek" .

2) Microbial isolation and identification at BPPT
More than 1,000 identified isolates are expected from the newly collected samples at BPPT in 2018

3) Extracts preparation at BPPT
More than 3,000 extracts are expected to be prepared for screening using microbial isolates from the collection, as well as from newly isolated microbes as mentioned in 2) above.

4) Screening of active extracts
More than 5,000 extracts expected to be screened in both fields of antimalarial and antiamoeba

5) Purification at BPPT in 2018
The target in 2018 is to get 4 purified and structure-elucidated compounds

6) Animal Test
More than one compound is expected to have animal test after in vitro assessment.

7) Publications
More than two (2) of scientific papers is expected to be submitted to the international peer-reviewed journals.

8) Eleven (11) Indonesian scientists among research members (BPPT, AU & LIPI) are planned to participate in the training course organized in Japan,

9) Dispatching Japanese researchers in 2018
Around twenty four (24) turns of dispatching Japanese researchers are planned tentatively

10) Laboratory equipment
Now two (2) items of equipment (BPPT: 1, AU: 1) are being procured and the project is proposing budget for 2018 to JICA including cost of equipment required

3. Administrative Issues

1) Implementation Agreement including scheme for MTA
To define detail of cooperation scheme between BPPT and U Tokyo, both sides signed on the "Implementation Agreement" in October 2017 .

2) Alteration of Japanese coordinating institute
The Japanese coordinating institute for the SLeCAMA Project was changed from University of Tuskuba to the U.Tokyo in April 2017. The Memorandum of Understading concerning the SLeCAMA Project was made and entered by and between BPPT and U.Tokyo at this moment.

3) Handing over of equipment
There is no progress on the transaction of handing over since the last JCC meeting, the property right of provided equipment should be handed over to Indonesian side from JICA right after its provision. Therefore, BPPT will inform JICA the required transaction for official hand over as soon as possible.

4) Update on research members
Some members of the SLeCAMA Project were updated, the detail as of January 2018 is shown in the ANNEX 1 "List of Researchers as of January 31, 2018"

5) Punctual submission of "Project Monitoring Sheets"
SATREPS project should submit periodical monitoring report "Project Monitoring Sheets" to JICA biannually. The delay of the submission will reduce the significance of its monitoring functions, therefore JICA requests SLeCAMA Project punctuality

on the submission.

4. **Other Needs**

1) Enhancement of researchers on taxonomy
To establish the libraries as open source, the capacity building on characterization and archiving microbial strains are required

2) Introduction of new screening platform
3 to 4 new enzyme targets have been selected and will be explored.

3) Requirement of prioritization among identified hits for purification
To purify potential compounds efficiently, prioritization should be strengthened by hit ranking of selectivity index, counter screening, taxonomy of isolates, preliminary extract test, etc.

4) Additional Purifying Stations
The bottlenecks on processes due to limited capacity of purification/elucidation should be solved by introducing purification process into AU and U. Tokyo in addition to the laboratory of BPPT.

5) Coordination among BPPT, AU and LIPI
Periodical mutual visits and joint meetings are expected to be more frequent in order to share data and method and for cross depositing of microbes

6) Strengthening the consortium of drug development
The drug development networking should be strengthened effectively by utilizing various types of occasions such as symposium and JCC meeting

7) Broadening of target diseases
To utilize outcome of the research effectively for global issues, other diseases out of malaria and amebiasis might be targets of drug development by the SLeCAMA Project

8) Strengthening sustainability
In order to capacitate drug development even after the SLeCAMA Project, Continuous supports, such as research funding and international collaborations, will be required for sustainable activities on drug development by utilizing biodiversity in Indonesia

ANNEX

1. List of Researchers as of as of January 31, 2018
2. Progress 2017 and Planning 2018 (BPPT)
3. Report activities of ITD-AU, January 31, 2018
4. Identified Problems/Needs and Solutions (Chief Advisor)

Annex I

List of Researchers (version #3 as of 2018-01-31)

Reaserch Subject	The Indonesian Side	The Japanese Side
Output 1: Compounds with anti-malarial activity are identified from the extracts on Indonesian biological resources (microorganism, plants, etc.		
1.1. Primary screening for inhibitory activity of extracts to the plasmodium-derived recombinant enzyme	• Erwahyuni E. Prabandari (BPPT) • Endah Dwi Hartuti (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Wan Xinying (NagasakiUniv) • Youichi Matsuo (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
1.2. Secondary screening for selective inhibitory activity of the extracts to the proliferation of Plasmodium falciparum	• Danang Waluyo (BPPT) • Dian Japany Puspitasari (BPPT) • Nadia Adipratiwi (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Takaya Sakura (Nagasaki Univ) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
1.3. Screening for selective inhibitory activity of extracts to the proliferation of *Plasmodium falciparum*, in parallel with Activity 1-1- and 1-2	• Danang Waluyo (BPPT) • Dian Japany Puspitasari (BPPT) • Nadia Adipratiwi (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Takaya Sakura (Nagasaki Univ) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
1.4. Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	• Anis H. Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT) • Evita Chrisnayanti (BPPT) • Kesi Kurnia (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita (U.Tokyo) • Kazuyuki Dobashi (KU)
1.5. Establishment of mass production system of the lead compounds candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BPPT)	• Azuma Watanabe (MBJ)

	• Kristiningrum(BPPT) • Kiki RizkiaAfrianti (BPPT) • Suryani (BPPT)	
1.6. Determination of chemical structures of the lead compound candidate	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT) • Evita Chrisnayanti (BPPT) • Kesi Kurnia (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita (U.Tokyo) • Kazuyuki Dobashi (KU)
1.7. Selection of lead compound(s) through *in vitro* assessment and subsequent animal testing	• Agung Eru Wibowo (BPPT) • Kurnia Agustini (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ)
1.8. Discussion of future direction of derivatization on the basis of the structure biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT) • Agung Eru Wibowo (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Tomoyoshi Nozaki (U.Tokyo) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 2: Compounds with anti-amebic activity are identified from the extracts of Indonesian biological resources (microorganism, plants, etc)		
2.1. Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica-derived* site-specific recombinant enzyme	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU) •	• Tomoyoshi Nozaki (U.Tokyo) • Ghulam Jeelani (U. Tokyo) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)
2.2. Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (U.Tokyo) • Ghulam Jeelani (U.Tokyo) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)

2.3. Screening for selective inhibitory activity of extracts to the extracts of *Entamoeba histolytica*, in parallel with Activity 2-1 and 2-2	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (U.Tokyo) • Ghulam Jeelani (U.Tokyo) • Kumiko Tsukui(NIID) • Herbert Santos(NIID)
2.4. Isolation and purification of chemical compounds with inhibitory to the proliferation against *Entamoeba histolytica*	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT) • Evita Chrisnayanti (BPPT) • Kesi Kurnia (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita (U.Tokyo) • Kazuyuki Dobashi (KU)
2.5. Establishment of mass production system of the lead compound candidates	• Diana Dewi (BPPT) • Suyanto (BPPT) • Anna Safarrida (BPPT) • Dyah Noor Hidayati (BBPT) • Kristiningrum(BPPT) • Kiki RizkiaAfrianti (BPPT) • Suryani (BPPT) • AviNurulOktaviani (BPPT)	• Azuma Watanabe (MBJ)
2.6. Determination of chemical structures of the lead compound candidates	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT) • Evita Chrisnayanti (BPPT) • Kesi Kurnia (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita(U.Tokyo) • Kazuyuki Dobashi (KU)

2.7. Selection of lead compound(s) through in vitro assessment and subsequent animal testing	• Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni(AU) • Dwi Peni Kartikasari(AU) • Hikatul Ilmi(AU) • Lidya Tumewu(AU) • Aty Widyawaruyanti (AU) • Lidya Tumewu(AU) •	• Tomoyoshi Nozaki (U.Tokyo) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID) • Herbert Santos(NIID) •
2.8. Discussion on future direction of derivatization on the basis of the structure biology assessment	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Chaidir (BPPT) • Agus Supriyono (BPPT) • Agung Eru Wibowo (BPPT)	• Daniel Ken Inaoka (Nagasaki Univ) • Tomoyoshi Nozaki (U.Tokyo) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)
Output 3: Technologies and research system for drug discovery using biological resources are established at the Indonesian research institute		
3.1. Sample collection and additional registration of newly-obtained extracts to the biological resources library	• Puspita Lisdiyanti (LIPI) • Atit Kanti, (LIPI) • Muhammad Ilyas (LIPI) • Ade Lia Putri(LIPI) • Arif Nurkanto (LIPI) • Dyah Noor Hidayati (BPPT) • Suryani (BPPT) • Kristiningrum(BPPT) • AviNurulOktaviani (BPPT)	• Atsuko Matsumoto (KU) • Ken-ichi Nonaka (KU) • Kazuro Shiomi (KU) • Mihoko Mori (KU) • Kazuyuki Dobashi (KU) • Toshiyuki Tokiwa (KU) • Azuma Watanabe (MBJ) • Tomoyoshi Nozaki (U.Tokyo) • Daniel Ken Inaoka (Nagasaki Univ)
3.2. Establishment of screening systems	• Erwahyuni E. Prabandari (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU) • Dwi Peni Kartikasari(AU)	• Tomoyoshi Nozaki (U.Tokyo) • Daniel Ken Ianoka (Nagasaki Univ) • Takaya Sakura (Nagasaki Univ) • Wan Xinying (Nagasaki Univ) • Yukiko Miyazaki (Nagasaki Univ)

		• Youichi Matsuo (Nagaski Univ) • Kota Mochizuki (Nagasaki Univ)
3.3. Establishment of culture and evaluation system	• Danang Waluyo (BPPT) • Dian Japany Puspitasari (BPPT) • Nadia Adipratiwi (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU) • Dwi Peni Kartikasari (AU)	• Tomoyoshi Nozaki (U.Tokyo) • Daniel Ken Inaoka (Nagasaki Univ) • Takaya Sakura (Nagasaki Univ) • Yukiko Miyazaki (Nagasaki Univ) • Kota Mochizuki (Nagasaki Univ)
3.4. Introduction of technologies of isolation and purification	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT) • Evita Chrisnayanti (BPPT) • Kesi Kurnia (BPPT) • Achmad Fuad Hafid (AU) • Aty Widyawaruyanti (AU) • Lidya Tumewu (AU)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita (U.Tokyo) • Kazuyuki Dobashi (KU)
3.5. Introduction of technologies of chemical structure elucidation	• Anis H Mahsunah (BPPT) • Amila Pramisandi (BPPT) • Eka Siska (BPPT) • Nuki Bambang Nugroho (BPPT) • Nurlaila (BPPT) • Sasmito Wulyoadi (BPPT) • Evita Chrisnayanti (BPPT) • Kesi Kurnia (BPPT)	• Kazuro Shiomi (KU) • Mihoko Mori (KU) • Michio Yamashita (U.Tokyo) • Kazuyuki Dobashi (KU)

3.6. Establishment and enhancement of a research network in Indonesia	• Tarwadi (BPPT) • Danang Waluyo (BPPT) • Agung Eru Wibowo (BPPT) • Ahmad Fuad Hafid (AU) • Puspita Lisdyanti (LIPI) • Atit Kanti, (LIPI)	• Tomoyoshi Nozaki (U.Tokyo) • Daniel Ken Ianoka (Nagasaki Univ) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)

Institution Abbreviation:

- BPPT: Agency for the Assessment and Application Technology
- AU: Institute for Tropical Disease, Airlangga University
- LIPI: Indonesia Institute of Science
- U. Tokyo: the University of Tokyo
- KU: Kitasato University
- MBJ: MicroBiopharm Japan, Co., Ltd.
- NIID: National Institute of Infectious Diseases of Japan

The 3rd Joint Coordinating Committee Meeting

The Project for Searching Lead Compound of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

SATREPS SLeCAMA Project

Progress 2017 and Planning 2018

Danang Waluyo
Project Co-manager

BPPT Main Office, Jakarta
January 31th, 2018

Content

2

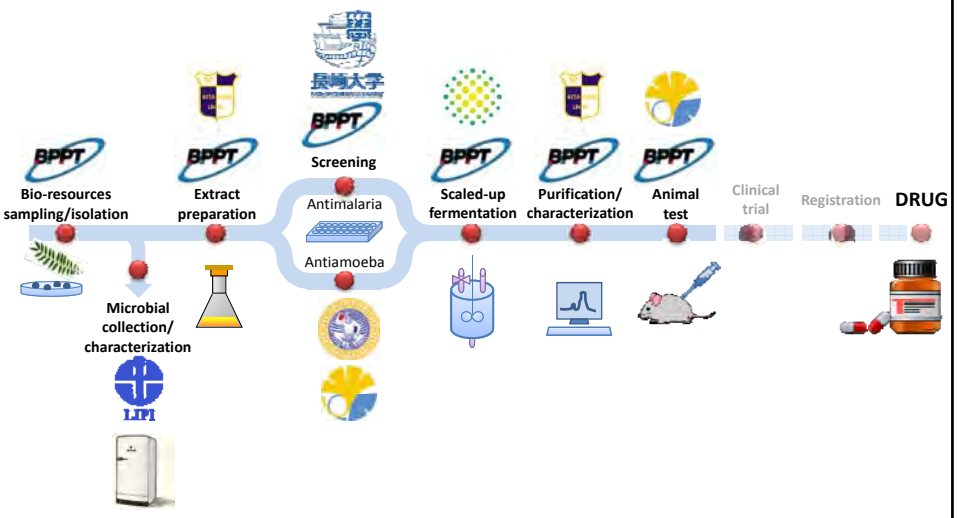
Target Review

Project purpose/Outputs	Indicator	Time achievement (est. time)
Project Purpose: Research capacity is enhanced	• 1< lead compound (antimalaria) • 1< lead compound (antiamoeba) • 2< papers	• 5th year (Mar 2020) • 5th year (Mar 2020) • 5th year (Mar 2020)
Output 1. Compounds with anti-malarial activity are identified	1-1. 1< isolated and purified compound 1-2. 1< structure elucidated compound 1-3. 1< efficacy tested compound	1-1. Mid-term review (Jan 2018) 1-2. Terminal evaluation (Oct 2019) 1-3. 5th year (Mar 2020)
Output 2. Compounds with anti-amebic activity are identified	2-1. 1< isolated and purified compound 2-2. 1< structure elucidated compound 2-3. 1< efficacy tested compound	1-1. Mid-term review (Jan 2018) 1-2. Terminal evaluation (Oct 2019) 1-3. 5th year (Mar 2020)
Output 3. Technologies and research system for drug discovery using biological resources are established	3-1. 10,000< microbes, plants, extracts are registered 3-2. Enzyme-based screening system are established 3-3. Cell-based screening system are established 3-4. Technologies of Isolation and purification are introduced 3-5. Technologies of chemical structure analysis are introduced 3-6. 2< international symposium are held	3-1. 3rd year (Mar 2018) 3-2. 2nd year (Mar 2017) 3-3. 3rd year (Mar 2018) 3-4. Terminal evaluation (Oct 2019) 3-5. Terminal evaluation (Oct 2019) 3-6. 3rd and 5th year (Aug 2017 and Aug 2019)

Red: already achieved
Blue: partially achieved

3

Research Flowchart



4

Progress 2017

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

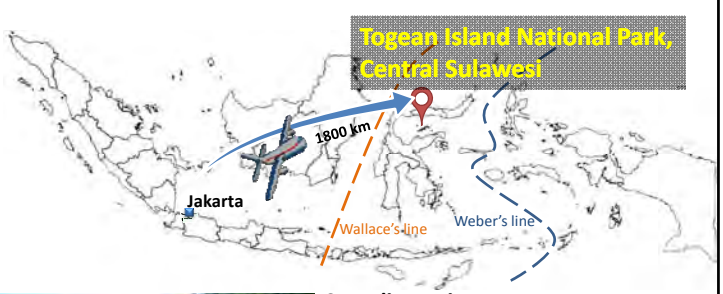
Other Activities

Budget Arrangement

5

Progress 2017 — Field Exploration

Objective: To collect sources for microbial isolation



Sampling point

Location : Togean Island National Park
Coordinate : -2.9225529, 111.5064353
Date : May 15-19, 2017
Temp./RH : 29-31°C, 68-75%

Sample obtained

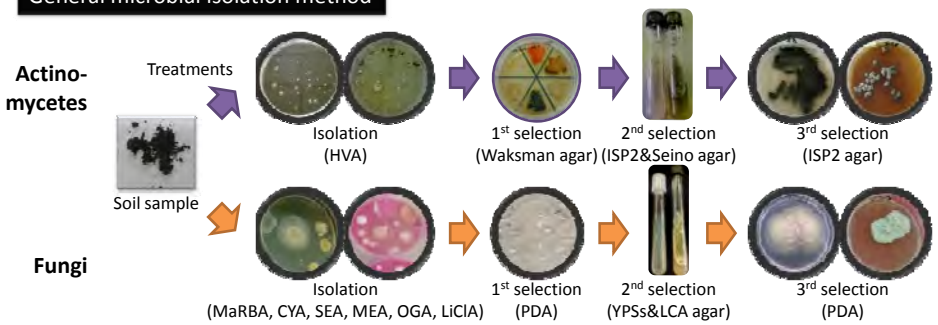
Type : Soil, plant litter, mushroom
Location : Terrestrial, shore side, river side
Total number : 92 samples

6

Progress 2017 — Microbial Isolation

Objective: To isolate microbial strain from source samples

General microbial isolation method



Result

Target	Isolation sources	Number of isolated sources	Number of isolates*
Fungi	Soil	20	155
	Plant litter	12	10
Actinomycetes	Soil	25	295
	Plant litter	14	17
	Mushroom	12	8
		TOTAL	**485**

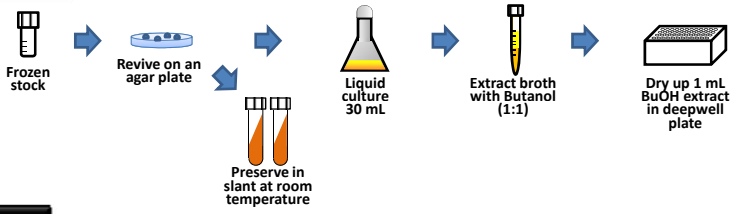
* Currently isolation is still on going

7

Progress 2017 — Extract production

Objective: To produce extracts of natural resources (microbes, plants) for screening

Method



Result

First Screening Extract Production (2017)	
Extract sources	**Number of extract**
Actinomycetes	1740
Fungi	2640
Total	**4380**

8

Progress 2017

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

9

Progress 2017

Screening of Active Extract

Anti-malaria

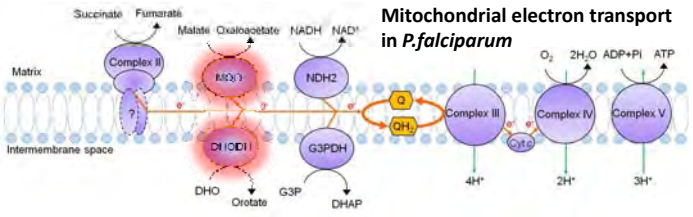
Objective: To search extract with antimalarial activity

Enzyme-based screening

Screening target: extracts with inhibitory activity for PfDHODH and PfMQO

DHODH : Dihidroorotate dehydrogenase

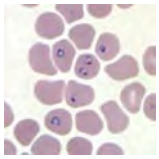
MQO : Malate-quinone oxidoreductase



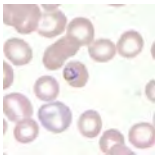
Cell-based screening

Screening target: extracts with inhibitory activity for proliferation of *Plasmodium falciparum* 3D7

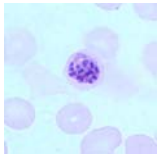
Life-stage of *Plasmodium falciparum*



Ring-form trophozoites



Trophozoites



Schizonts

10

Progress 2017

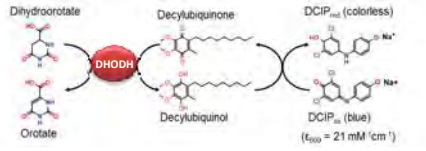
Screening of Active Extract

Anti-malaria

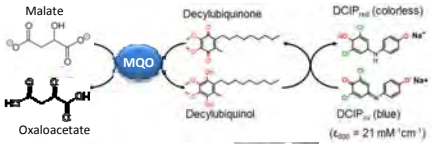
Enzyme-based Screening

Objective: To search extract with inhibitory activity against malaria parasite specific target enzymes

Target enzyme: *Pf*DHODH



Target enzyme: *Pf*MQO



Calculation of inhibition rate

$$\text{Inhibition rate (\%)} = \left(1 - \frac{A_s - A_p}{A_n}\right) \times 100\%$$

As = Sampel absorbance, Ap = Positive control absorbance (no substrate), An = Negative control absorbance (with substrate)

Result (PfDHODH) **Total extract screened = 12.028**

Year		Sample			
		Fungi	Actino-mycetes	Plant	Total
2015	Screened	640	640	0	1280
	Hit	11	6	0	17
	Hit rate (%)	**1.7**	**0.9**	**0**	**1.3**
2016	Screened	3200	2880	120	6200
	Hit	76	31	0	107
	Hit rate (%)	**2.3**	**1.1**	**0**	**1.7**
2017	Screened	2615	1825	108	4548
	Hit	36	0	5	41
	Hit rate (%)	**1.4**	**0**	**4.6**	**0.9**

Result (PfMQO) **Total extract screened = 11.148**

Year		Sample			
		Fungi	Actino-mycetes	Plant	Total
2015	Screened	240	240	0	480
	Hit	53	21	0	74
	Hit rate (%)	**22.0**	**8.7**	0	**15.4**
2016	Screened	2400	2080	120	4600
	Hit	106	73	29	208
	Hit rate (%)	**4.4**	**3.5**	**24.2**	**4.5**
2017	Screened	3095	2865	108	6068
	Hit	89	22	52	163
	Hit rate (%)	**2.5**	**0.7**	**48.1**	**2.7**

Progress 2017

Screening of Active Extract

Anti-malaria

Screening (Enzyme-based screening performance)

Z-factor: a statistical tool for comparison and evaluation of the quality of high-throughput screening assay (Zhang et.al., 1999)

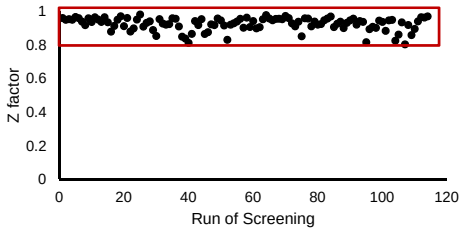
$$Z = 1 - \frac{(3\sigma_s + 3\sigma_c)}{|\mu_s - \mu_c|}$$

σ_s : standard deviation of positive control
σ_c : standard deviation of negative control
μ_s : mean of positive control
μ_c : mean of negative control

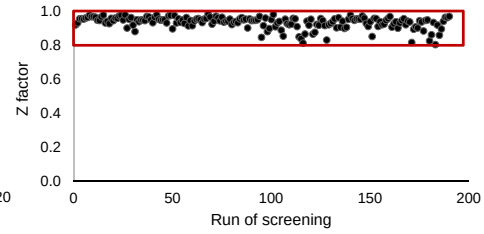
Screening assay quality evaluation

Z = 1 ideal assay
1 > Z ≥ 0.5 excellent assay
0.5 > Z ≥ 0 marginal assay

Z-factor of PfDHODH inhibitor screening



Z-factor of PfMQO inhibitor screening



Z factor of both enzyme inhibitory screening was higher than 0.8

→ **The quality of screening data was good and reliable**

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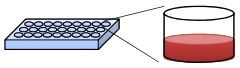
Progress 2017

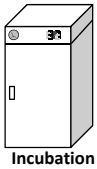
Screening of Active Extract

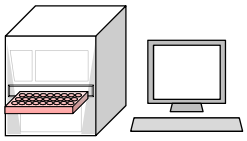
Anti-malaria

Cell-based Screening

Objective: To search extract with inhibitory activity to proliferation of malaria parasite cell



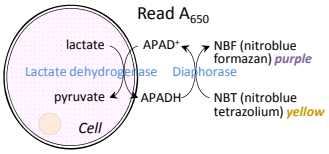




Sorbitol-treated *P.falciparum* cell culture + extract
0.3% parasitemia, 3% hematochrit (blood type=0(+)) in RPMI (+) (10% Albumax®), culture volume 100 µl

Incubation
37°C, 5% CO_2, 5% O_2, 48 h

LDH assay
Read A_{650}



Calculation of inhibition rate

$$\text{Inhibition rate (\%)} = \left(1 - \frac{A_s - A_p}{A_n - A_p}\right) \times 100\%$$

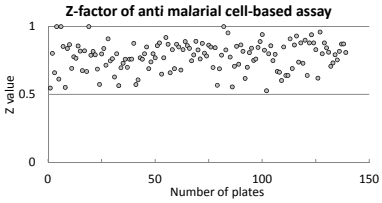
As = Sampel absorbance
Ap = Positive control absorbance (atovaquone)
An = Negative control absorbance (DMSO)

Screening result

Source	Number of extract	Number of Hit	Hit rate (%)
Actinomycetes	3080	497	16.1
Fungi	2640	200	7.6
Total	**5720**	**697**	**12.2**

Hit is considered as extract with inhibition rate ≥ 50%
Atovaquone concentration: 1µM

Screening performance



Z value of screening > 0.5
→Screening result is reliable

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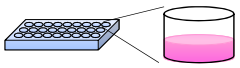
Progress 2017

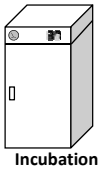
Screening of Active Extract

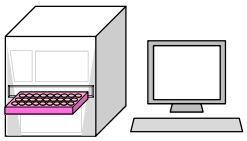
Toxicity Assay

Toxicity Assay

Objective: To evaluate toxicity of active extract that inhibit proliferation of malaria parasite against mammalian cell



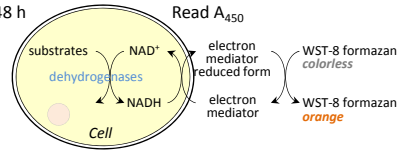




Human colon cancer DLD-1 cell culture + extract
Initial cell number 2.5 x 10^4, DMEM medium (10% FBS, 1% Pen/Strep), culture volume 200 µl

Incubation
37°C, 5% CO_2, 48 h

WST-8 assay
Read A_{450}



Calculation of survival rate

$$\text{Survival rate (\%)} = \left(\frac{A_s - A_b}{A_c - A_b}\right) \times 100\%$$

As = Sampel absorbance
Ab = Positive control absorbance (Blank)
Ac = Negative control absorbance (DMSO)

Screening result

Source	No. of extract	1st screening hit	Hit rate (%)	Non toxic hit	Hit rate*(%)
Actinomycetes	2000	363	18.2	306	15.3
Fungi	1200	88	7.3	80	6.7
Total	**3200**	**451**	**14.1**	**386**	**12.1**

Toxicity assay reduced hit rate from 14.1% to 12.1%

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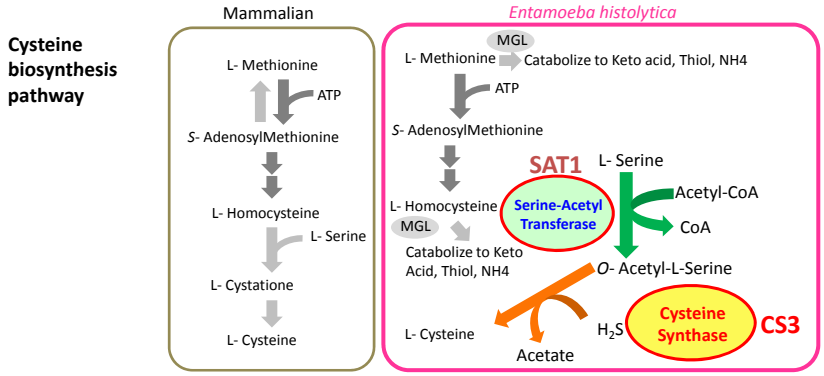
Progress 2017 — Screening of Active Extract — Anti-amoeba

Objective: To search extract with antiamebic activity

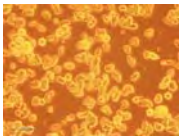
Enzyme-based screening

Screening target: extracts with inhibitory activity for SAT1 and CS3



Cell-based screening

Screening target: extracts with inhibitory activity for proliferation of *Entamoeba histolytica* HM-1:IMSS cl6



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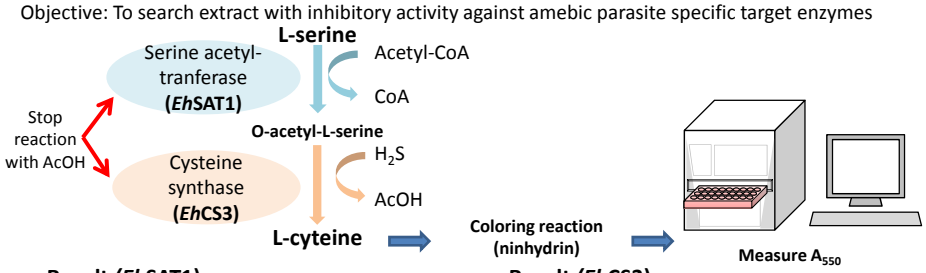
Progress 2017 — Screening of Active Extract — Anti-amoeba

Enzyme-based Screening

Objective: To search extract with inhibitory activity against amebic parasite specific target enzymes



Result (*Eh*SAT1)

Year		Sample		
		Fungi	Actino-mycetes	Total
2015	Screened	0	0	0
	Hit	0	0	0
	Hit rate (%)	**0**	**0**	**0**
2016	Screened	280	480	760
	Hit	7	24	31
	Hit rate (%)			
2017	Screened	420	1040	1460
	Hit	7	3	10
	Hit rate (%)			

Total extract screened = 2.220

Result (*Eh*CS3)

Year		Sample		
		Fungi	Actino-mycetes	Total
2015	Screened	240	80	320
	Hit	1	0	1
	Hit rate (%)		**0**	
2016	Screened	200	240	440
	Hit	4	0	4
	Hit rate (%)			
2017	Screened	2000	1840	3840
	Hit	64	122	186
	Hit rate (%)			

Total extract screened = 4.600

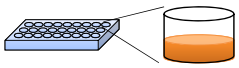
Progress 2017

Screening of Active Extract

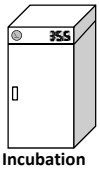
Anti-amoeba

Cell-based Screening

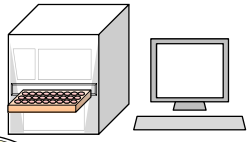
Objective: To search extract with inhibitory activity to proliferation of amebic parasite cell



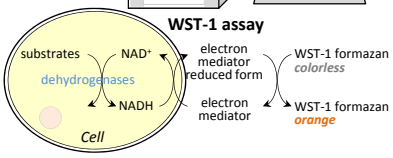
***E.histolytica* cell culture + extract**
Initial cell number = 8000 cells in BIS medium, culture volume 200 µl



Incubation
35.5°C,
24 or 48 h



WST-1 assay



Result

Year		Sample		
		Fungi	Actinomycetes	Total
2015	Screened	0	0	0
	Hit	0	0	0
	Hit rate (%)	**0**	**0**	**0**
2016	Screened	320	560	880
	Hit	8	31	39
	Hit rate (%)	**2.5**	**5.5**	**4.4**
2017	Screened	2480	2640	5120
	Hit	82	131	213
	Hit rate (%)	**3.3**	**4.9**	**4.1**

Total extract screened = 6.000

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Progress 2017

Microbial Isolation, Identification, and Extract Production
Screening of Active Extract
Purification of Active Compound
Other Activities
Budget Arrangement

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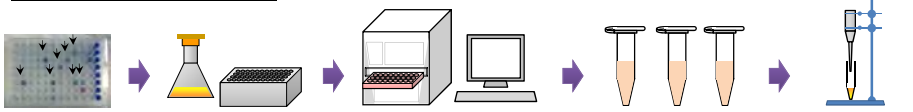
Progress 2017 Purification

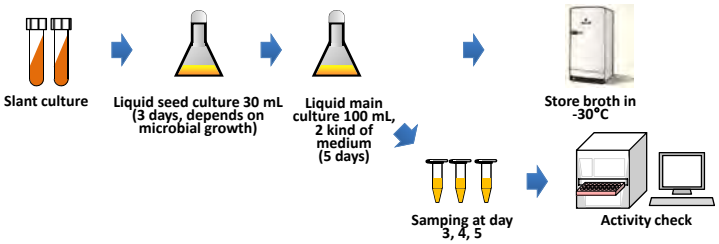
Objective: To obtain purified compound with antimalarial/antiamebic activity

General purification flow



Reconfirmation extract production

Objective: To prepare *hit* extracts by reculturing the producer (microbial strain) for confirming their activities and for preliminary purification process



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Progress 2017 Purification

Reconfirmation extract production

Reconfirmation Extract Production (2017)	
	Number of extract
I Antiamebic	
- *Eh* SAT	-
- *Eh* CS3	32
- *E.histolytica*	104
- *Eh* PanK	14
II. Antimalaria	
- *Pf* DHODH	158
- *Pf* MQO	222
- *P.falciparum*	56
- *Pf* PanK 1.2	18
- *Pf* DHODH&*P.falciparum*	8
- *Pf* MQO&*P.falciparum*	14
Total	**626**

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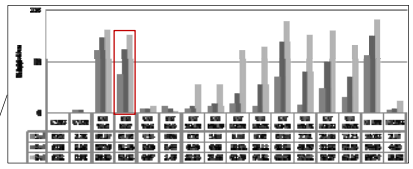
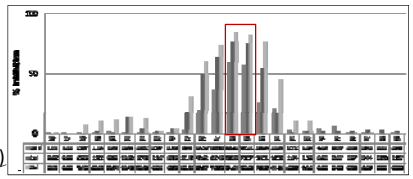
Progress 2017 Purification Anti-malaria

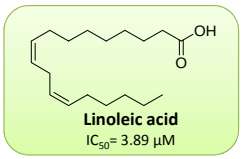
PfMQO

Extract code : F.0267
Producer : Fungus, BioMCC-f.I.1004 (*Trematosphaeria biappendiculata*)
Source : Insect gut (termite)
Sampling point : Pangandaran, West Jawa

5 L of microbial broth
↓ **Extracted with BuOH (1:1)**
7.36 g (brown oily, IC_{50} 30.9 µg/ml)
↓
0.98 g
↓ **Partition in hexane-MeOH**
MeOH part (733 mg, IC_{50} 25.1 µg/ml)
↓ **Silica gel open column**
$CHCl_3$-MeOH 1:0, 98:2, 95:5, 9:1, 8:2, 6:4, 5:5, 0:1
$CHCl_3$-MeOH (98:2)-2 (59.9 mg, IC_{50} 19.0 µg/ml)
↓ **Sephadex LH-20 open column (MeOH)**
3 ml x 120 fractions
Fr. 28 + Fr. 29 (11.1 mg)
↓ **^{1}H-NMR**







Linoleic acid
IC_{50}= 3.89 µM

(Amila et.al., 2017)

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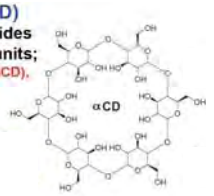
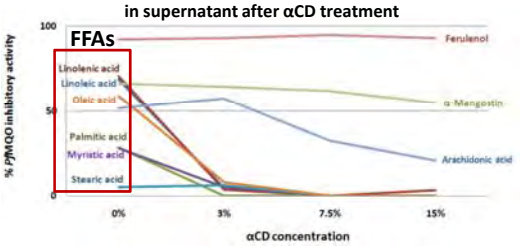
Progress 2017 Purification Anti-malaria

PfMQO **Dereplication of Free Fatty Acids (FFAs) from extracts**

Cyclodextrin (CD)
Cyclic oligosaccharides containing glucose units;
6 units (αCD), 7 units (βCD), 8 units (γCD)

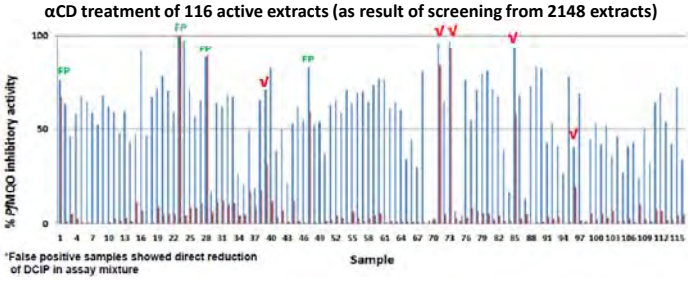


PfMQO inhibitory activity of FFAs and other inhibitors in supernatant after αCD treatment



αCD binds with free fatty acid selectively to form an inclusion complex

αCD treatment of 116 active extracts (as result of screening from 2148 extracts)



*False positive samples showed direct reduction of DCIP in assay mixture

- **αCD significantly reduced number of active extracts (116 to 5)**
- **αCD is effective to dereplicate FFAs from microbial extracts in PfMQO inhibitor screening system**

No αCD treatment
15% αCD treatment
FP False positive
√ Candidate

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(Amila et.al., 2017)

Progress 2017 Purification Anti-malaria

PfMQO

List of PfMQO Inhibitory Active Extract to be Purified

No	Extract Code	100 ml cultivation	Large scale cultivation	α-CD Treatment	Remark
1	F15.1645	√	5 L (2x)	√	
2	P3	√	2 L	√	
3	F15.0538	√	5 L	√	
4	F.1688	√	-	√	STOP
5	F.0538	√	-	√	STOP
6	F.1645	√	-	√	STOP
7	F15.1645	√	-	√	STOP
8	F.1676	√	5 L	√	STOP
9	F.0492	√	5 L	√	STOP
10	F15.0492	√	5 L	√	STOP
11	F15.1794	√	-	√	STOP
12	F15.1676	√	5 L	√	STOP
13	F15. 1706	√	-	√	STOP
14	F.0174	√	-	√	STOP
15	F.0142	√	-	ND	
16	F.0143	√	-	ND	
17	F. 0193	√	-	ND	
18	F.0267	√	-	ND	
19	F15. 0174	√	-	ND	
20	F. 0194	√	-	ND	
21	F. 0159	√	-	ND	

Currently being purified

Not continued due to free fatty acid content

Will be proceeded for purification

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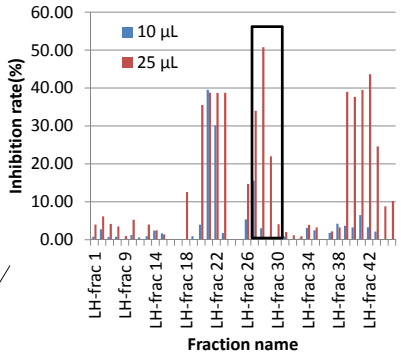
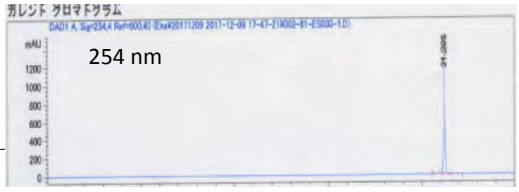
Progress 2017 Purification Anti-malaria

PfMQO

Extract code : **F15.1645**
Producer : Fungus, BioMCC-f.PL.142
Source : Plant litter
Sampling point : Kupang, Nusa Tenggara Timur

2.7 L microbial Broth
↓ Separate mycelia and supernatan
mycelia
↓ Extracted with MeOH (1:1)
Dried mycelia extract (16 gram)
↓ Extracted with Hexane
MeOH part (12.74 gr)
↓ Silica gel open Column
CHCl3-MeOH (8:2), 169.2 mg
↓ Sephadex LH-20 open
Fraction 27- 33, 12 mg
↓
Preparative HPLC





Progress 2017

Purification

Anti-malaria

PfDHODH

List of PfDHODH Inhibitory Active Extract to be Purified

No	Extract Code	PET	Polarity Open Column (ODS/HP-20/ Silica)	Open column LH-20	HPLC profile	HPLC-prep	LC-MS	Remark	
1	F15.1158	√	√ (HP-20)	√	√	√			Currently being purified
2	F.2182	√	√ (Silica)	√	√	√			
3	F15.2274	√	√ (Silica)	√	√	√			
4	F15.2383							No activity	
5	F15.2236	√							Will be proceeded for purification
6	F.2046	√							
7	F15.2179	√							
8	F15.2584	√							
9	F.2182	√							
10	F15.2299	√							
10	F15.2299								
11	F15.2274	√							

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Progress 2017

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

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Progress 2017

International Symposium on Natural Resources-based Drug Development

Date : August 21-22, 2017
Venue : BPPT Building II 3rd F, Jl. MH Thamrin No.8, Jakarta
Invited speakers : 17 persons
Attendance : 116 persons (EoJ, JICA, governmental officials, researchers from universities, research institutes, pharma industries)

Objective

1. To promote and strengthen local and international network and collaboration on drug development
2. To promote research on utilization of Indonesia natural resources for drug development
3. To build capacity of Indonesian researcher on drug development
4. To accelerate the application of innovation on drug development

Prof. Eniya LD received a letter from Prof. Satoshi Omura, which is delivered by Prof. K.Shiomi

Dr. Unggul Priyanto (BPPT Chairperson) delivered opening remark

Prof. K. Kita gave keynote speech

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Progress 2017

Research Networking

Among Project Related Institutes

Airlangga University
- Discussion on project progress (May 2017)

LIPI
- Discussion on project progress (October 24, 2017)
- LIPI shared 200 microbial strains to BPPT to be used as resource for screening

Kitasato University
- Discussion on Material Transfer Agreement (September 29, 2017)

Nagasaki University
- Courtesy visit and introduction of project activities (Oct 4, 2017)

Among Other Institutes

Obihiro University of Agriculture and Veterinary Medicine, Japan
- Joint research on drug development for anti-toxoplasmolysis
- More then 3000 microbial extracts are currently being screened

National Islamic University (UIN) Syarif Hidayatullah, Jakarta
- UIN provided local plant extracts to be assayed for anti-malarial activity
- Currently, 2 active plant extracts are being purified (with inhibitory activity against PfMQO)

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Progress 2017

Publication and Seminar

Publication on BBA Bioenergetics

- First publication written by Indonesian researcher as the first author

Presentation in The 9th International Seminar of Indonesian Society for Microbiology, Palembang (November 14-15, 2017)

- Presented 4 topics related to the project achievements



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Progress 2017

Training

Training in Japan

Name	Topic	Period	Place
Short term training			
Endah Dwi Hartuti	Expression, Purification, Activity Measurement of Plasmodium falciparum Enzymes	12 June 2017 – 14 July 2017	School of Tropical Medicine and Global Health, Nagasaki University
Erwahyuni E. Prabandari	Production of enzyme for screening of antiparasitic active compounds	18 September 2017 – 14 October 2017	University of Tokyo dan University of Nagasaki
Nurlaila	Purification of active compound	18 September 2017 – 14 October 2017	Department of Drug Discovery Sciences, Kitasato Institute for Life Sciences, Kitasato University
Eka Siska	Structure elucidation of active compound	09 October 2017 – 02 December 2017	Department of Drug Discovery Sciences, Kitasato Institute for Life Sciences, Kitasato University
Kristiningrum	Isolation, Identification and characterization of Fungi	30 October 2017 – 23 December 2017	Department of Drug Discovery Sciences, Kitasato Institute for Life Sciences, Kitasato University
Nadia Adipratiwi	Amebic Culture and Amebic Cell-based Assay, MRC_5 Cell-based Assay, and Plasmodium Cell-based Screening	30 October 2017 – 23 December 2017	Nagasaki University and University of Tokyo
Long term training			
Amila Pramisandi	Drug Discovery Sciences: Isolation and structure elucidation of antiprotozoal antibiotics	03 April 2017 – 19 March 2020	Graduate School of Infection Control Sciences, Kitasato University, Tokyo Japan
Endah Dwi Hartuti	Metabolism-based drug discovery against plasmodium	22 Augusst 2017 – 21 August 2021	School of Tropical Medicine and Global Health, Nagasaki University

Progress 2017

Training

Training in Indonesia by Japanese Expert

Nama	Expertise	Period	Institution
Prof. Tomoyoshi NOZAKI	Tropical Medicine Research	16 – 24 May 2017 14 – 24 August 2017 10 – 18 October 2017 21 – 29 December 2017	University of Tsukuba & University of Tokyo
Prof. Kazuro SHIOMI	Isolation, Purification, and Structure Analysis of Chemical Compounds	20 – 22 August 2017	Kitasato University
Dr. Mihoko MORI	Isolation, Purification, and Structure Analysis of Medical Compounds	09 – 26 May 2017	Kitasato University
Dr. Toshiyuki TOKIWA	Isolation, Purification, and Structure Analysis of Medical Compounds	09 – 13 May 2017	Kitasato University
Dr. Kazuyuki DOBASHI	Isolation, Purification, and Structure Analysis of Medical Compounds	21 – 24 May 2017 12 November 2017 – 08 December 2017	Kitasato University
Dr.Michio YAMASHITA	Isolation, Purification, and Structure Analysis of Medical Compounds	21 – 25 May 2017 13 August 2017 – 09 September 2017	University of Tokyo
Dr. Ken Daniel INAOKA	Malaria (Investigation and Analysis)	09 – 25 August 2017 11 – 21 November 2017	Nagasaki University
Dr. Yukiko MIYAZAKI	Malaria (Investigation and Analysis)	15 – 22 August 2017	Nagasaki University
Dr. Azuma WATANABE	Isolation, Purification and Structure Analysis of Chemical Compounds	20 – 26 August 2017	MicroBioFarm Japan
Dr. Takaya SAKURA	Malaria (Investigation and Analysis)	11 November 2017 – 06 December 2017	Nagasaki University

Progress 2017

Microbial Isolation, Identification, and Extract Production
Screening of Active Extract
Purification of Active Compound
Other Activities
Budget Arrangement

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Progress 2017

Disimbursment

BC for SLeCAMA project 2017

- Initial budget = Rp. 500.000.000
- After budget optimization = Rp. 476.930.000

Insinas MoRTHE 2017

- Budget = Rp. 258.175.000

Other BC fund 2017

- Budget = Rp. 151.510.000

→ **Total = 886.615.000**

Description	Realization (Rp.)	Note
Chemical & laboratory supplies	361,418,850	Incl. gases and liquid gases
Salary	233,316,000	Salary for not permanent BC member
Office supplies	8,078,250	Stationaries
Travel	54,391,246	Field trip, visit AU&LIPI
Mantenance & repair	1,775,000	
Meeting	125,030,080	JCC Meeting, International Symposium, etc.
Equipment	33,029,150	AC, Printer
Other	5,557,000	Seminar registration fee, delivery fee
TOTAL	**822,595,576**	

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Planning 2018

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Planning 2018

Research

1. **Field expedition**
   - Location: Puspiptek Area
2. **Microbial isolation and identification**
   - Target: 1000 identified isolates
3. **Extract preparation**
   - Target: 3000 extracts for 1st screening
4. **Screening of active extract**
   - Target:
     a. Anti-malaria : 5000 extracts
     b. Anti-ameba : 5000 extracts
5. **Purification of active compound**
   - Target: 4 purified and structure-elucidated compounds
6. **Animal test**
   - Target: 1 compound
7. **Publication**
   - Target: submission of 2 international peer-reviewed papers

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Planning 2018

Budget Arrangement

Budget Arrangement

- BPPT allocated budget for FY 2018 as much as **Rp. 418.444.000**
- BPPT is currently applying some proposals to several funding agency, including to Ministry of Research, Technology and Higher Education, with total of proposed budget is as much as **Rp. 800.000.000**

Description	BPPT Budget (Rp.)	Note
Salaries	198.911.000	Salary for not permanent BC member
Reagents and consumables	62.757.000	Incl. gases and liquid gases
Travel	17.976.000	Transportation (airfare, sea, ground), accomodation, daily allowance
Equipment	138.800.000	Laboratory bench, etc.
TOTAL	**418.444.000**	

Planning 2018

Training

List of Proposed Researcher for Training in Japan

	Name	Topic	Destination	Length	Period (2018)
1	Danang Waluyo	Searching of new target for drug discovery	UTo	1 month	September
2	Erwahyuni E. Prabandari	Searching of new target for drug discovery	NU	1 month	November
3	Eka Siska	Purification of active compound	KU	1 month	June
4	Nurlaila	Purification of active compound	KU	1 month	July
5	Evita Chrisnayanti	Purification of active compound	KU	1 month	August
6	(Tentative)	Isolation and identification of actinomycetes	KU	1 month	August
7	Kristiningrum	Isolation and identification of fungi	KU	2 month	July-Sep
8	Dian Japany Puspitasari	Searching of new target for drug discovery	UTo/NU	1 month	October
9	(Tentative)	Mass production of active compound	KU	1 month	September
10	(LIPI)	Isolation and identification of fungi	KU	1-2 month	September
11	(LIPI)	Isolation and identification of actinomycetes	KU	1-2 month	October

Planning 2018

Project Management

Implementing unit	**Laboratory for Biotechnology-BPPT (Biotech Center)**
Project Director	**Prof. Dr. Eng. Eniya Listyani Dewi, B.Eng., M.Eng.** (Deputy Chairperson of Technology for Agroindustry and Biotechnology, BPPT)
Project Manager	**Dr. Agung Eru Wibowo, Apt.** (Head of Laboratory for Biotechnology, BPPT)
Project Co-manager	**Danang Waluyo, M.Eng.** (Program Head, BPPT)
Project Co-manager	**Prof. Maria Inge Lusida, M.Kes., Sp.MK(K), Ph.D.** (Head of Institute of Tropical Disease, Airlangga University)
Project Co-manager	**Dr. Atit Kanti, M.Sc.** (Head of InaCC, LIPI)

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Thank You

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Report activities of ITD-UNAIR

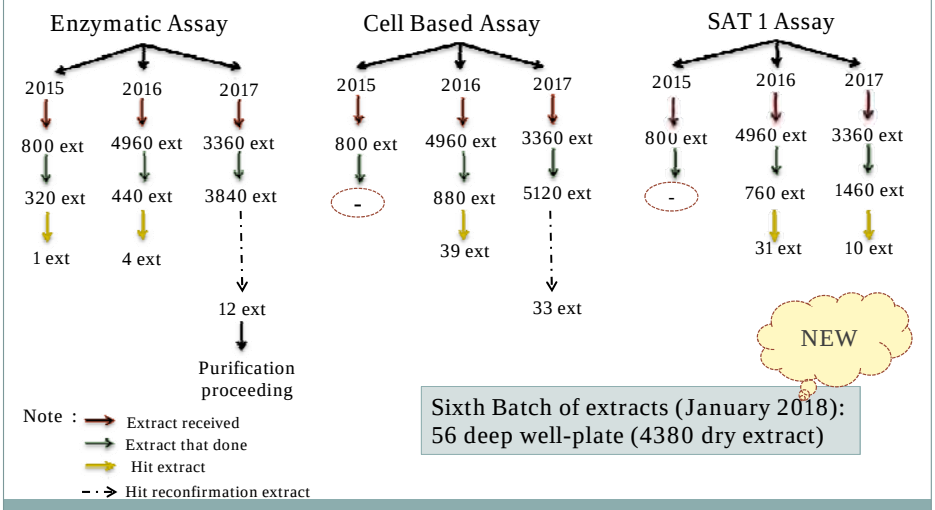
"Project for Searching Lead Compounds of anti-Malarial and Anti-Amebic Agent by Utilizing Diversity of Indonesian Bio-Resources"

January 31, 2018

Third Year Activities

- Training in Japan:
  - Mrs. Peni
    - Training January 2017
    - Long term for doctoral program start from April 2017
- Laboratory Set up
- Laboratories Activity
- Consumables (reagents, plasticware and glassware)
- Training from Japanese researcher to ITD-UNAIR for anti malarial and enzymatic assay
- Screened dried extract from BPPT (Cell culture based and enzymatic based screening)
- Assay for Confirmation Extract from BPPT
- Preliminary run for fractionation
- Chemical Analysis

Extract from BPPT & Result



Sixth Batch of extracts (January 2018):
56 deep well-plate (4380 dry extract)

Future Plan

- Training in Japan :
  - Ms. Lidya (July 2018)
  - Mrs. Myrna (January 2018 and July 2018)
- Purification for hit extracts
- AntiAmoeba activity assay guided for purification and fractionation extracts
- Primary screening and secondary screening of BPPT samples (56 deep well-plate (4380 dry extract))
- Primary screening with different enzymes (CS3 and SAT1)

Counter Budget 2017

Item	Detail	Disebursed amount in Rupiah	Remarks Kick off / JCC Meeting
Travel cost	Airfare SUB and JKT (Feb 2017,13 dr. Dwi Peni, M.Imun)	2.674.000	Training JICA
	Airfare SUB and JKT, taxi & Accomodation 2D2N(August 2017,25 Prof. Achmad Fuad)	6.483.332	Progress meeting
	Airfare SUB and JKT, taxi & Accomodation 2D2N(August 2017,25 Dr. Myrna Adianti, Ph.D)	5.193.271	Progress meeting
	Airfare SUB and JKT, taxi & Accomodation 2D2N(August 2017,25 Defi Kartikasari, S.Si)	3.869.531	Progress meeting
Consumables	10ul tips extra long, sterile, Rnase and Dnase Free, 1000/bag	2.062.500	
	NaOH 1000 gram	572.000	
	yellow tips 200 ul	3.850.000	
	50 ml centrifuge tube	1.980.000	
	15 ml centrifuge tube	2.640.000	
	microcrystal tips 0.5-10ul	2.750.000	
	90mm Petri Dish	2.475.000	
	Yellow tips 200 ul	1.375.000	
	microcrystal tips 0.5-10ul	1.925.000	
	4-way flipper racks	715.000	
	microtube 1.5 ml	1.650.000	
	metal enhanced dab substrate kit	4.000.000	
	pipet tips 1-1000ul	3.960.000	
Technition Lab and Honorarium	maintanance laboratory	~~107.552.400~~	
	Total	155.727.034	

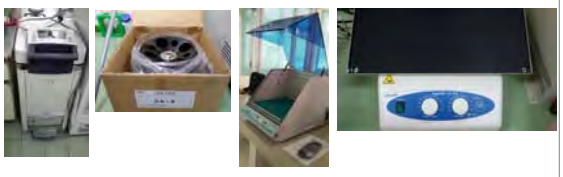
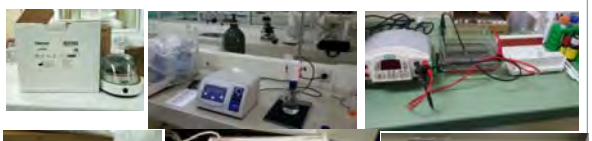
Future Plan Counter Budget 2018

Item	Detail	Diserbursed amount in Rupiah	Remarks Kick off/ JCC meeting
Travel cost	Airfare SUB-JKT , taxi & acomodation 3D2N (Jan & August 2018 Prof. Achmad Fuad)	18.000.000	JCC meeting & progress meeting
	Airfare SUB-JKT , taxi & acomodation 3D2N (Jan 2018 Dr. Aty Widyawaruyanti)	9.000.000	JCC meeting
	Airfare SUB-JKT , taxi & acomodation 3D2N (Jan & August 2018 Myrna Adianti, Ph.D)	15.000.000	JCC meeting & progress meeting
	Airfare SUB-JKT , taxi & acomodation 3D2N (Jan 2018 Lidya Tumewu, M.Farm, Apt.)	6.000.000	JCC meeting
	Airfare SUB-JKT , taxi & acomodation 3D2N (Jan & August 2018 Defi Kartika Sari, S.Si)	10.000.000	JCC meeting & progress meeting
	Airfare SUB-JKT , taxi & acomodation 3D2N (Jan & August 2018 Yulia Rahmawati, S.Si)	10.000.000	JCC meeting & progress meeting
Consumables	10ul tips extra long, sterile, Rnase and Dnase Free, 1000/bag	2.062.500	
	NaOH 1000 gram	572.000	
	AlbuMAX 25 g	3.500.000	
	50 ml centrifuge tube	1.980.000	
	15 ml centrifuge tube	2.640.000	
	microcrystal tips 0.5-10 ul	2.750.000	
	90mm Petri Dish	2.475.000	
	Yellow tips 200 ul	2.500.000	
	microcrystal tips 0.5-10 ul	3.500.000	
	4-way flipper racks	715.000	
	microtube 1.5 ml	3.500.000	
	metal enhanced dab subtrate kit	4.000.000	
	pipet tips 1-1000 ul	3.960.000	
	maintanance laboratory	~~107.552.400~~	
	Total	**209.706.900**	

Thank you

Lab Equipment

ITEM NO.	EQUIPMENT NAME
1	Autoclave
2	Rotor
3	Incu Shaker
4	Rocking Platform Shaker
5	Mini Sentrifuge MySpin 6
6	Sonic Ruptor 400
7	PCR-T 100
8	Pippet Aid
9	Gel documantation system
10	Cell GT Basic System
11	Mini Gel Caster
12	Gel Try
13	Basic Power Suply
14	Single pippet
15	Finnpipette


Laboratories Activity

Cell Based Assay

Training Anti-Malarial

Purification process

Enzymatic assay

Reconfirmation extract

Consumables-Reagens

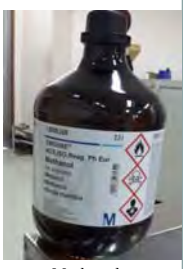
DMSO

Acetyl CoA

HCl 37%

Acetic Acid

Ethanol



Methanol

WST-1

Ninhydrin

L-serine

Bovine Serum

OPTI-MEM

Consumables-Plasticware

Well Plate-Round Bottom


Autoclave Bag


Microtube Racks


Hands glove


Well Plate-Flat Bottom

50 mL Centrifuge Tube


PCR tube Racks

PCR tube 8 strips

Consumables-Glassware


1000 mL bottle


500 mL bottle


250 mL bottle


100 mL bottle


1000 mL beaker


500 mL beaker

Visiting Japanese Researcher

Tomoyoshi Nozaki

Time Duration	Subject
March 15&16, 2017	Freeze thawing & Cell Based Assay
May 17-19, 2017	SAT 1 Assay
August 15&16, 2017	Enzymatic Assay and Cell Based Assay
October 11-13, 2017	Cell Based Assay and DNA extraction
December 27&28, 2017	Discussion result Cell Based and Enzymatic Assay

Daniel Ken Inaoka

Time Duration	Subject
February 6-10, 2017	Enzymatic Assay
August 12-15, 2017	Enzymatic Assay
November 20&21, 2017	Training Anti Malarial

Takaya Sakura

Time Duration	Subject
Nov 20- Des 3, 2017	Training Anti Malarial

Kazuyuki Dobashi

Time Duration	Subject
January 15-21, 2018	Purification Process

Fractionation of hits no.12 (CS3.F15.0803.R2.12)

1 BuOH Extract (100 mg)

+MeOH, fractionated with Hexane

2 Hexane fraction (H: 16.6 mg)

3 MeOH fraction (M)

4 MeOH Precipitate fraction (Mp)

Suspended in Aquadest, Fractionated with ethyl acetate (EA)

5 EA fraction (EA: 11.8 mg)

Aquadest layer

6 EAp fraction (EAp: 5.9 mg)

Aquadest layer

Fractionated with BuOH

Fractionated with BuOH

7 BuOH fraction (B: 14.1 mg)

8 Aquadest fraction (A: 30.2 mg)

9 BuOH p fraction (Bp: 13.2 mg)

10 Aquadest p fraction (Ap: 3.1 mg)

Samples 1-10 → Enzymatic assay

JCC THIRD YEAR

The Project for Searching Lead Compounds of
Anti-Malarial and Anti-Amebic Agents
by Utilizing Diversity of Indonesian Bio-resources

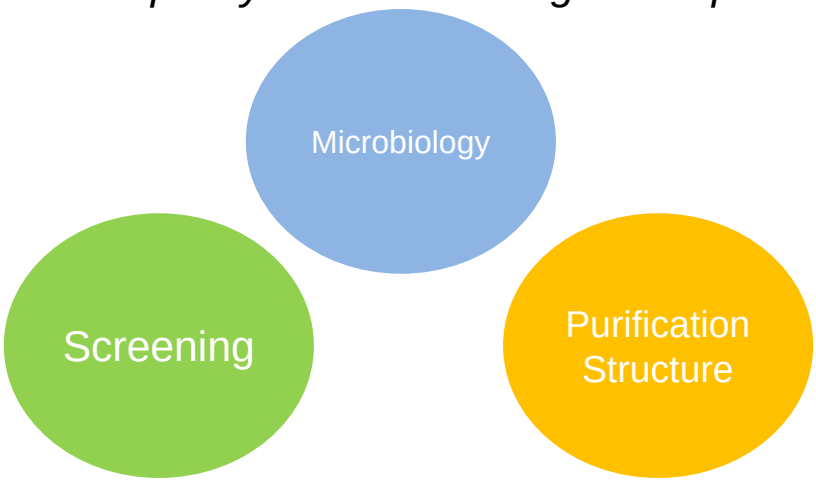
Identified problems/needs and solutions

Tomo NOZAKI
The University of Tokyo
CHIEF ADVISOR

BPPT, Jakarta, January 31th, 2018

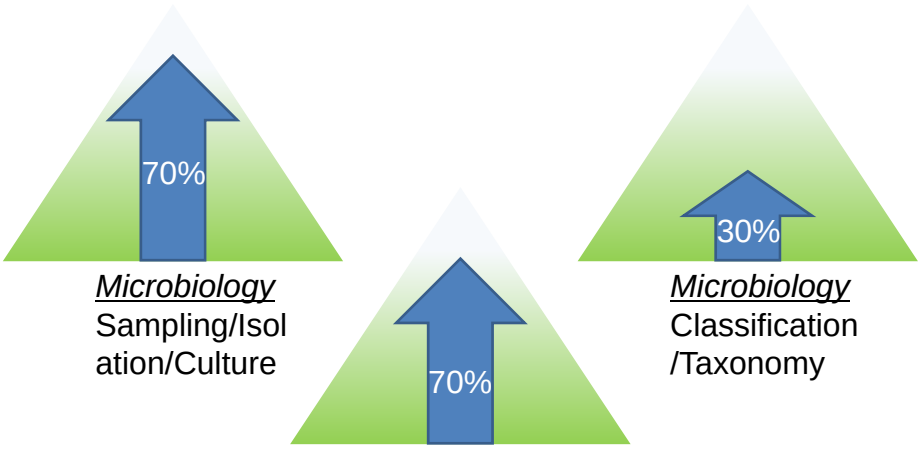
Goals of the project

1. *Identify >1 lead compounds with anti-malarial and anti-amebic activities in vivo*
2. *Build capacity needed for drug development*



Accomplishment of goals

Research Capacity Building – Microbiology



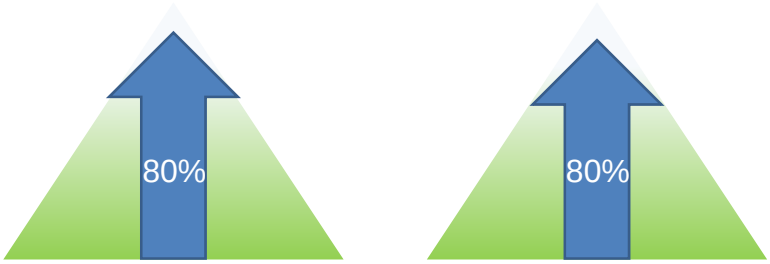
Microbiology
Sampling/Isolation/Culture

Microbiology
Classification/Taxonomy

Microbiology
Extract production
Data management

Accomplishment of goals

Research Capacity Building – Screening

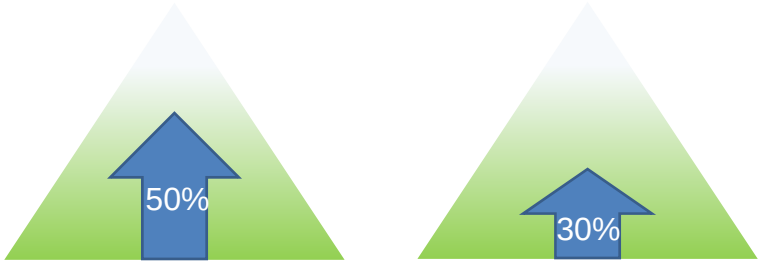


Screening
Enzyme-based assay

Screening
Cell-based (phenotypic) assay

Accomplishment of goals

Research Capacity Building
– Purification and structural elucidation

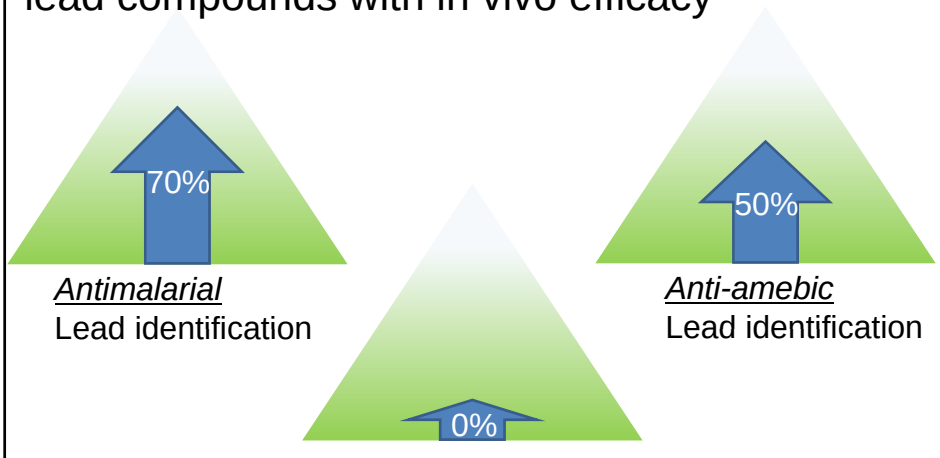


Purification
Liquid partition
Chromatography

Structural elucidation
Mass spectrometry
Nuclear magnetic resonance

Accomplishment of goals

Identification of anti-malarial and anti-amebic lead compounds with in vivo efficacy



Antimalarial
Lead identification

Anti-amebic
Lead identification

In vivo Efficacy confirmation

Problems / needs

1. Characterization/archiving of microbial strains…..Critical for future use of the libraries as open source
2. Exploitation of new targets and introduction of new screening platforms
3. Prioritization of identified hits for purification
4. Broadening of the bottleneck process(es) (purification/structure elucidation)

5. Cordination between BC/Airlangga U/InaCC……Sample transfer/record
6. Establishment and development of a drug develop consortium (networking)
7. Broadening of disease areas
8. Sustainable development of the capacity

Solutions to problems/needs

1. Characterization/archiving of microbial strains…..Enhance training for taxonomy
2. Exploitation of new targets and introduction of new screening platforms…3-4 new enzyme targets have been selected and will be explored
3. Prioritization of identified hits for purification…Ranking of hits by selectivity index, counter-screening, taxonomy of isolates, preliminary extraction test
4. Broadening of the bottleneck process(es) (purification/structure elucidation)…Inclusion of additional purification stations (Unair and UTokyo)

Solutions to problems/needs

5. Cordination between BC/Airlangga/InaCC……Periodical mutual visits / joint meetings for data and method sharing; cross depositing of microbes
6. Establishment and development of a drug develop consortium (networking)……Utilization of next JCC meeting or International Symposium
7. Broadening of disease areas….toward other infectious diseases (e.g., Helicobacter/TB/HIV/hepatitis) and non-communicable diseases (e.g., cancers/obesity/hypertension….)
8. Sustainable development of the capacity…..Continuous funding >5 years, continuous oversea collaboration/exchange

Other general difficulties/problems

Academic/Governmental systems for research

- Paucity and stability of academic/governmental research positions
- Gender bias of opportunities (e.g. degrees)
- Lack of incentive of being in academia
- Lack of incentive of high achievement
- Heavy administrative responsibilities
- Limited resources for funding

School education systems

- Low mathematics/science knowledge at high school and college levels

Social behaviors

- Indifference to others' activities
- Lack of spontaneity (too obedient)
- Lack of atmosphere of healthy mutual criticisms

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Plan for capacity building in 2018

Training in Japan

- 6 or more long-term (3-5 years) trainees (incl. other funding sources)
- 11 short-term (1-2 months) trainees

Training in Indonesia

- 20 dispatch of Japanese experts (1-8 weeks)