MINUTES OF MEETING

OF

THE 4th 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 4th 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 Hotel Sari Pacific Jakarta, Jakarta, Indonesia on 29th January 2019.

As a result of the discussions, both Indonesian side and Japanese side agreed upon the matters in the document attached hereto.

Jakarta, 29th January 2019

Dr. Kaname KANAI
Executive Technical Advisor to the Director
General Human Development Department
Japan International Cooperation Agency

Dr.Soni Solistia Wirawan. M.Eng.
Deputy Chairperson for Agricultural
Technology and Biotechnology,
Agency for the Assessment and Application
of Technology (BPPT)
The Republic of Indonesia

At 10 am Ms. Suryani (MC) called the meeting to order.

I. Welcome address and Opening remarks

Dr. Ir. Soni Solistia Wirawan (Project Director/Deputy Chairperson for Agroindustrial Technology and Biotechnology, BPPT) officially welcomed participants and special thanks for attend Dr. Kanai from JICA HQ, Prof. Fuad Representative Airlanga University and Prof. Nozaki from University of Tokyo to the meeting.

This collaboration project between Japan and Indonesia "Searching Lead Compound of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources (SLeCAMA) project" has made tremendous progress for these four years. This project has great programmes of capacity building for technical skill and human resources for drug development against tropical diseases like malaria and amebiasis by utilizing bio resources in Indonesia. During these four years of the project, more than 15,000 of extracts from natural resources in Indonesia had done 1st screening, and more than 600 extracts had been produced for reconfirmation of inhibition activity against Malaria and Ameba. Six anti-Malaria and one anti-Ameba extracts were purified from large scale microbial culture under appropriate quality control.

Biotech center BPPT also built various network including with Gadjah Mada University, Brawijaya University and Obihiro University of Agriculture and Veterinary Medicine in addition to current relationship of this project now.

Following Dr. Ir. Soni's welcome remarks, Dr. Kanai (Executive Technical Advisor to the Director General Human Development Department, JICA HQ) delivered his opening remarks. He also thanked to Dr. Ir. Soni Wirawan, Prof. Fuad and all of participants for attending this JCC meeting. This project is technical cooperation project between Indonesia and Japan supported by Japan International Cooperation Agency (JICA) and Agency of Medical Research and Development (AMED) named SATREPS and focused on technical partnership in public health and infectious diseases field. Start from SATREPS project, JICA and AMED are conducting many projects and currently10 projects are going now all over the world. On the earth there are huge number of useful natural resources for human life especially in Indonesia and Brazil and this collaboration will support sustainability of research related to natural resource development. He expected this project should be a great seed of good products for this country in near future.

Dr. Agung Eru Wibowo was selected as the JCC meeting Chairperson and invited person who would deliver next presentation.

II. Progress Review and Planning

1. 2018 Biotechnology Centre, BPPT

Firstly Mr. Danang Waluyo (Project Co-manager) from BTC, BPPT presented summary of the project progress in 2017. Then he expressed result of capacity development Isolation number, screening precision and extract purification were extremely increase.

After that he presented following progress in 2018.

122 Samples from the area of puspiptek and samples from Togean they were collected in 2017 had done for Microbial Isolation and 29 genus 459 microbes from puspiptek and 13 genus 220 microbes from Togean were isolated.

BioMCC-f.PL.142 from Fungi and BioMCC-a.T.2931 from Actinomycetes were identified as Identification of interest microbial isolates in 2018. 5120 extracts 2320 from Fungi and 2800 from Actinomycetes produced by 1st screening then 46 extracts though reconfirmation and pre scaleup procedures had produced in large scale.

Reproducibility of active extract for purification was increased significantly after Pre Scaleup extract production was introduced in July 2018.

PfDHODH, PfMQO and PfNDH2 were targeted for Enzyme based screening of Active Extracts against anti-malarial, 5120 extracts for PfDHODH, 3520 extracts for PfMQO and 5200 extracts for PfNDH2 have screened according to each method and gained each 5extracts, 5extracts and 1extract for large scale.

10160 extracts have screened by Cell-based screening and 83 extracts dereplicated though toxicity assay on 0.82% hit rate, then 6 active extracts gained from 35 reconfirmed extracts.

41 extracts were purified activity on PfDHODH, PfNDH2, PfMQO and PfCells and 6 structure were elucidated for active compounds.

6 project members from BTC were trained in Japan and more than 20 times of on-site training conducted by Japanese experts had held. Result of these training, all of important skills such as isolation, identification, screening and purification were improved extremely. 5 times Scientific meeting, Weekly meeting and twice Annual meeting in BTC were good opportunities of our technical and logical exchange for progress of their research. Also new networks with other universities and institutes should enhance BTC's motivation.

In 2019, BTC have plan for microbial sampling at Bawean Island for isolation and identification. Extract production team will improved the reproducibility of active extracts, Screening team will continue screening and considering to add another target and purification team will establish new dereplication method. And then active compound will be tested in animal.

2. 2018 Airlangga University

Prof. Achmad Fuad (Representative of Airlanga University) from Institute of Tropical Disease (ITD), Airlanga University presented progress in ITD 2018.

ITD-Airlanga University implement the part of Anti-amebic screening in this project.

In 2018, 7260 extract from BTC were screened against Entamoeba cell and gain 2 active extracts. One extract out of two large scaled extract confirmed nontoxic and it is on progress for purification. 7260 extract from BTC were done for Enzymatic assay against NADK/NO1 coupled enzymes and gain 50 hits. Then continual Enzymatic assay from 2016 against CS3/SAT1 was done against 4380 extracts and gain 26 hits. 2 hit extracts out of 26 extracts were gained though reconfirmation and PSU.

3. Overview of the project progress in 2018

Prof. Tomiyoshi Nozaki (Project Chief Advisor) from University of Tokyo presented the Achievement, needs and solutions of the project. There are 3 components which were Microbiology, Screening and Purification structure toward project goal capacity building for drug development. He indicated almost of project activities accomplished around 70 to 90% except molecular on Microbiology and In vivo Efficacy confirmation. He showed his suggestion in last JCC meeting and explained the current status. Some of suggestions from last JCC meeting were not achieved yet. " Dereplication step during screening" was introduced to solve problems finding common active compound.

In 2019 5-6 short term trainees were funded from this project and 7 long term trainees were funded from other sources will be invited to Japan. 20 Japanese experts will be despatched for on-site training. Then in October International Symposium will be held.

Ⅲ. Open Discussion and Comments

After presentations, Dr. Agung Eru Wibowo invited participants for their comments, inputs and suggestions.

Mr. Danang Waluyo expressed additional comments for the condition in progress, Appropriate strategical plan for Isolation process and compound development will certainly necessary, we should consider progressive design such as introducing pre identify and establishment of natural products data base.

Dr. Shiomi, Team Leader, Kitasato University indicated that there are many factors affected new compound purification. So remarkable compound finding will depend on appropriate or accurate Assay system. Even one species has many factors for possibility of active compound and new compound. However, reliable assay system and purification method are necessary.

Dr. Dobashi, Researcher, Kitasato University indicated that searching new screening approach from Biological Method or Unique purification Method for interesting and good compound will take long period. It is also difficult to find unique new active compound from one microbe strain from huge

national resources. For example, we can purify many compounds from one unknown strain, however, the point is whether they are actuary new or nontoxic.
Dr. Kanai gave his comment, SATREPS project has aims of utilization the research outcomes to the benefit of the society. Under SATREPS research collaboration, there are many successful projects such like developing rapid diagnosis kids for Ebola and Malaria. He supposed this project is implemented by all project members. Then he also expected useful drug from natural resources in Indonesia will be developed by continuous efforts from each members.

Update on research members
Some members of the SLeCAMA Project were updated, the detail as of January 2019 is shown in the ANNEX 1 "List of Researchers as January 29, 2019"

Ⅳ.Chair Person's Closing remarks

Dr. Agung Eru Wibowo commented this project is well organized between Japanese scientists and Indonesia scientists. This project resulted six (6) active compound against Matlaria and one (1) active compound against Ameba which were purified. He elaborated that project members should continue to work hard toward natural resources development in Indonesia.
Meeting closed at 12:30 PM.

Attendance List

Participants List　The4th JCC SLeCAMA

Sari-Pacific Hotel- Jakarta, 29th January 2019

	Name	Title, Institute
1	Dr. Soni Solistia Wirawan, M.Eng.	Project Director/Deputy Chairperson of Agroindustrial Technology and Biotechnology.BPPT
2	Dr. Agung Eru Wibowo, Apt.M.Si.	Project Manager/Head of Laboratory for Biotechnology,BPPT
3	Prof. Achmad Fuad	Representative, Airlangga University
4	Mr. Danang Waluyo, M.Eng.	Project Co-manager/Program Head, Laboratory for Biotechnology,BPPT
5	Ms. Nahoko Hirose	Representative, JICA Indonesia Office
6	Prof. Kaname Kanai	Executive Thechnical Advisor, JICA HQ

7	Prof. Tomoyoshi Nozaki	Chief Advisor/Professor, University of Tokyo
8	Prof. Kazuro Shiomi	JICA Expert/Professor, Kitasato University
9	Dr. Azuma Watanabe	JICA Expert/Advisor, MicroBiopharm Japan
10	Dr. Michio Yamashita	JICA Expert/Researcher, University of Tokyo
11	Dr. Kazuyuki Dobashi	JICA Expert/Researcher, Kitasato University
12	Dr. Mihoko Mori	JICA Expert/Researcher, Kitasato University
13	Dr. Takaya Sakura	JICA Expert/Researcher, Nagasaki University
14	Dr. Anis Herliyati Mahsunah	Division Head of Program and Biotechnology Application, Laboratory for Biotechnology, BPPT
15	Dr. Farida Rosana Mira	Division Head of Collaboratin and Technology Service, Laboratory for Biotechnology, BPPT
16	Ms. Irni Furnawanthi Hidanigrum, Msi.	Division Head of Administration, Laboratory for Biotechnology, BPPT
17	Bonny Agung Wahyuono, ST.	Division of Administration, Laboratory for Biotechnology, BPPT
18	Amin Pujianto, SP	Division of Administration, Laboratory for Biotechnology, BPPT
19	Wiwin Wihara, SE	Division of Administration, Laboratory for Biotechnology, BPPT
20	Mr. Nuki Bambang Nugroho, M.Si.	Researcher, Laboratory for Biotechnology, BPPT
21	Dr. Erwahyuni Endang Prabandari, M.Si.	Researcher, Laboratory for Biotechnology, BPPT
22	Dr. Chaidir, M.Si	Researcher, Center of Technology for Pharmaceutical and Medical, BPPT
23	Ms. Dyah Noor Hidayati,M.Si	Researcher, Laboratory for Biotechnology, BPPT
24	Ms. Suryani, S.Si	Researcher, Laboratory for Biotechnology, BPPT
25	Ms. Dian Japany Puspitasari, M.Biomed., Apt.	Researcher, Laboratory for Biotechnology, BPPT

26	Ms. Avi Nurul Oktaviani, M.Sc.	Researcher, Laboratory for Biotechnology, BPPT
27	Ms. Nurlaila, M.Si.	Researcher, Laboratory for Biotechnology, BPPT
28	Ms. Eka Siska, S.Si.	Researcher, Laboratory for Biotechnology, BPPT
29	Ms. Evita Chrisnayanti, M.Biotech., Apt.	Researcher, Laboratory for Biotechnology, BPPT
30	Ms. Titin Ariyani, S.Si	Researcher, Laboratory for Biotechnology, BPPT
31	Mr. Bayu Maulana, S.Kom	Division of Administration, Laboratory for Biotechnology, BPPT
32	Dr. Myrna Adianti	Researcher, Airlangga University
33	Ms. Lidya Tumewu, M.Farm.,Apt	Researcher, ITD, Airlangga University
34	Ms. Kristiningrum, S.Si	Research Assistant, Laboratory for Biotechnology, BPPT
35	Ms. Nadia Adipratiwi, S.Si	Research Assistant, Laboratory for Biotechnology, BPPT
36	Ms. Kiki Rizkia Afrianti, S.Si	Research Assistant, Laboratory for Biotechnology, BPPT
37	Mr. Denih	Research Assistant, Laboratory for Biotechnology, BPPT
38	Mr. Dedeng Taryana	Research Assistant, Laboratory for Biotechnology, BPPT
39	Mr. Wawan Hadiwijaya	Research Assistant, Laboratory for Biotechnology, BPPT
40	Ms. Putri Bernawati, SSi	Research Assistant, JICA
41	Ms. Defi Kartika Sari, Ssi	Research Assistant, JICA
42	Ms. Melinda, Ssi	Research Assistant, JICA
43	Ms. Sumiati Widodo	Project Secretary, JICA
44	Ms. Madoka Kurata	Project Coordinator, JICA

ANNEX

1. List of Researchers as of as of January 29, 2019
2. Progress 2018 and Planning 2019 (BPPT)
3. Report activities of ITD-AU, January 29, 2019
4. Identified Problems/Needs and Solutions (Chief Advisor)

List of Researchers (version #4 as of 2019-01-29)

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) • Titin Ariyani (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)	• 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) • Kristiningrum(BPPT)	• Azuma Watanabe (MBJ)

	• Kiki Rizkia Afrianti (BPPT) • Suryani (BPPT) • Avi Nurul Oktaviani	
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)	• 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) • Dian Japany Puspitasari (BPPT) • Danang Waluyo (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)	• 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 Rizkia Afrianti (BPPT) • Suryani (BPPT) • Avi Nurul Oktaviani (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)	• 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)	• Tomoyoshi Nozaki (U.Tokyo) • Ghulam Jeelani (NIID) • Kumiko Tsukui(NIID)

	• Dwi Peni Kartikasari(AU) • Hikatul Ilmi(AU) • Lidya Tumewu(AU) • Aty Widyawaruyanti (AU) • Lidya Tumewu(AU) • Hikatul Ilmi(AU)→Delete	• 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) • Avi Nurul Oktaviani (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) • Katsuhiko Ando (U.Tokyo)
3.2. Establishment of screening systems	• Erwahyuni E. Prabandari (BPPT) • Achmad Fuad Hafid (AU) • Myrna Adianti (AU) • Ratna Wahyuni (AU) • Dwi Peni Kartikasari(AU) • Titin Ariyani (BPPT) • Danang Waluyo (BPPT)	• 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) • 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)	• 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)	• Tomoyoshi Nozaki (U.Tokyo) • Daniel Ken Ianoka (Nagasaki Univ) • Kazuro Shiomi (KU) • Azuma Watanabe (MBJ)

	• Atit Kanti, (LIPI)	

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

BPPT

JICA
Japan International Cooperation Agency

LIPI
Indonesian Institue of Science

Airlangga University

東京大学 THE UNIVERSITY OF TOKYO

北里大学 KITASATO UNIVERSITY

長崎大学 NAGASAKI UNIVERSITY

MicroBiopharm Japan

AMED
Agency for Medical Research and Development

The 4th 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 2018 and Planning 2019

Danang Waluyo
Project Co-manager

Sari Pacific Hotel, Jakarta
January 29th, 2019

Content

1. **Target Review and Research Flowchart**

2. **Progress 2018**
   a. Microbes Isolation and Extract Preparation
   b. Screening of Active Extract
   c. Purification of Active Compound
   d. Other Activities
   e. Budget Arrangement

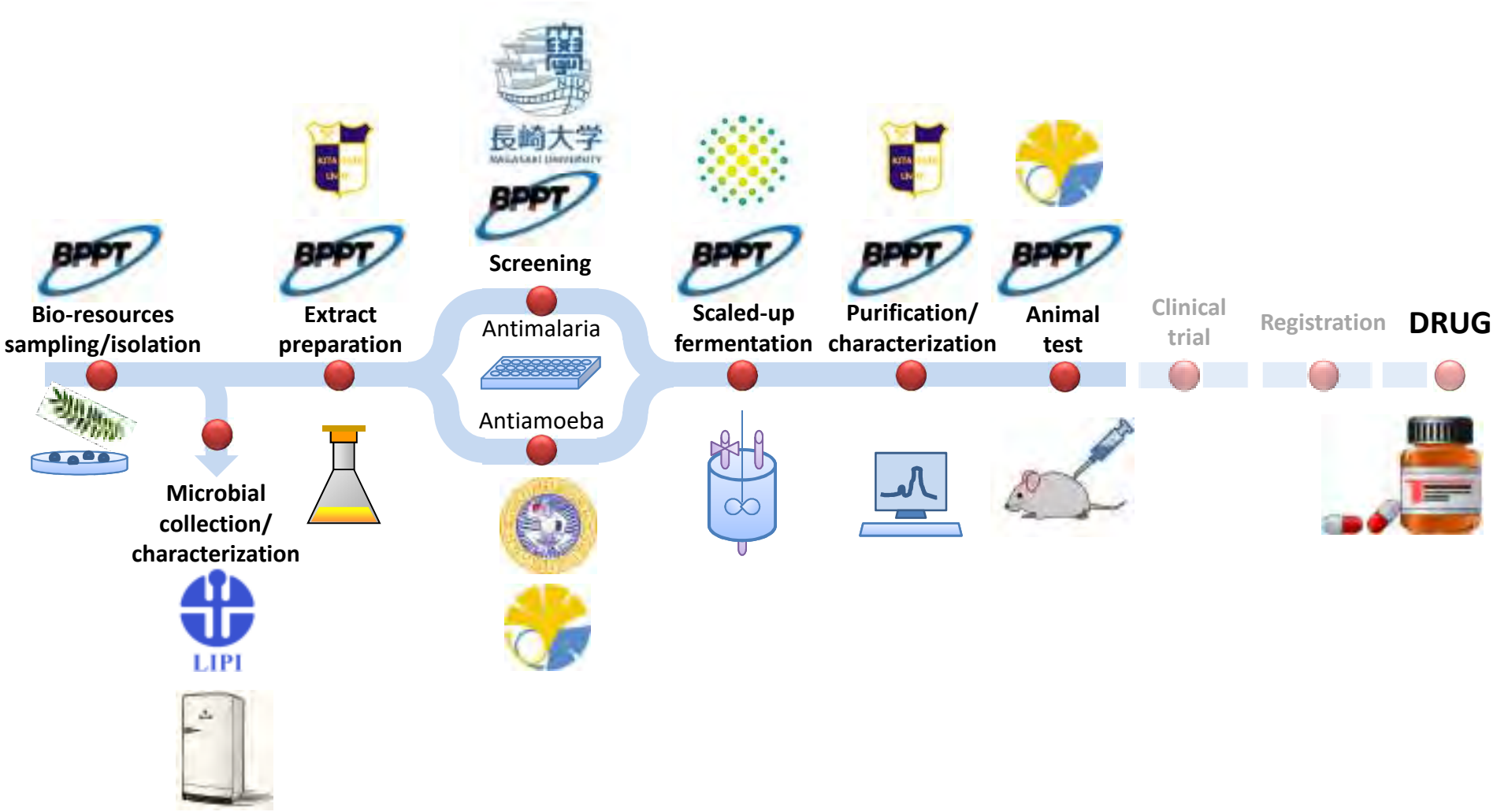
3. **Planning 2019**
   a. Research Activities
   b. Training
   c. Budget Arrangement
   d. Project Management

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 2017
Blue: partially achieved 2017

Research Flowchart



SATREPS Project 5 yrs
(FY 2015-2019)

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

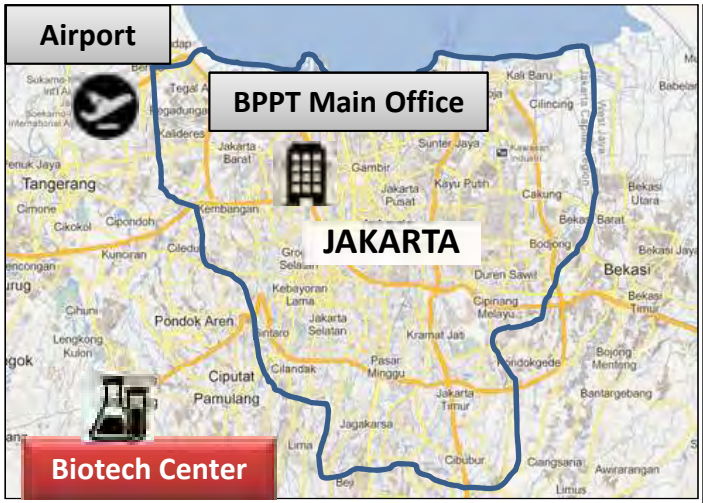
Purification of Active Compound

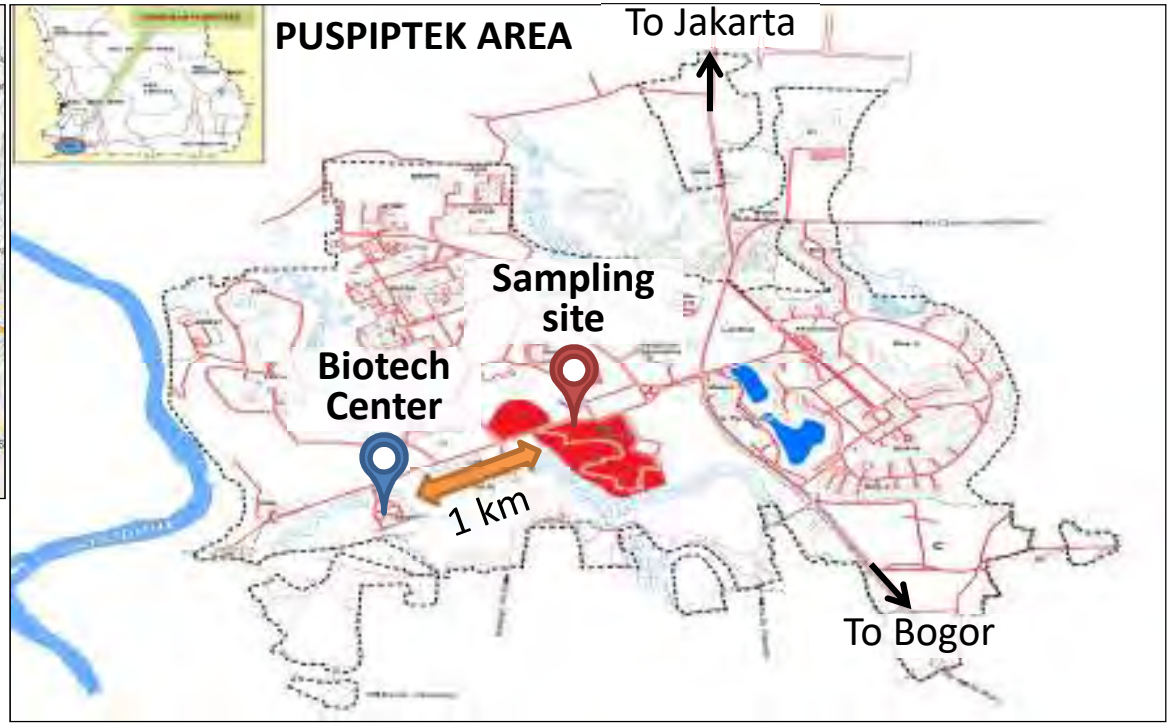
Other Activities

Budget Arrangement

Progress 2018

Field Exploration





Sampling point

Location : Puspiptek (Botanical Garden)
Coordinate : 6°20'36.7"S 106°40'39.4"E
Date : May 8-9, 2018
Temp./RH : 28-31°C, 80%

Sample obtained

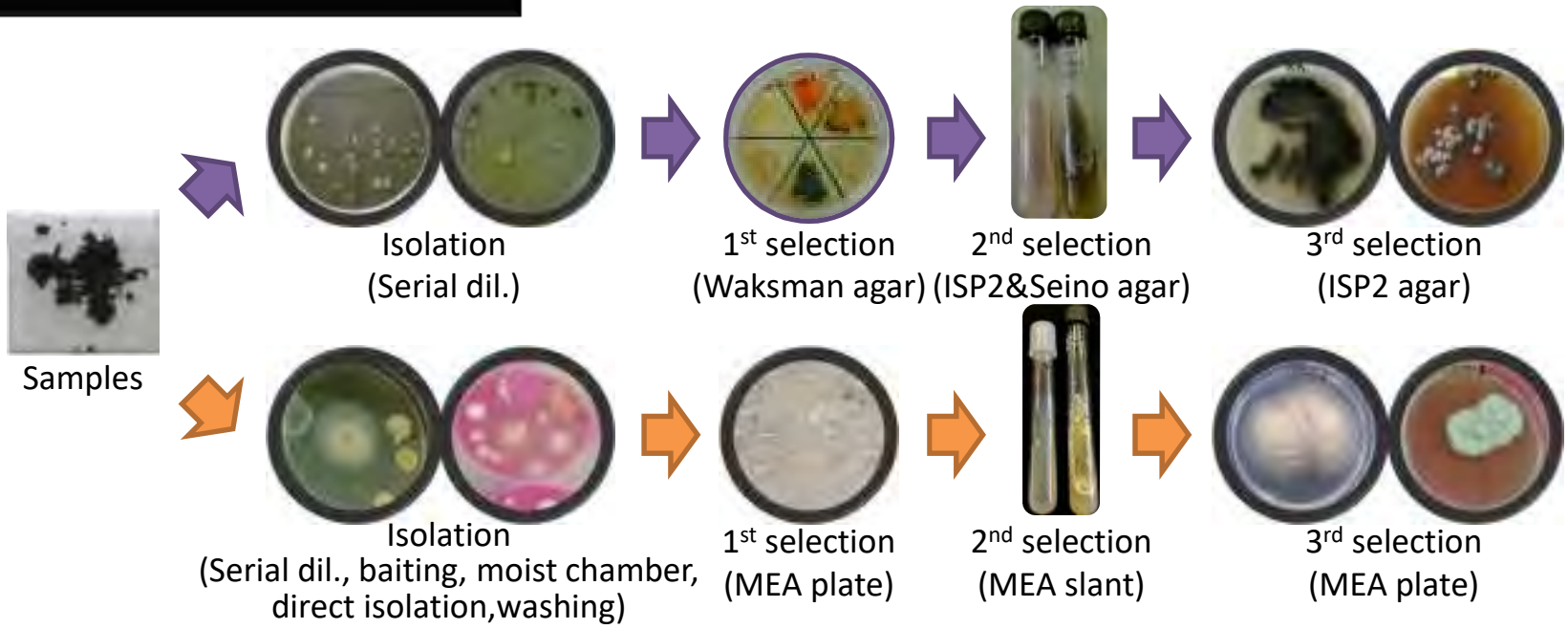
Type : Soil, litter, mushroom, insect dead body, etc.
Location : Terrestrial, wet surface, reservoir
Total number : 122 samples

Progress 2018

Microbial Isolation

Objective: To isolate microbial strain from source samples

General microbial isolation method



Result

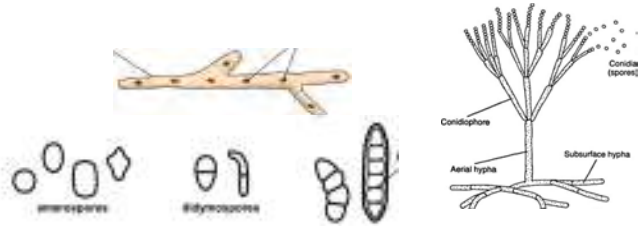
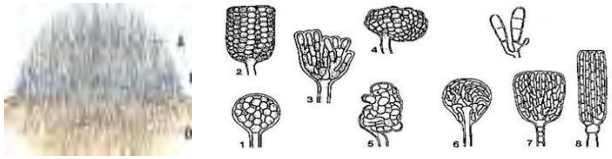
Target	Location	Number of isolated sources	Number of isolates*
Fungi	Puspiptek	83	632
	Togen (2017)	8	136
Actinomycetes	Puspiptek	37	444
	Togen (2017)	8	76
		TOTAL	**1288**

* Currently isolation is still continued

Progress 2018

Microbial Identification

Objective: To identify microbial strain (newly isolated, revived from frozen stock, interesting isolates, hit producing isolates)

	Fungi	Actinomycetes
Morphology-based	Shape of hyphae, conidial form, structure of conidiophore 	Chain of sporophore, aerial and agar hyphae, substrate mycelia, spore production within sporangia 
Molecular-based	16S rDNA	28S rDNA

Result

Target	Method	Number of Identified isolates*
Fungi	Morphology-based	1244
	Molecular-based	50
Actinomycetes	Morphology-based	793
	Molecular-based	2

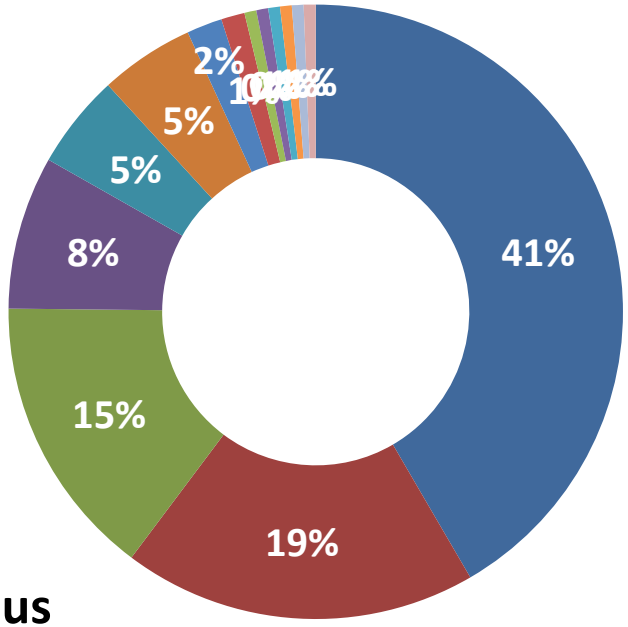
* Currently identification is still continued

Progress 2018

Microbial Identification

Result 2017

Sampling location: Togean

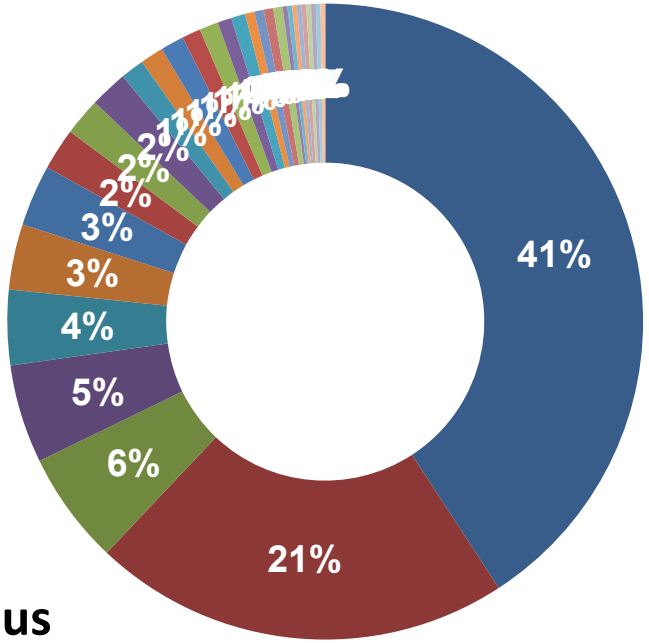


- No conidia
- *Paecilomyces*
- *Penicillium*
- *Aspergillus*
- *Fusarium*
- *Talaromyces*
- *Mortierella*
- *Verticillium*
- *Beauvaria*
- *Cephalosporium*
- *Metarhizium*

220 isolates, 13 genus

Result 2018

Sampling location: Puspiptek



- No conidia
- *Aspergillus*
- *Auerobasidium*
- *Cladosporium*
- *Paecilomyces*
- *Beauvaria*
- *Verticillium*
- *Nigrospora*
- *Dactilaria*
- *Gliocladium*
- *Pestalotiopsis*
- *Gilmaniella*
- *Idriella*
- *Penicillium*
- *Trichoderma*
- *Acremonium*
- *Fusarium*
- *Sphorotrix*
- *Rhizopus*
- *Phoma*
- *Curvularia*
- *Humicola*
- *Oidiodendron*
- *Geotrichum*
- *Gonytrichum*
- *Mammaria*

459 isolates, 29 genus

Progress 2018

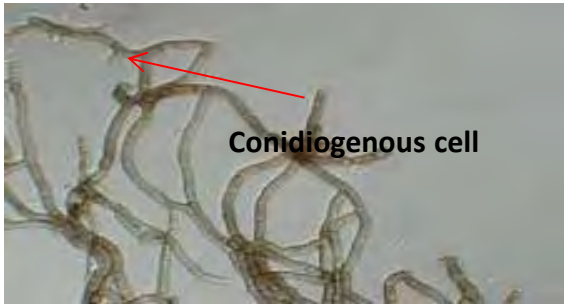
Microbial Identification

Identification of interesting microbial isolates | Fungi

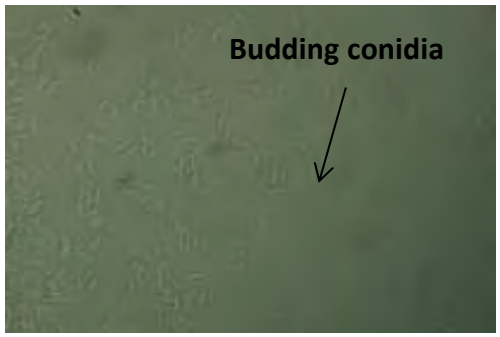
Isolate name	: BioMCC-f.PL.142	Sampling point	: Kupang
Isolation source	: Plant Litter (leaves)	Bioactivity	: MQO inhibitor
Isolation method	: Moist chamber method	Extract code	: F15.1645
Isolation time	: May 2, 2005	DNA analysis result	: 96% similarity to *Aureobasidium*

Micromorphology of BioMCC.f.PL.142 (Fungi)
MEA and PDA medium slide culture, incubate 25^0 C for 7 days

Chlamydospores Brown, 1-celled, cylindrical (young chlamydospores) and ellipsoidal and globose (old) shape,
size (3-)5-9(11)x(3-)5-7(-10) µm



- Conidiogenous cells blastic type, intercalary on hyphae.
size 1 – 2 (3)x 2-3,5 µm
- Hyphae thick, brown color, non septa , size (2,9) 3,9-5,9 µm

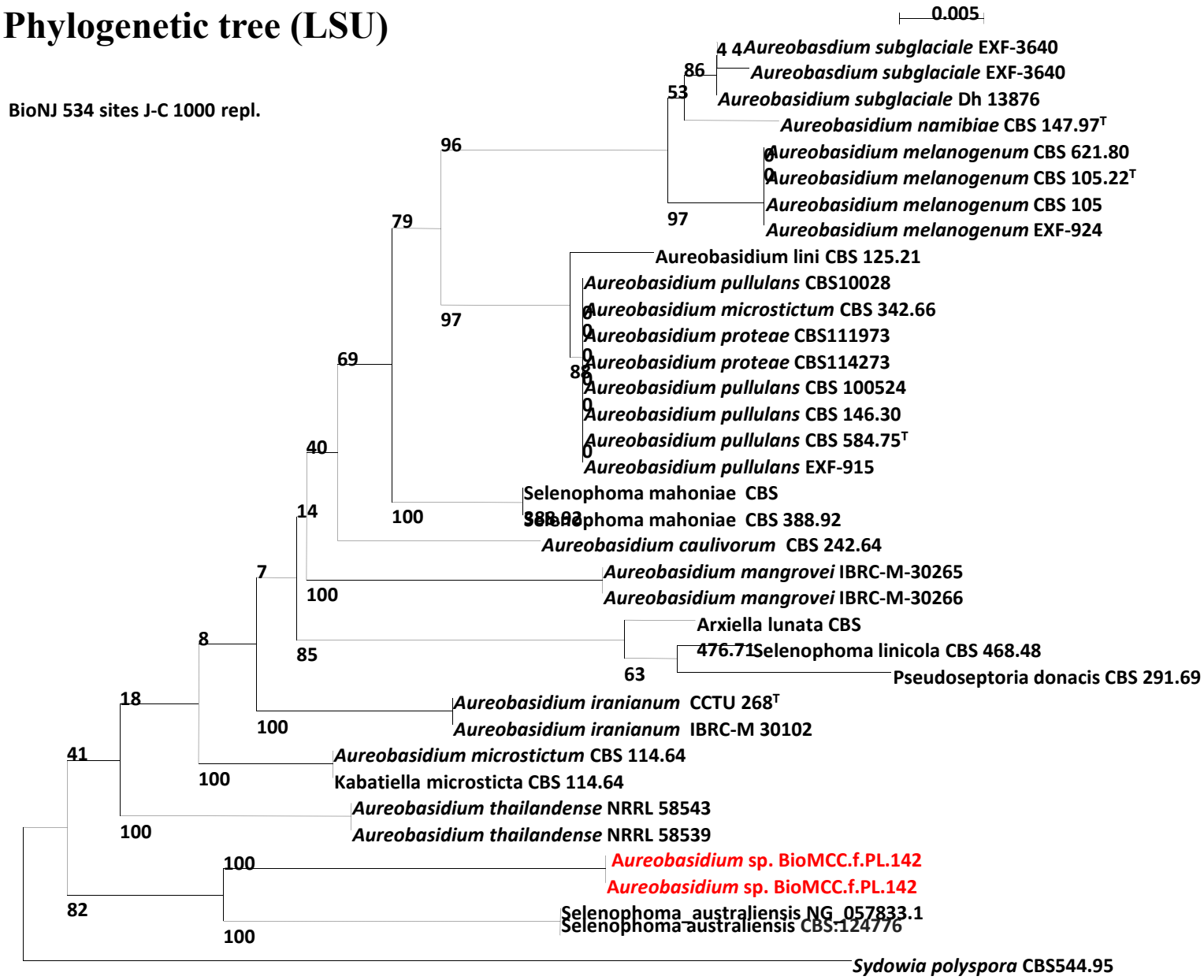


Conidia blastic, smooth, hyaline, 1-celled, ben or curved, typically lunate shaped or less often boomerang, very variable in size.
Size conidia (5-)7-9(-13)x 2-3 µm.
Budding conidia seen

Progress 2018

Microbial Identification

Phylogenetic tree (LSU)



Most probably new strain in genus *Aureobasidium*

Progress 2018

Microbial Identification

Identification of interesting microbial isolates | Actinomycetes

Isolate name	: BioMCC-a.T.2931	Sampling point	: Flores
Isolation source	: Soil	Bioactivity	: -
Isolation method	: Wet soil	Extract code	: -
Isolation date	: Sep 5, 2006	DNA analysis result	: 97% similarity to *Actinoplanes brasiliensis*

Chemotaxonomy of Strain BioMCC-a.T.2931

1. Major menaquinone is MK-9 (H_4) (79%) followed by MK-9 (H_6) (21%), analyzed by LC-MS
2. Cell wall DAP is *meso*-diamonipimelic acid (*meso*-DAP)
3. Whole cell sugars in the strain are glucose, xylose, and arabinose
4. Acyl type of the strain is glycolyl type
5. The strain contains phosphatidyl ethanolamine (PE), and phosphatidyl inositol (PI)
6. The strain doesn't contain mycolic acid

DNA-DNA HYBRIDIZATION

plate

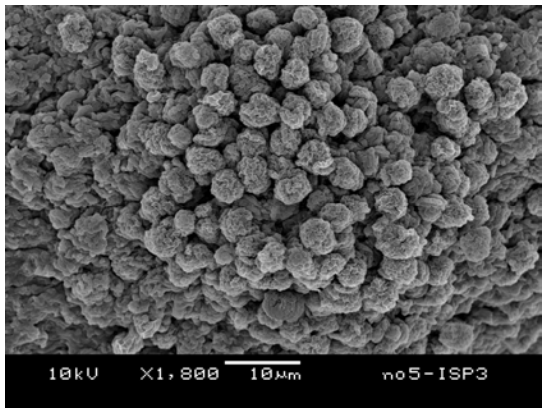
probe	3rd	5.5	nbrc 13938	nbrc 13994	nbrc 110975	nbrc 110796
	5.5	100	14.1	10.8	9.9	13.5
	nbrc 13938	216.7	100			
	nbrc 13994	22.8		100		
	nbrc 110975	10.7			100	
	nbrc 110796	7.5				100

2nd	5.5	nbrc 13938	nbrc 13994	nbrc 110975	nbrc 110796
5.5	100	40	38	67	36
nbrc 13938	30	100			
nbrc 13994	37		100		
nbrc 110975	65			100	
nbrc 110796	73				100

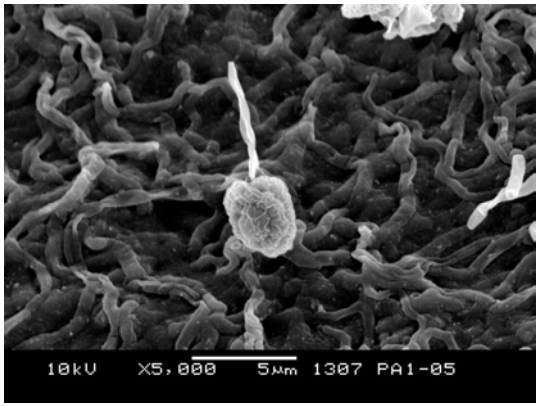
1st	5.5	nbrc 13938	nbrc 13994	nbrc 110975	nbrc 110796
5.5	100	16	27.4	14.9	15.6
nbrc 13938	6	100			
nbrc 13994	28.8		100		
nbrc 110975	8			100	
nbrc 110796	5				100

Most probably new species in genus ***Actinoplanes***

Scanning Electron Microscope



Immature sporangium (2 weeks, on ISP 3)

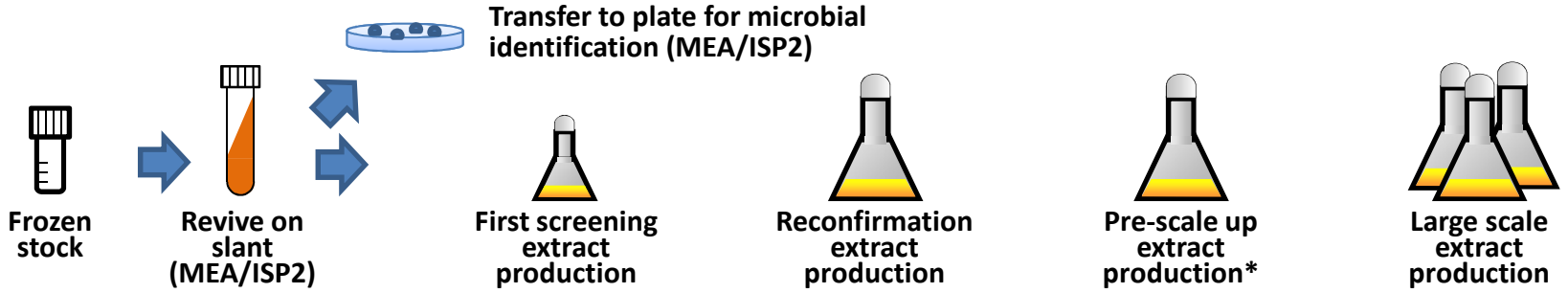


Mature sporangium (3 weeks, on ISP 7)

Progress 2018

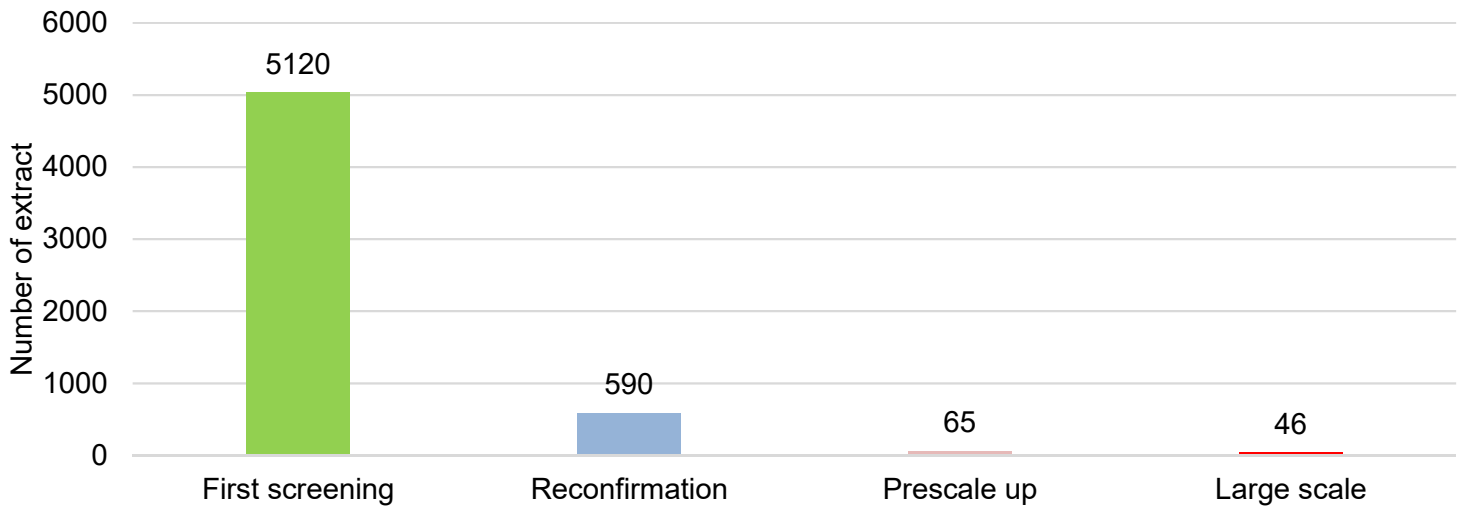
Extract Production

Objective: To produce extracts of natural resources for screening



Culture scale	30 mL	100 mL	100 mL	1000~5000 mL (100 mL x 10 / 50 flasks)
Extract volume	1 mL	600 µL	600 µL	1000~5000 mL
Number of medium	6 medium (fungi), 4 medium (actinomycetes)	2 medium	1 medium	1 medium
Temperature	28˚C			
Sampling point	-	Day 3,4,5	Day 3,4,5,6,7	Day 3 ~ harverst

Result



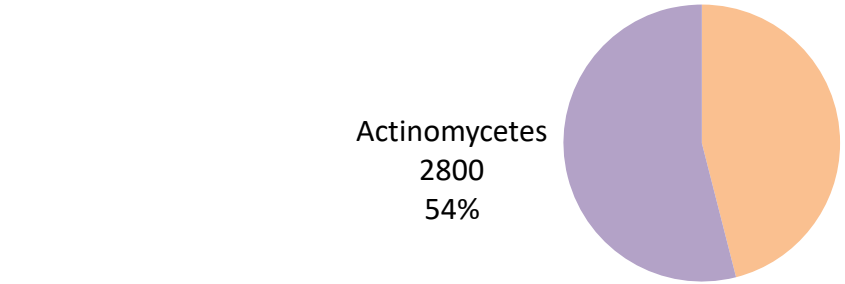
* Pre-scale up extract production was applied from July 2018

Progress 2018

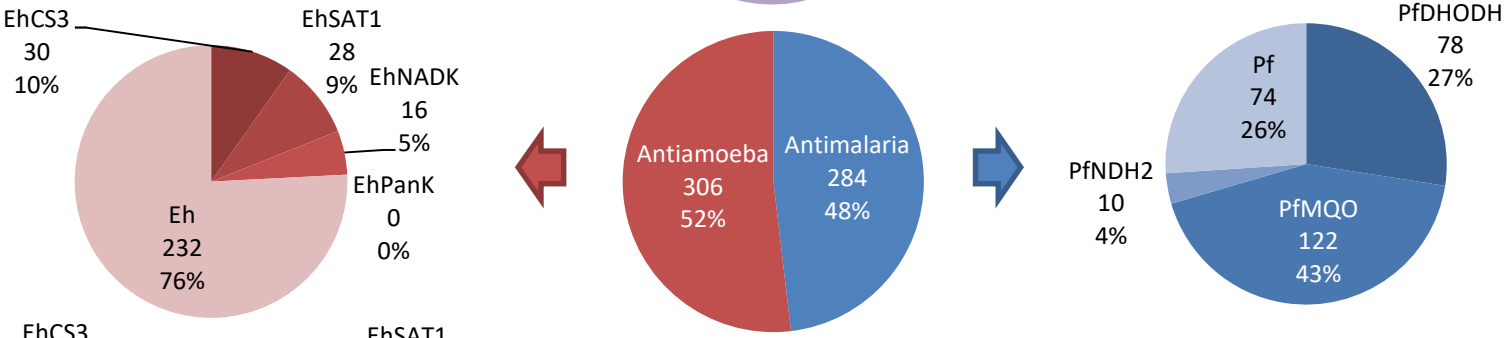
Extract Production

Result

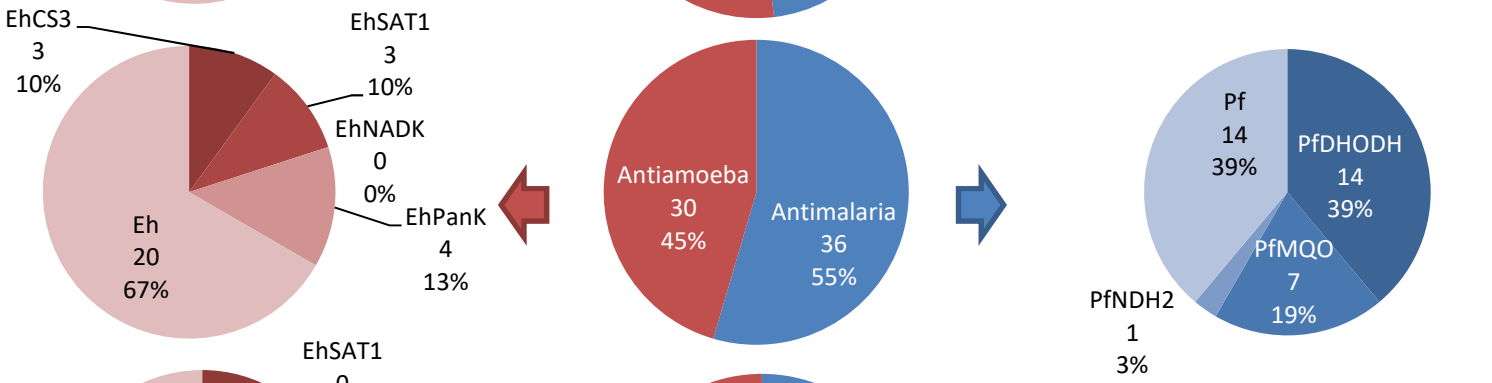
First screening extract production



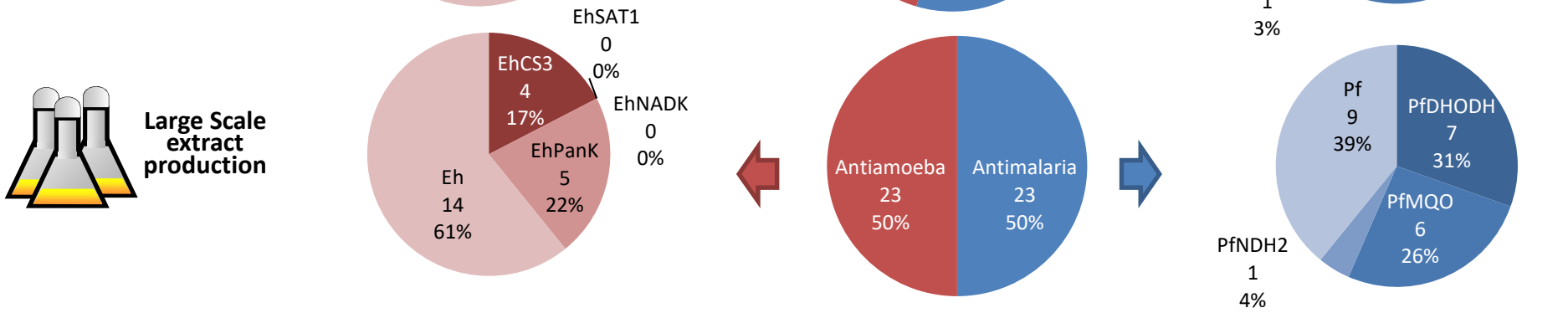
Reconfirmation extract production



Pre-scale up extract production



Large Scale extract production



Progress 2018

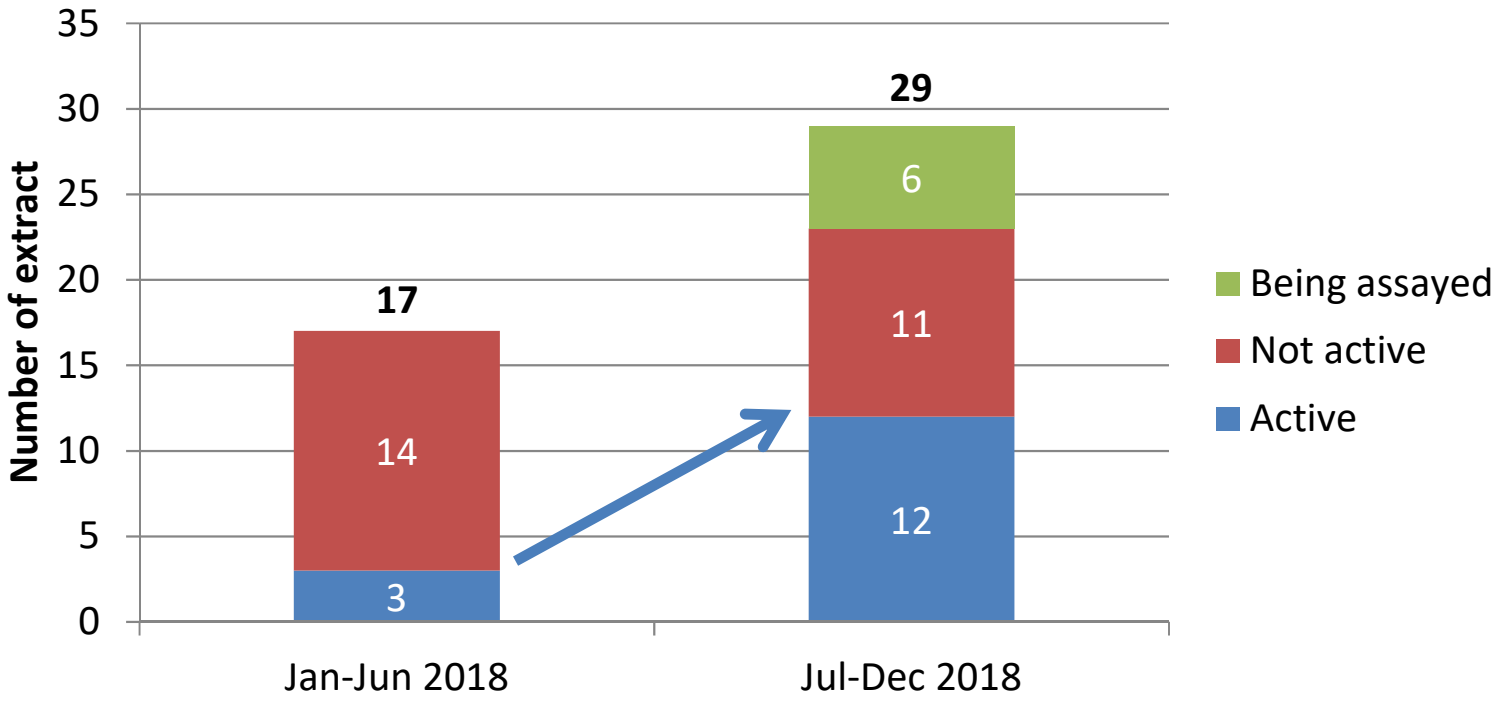
Extract Production

Pre-scale up (PSU) extract production

Objective: to improve reproducibility of active extract production

→Shorten time lag between small scale and large scale culture

→PSU extract production was introduced since July 2018



Reproducibility **17%** **41%**

Reproducibility of active extract for purification was **increased significantly** after PSU extract production was introduced

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

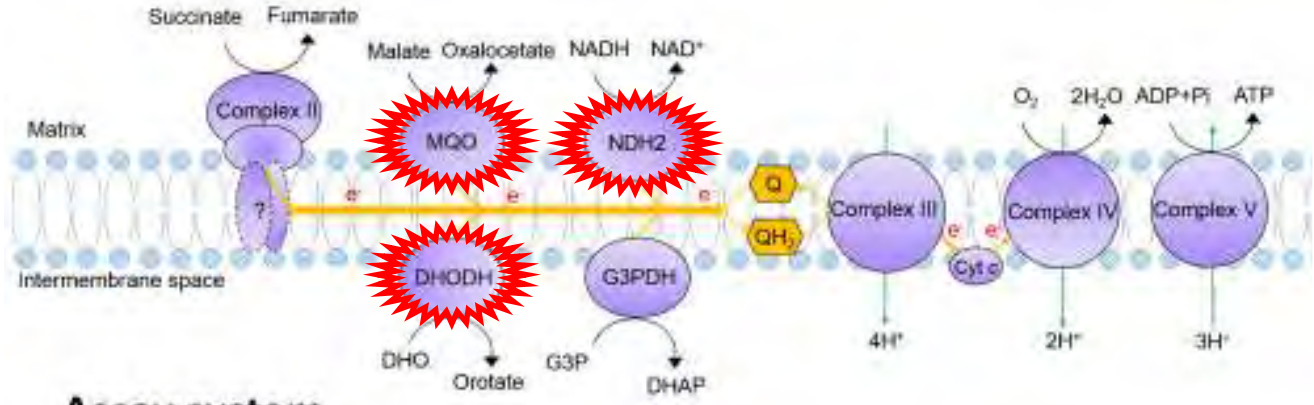
Budget Arrangement

Progress 2018

Enzyme-based screening | **Anti-malarial screening**

Objective: To obtain stable microbial extracts show selective antimalarial activity against target enzyme

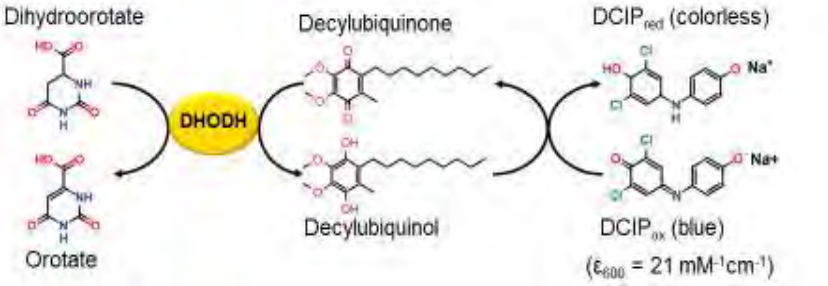
Electron transport chain in *P. falciparum*



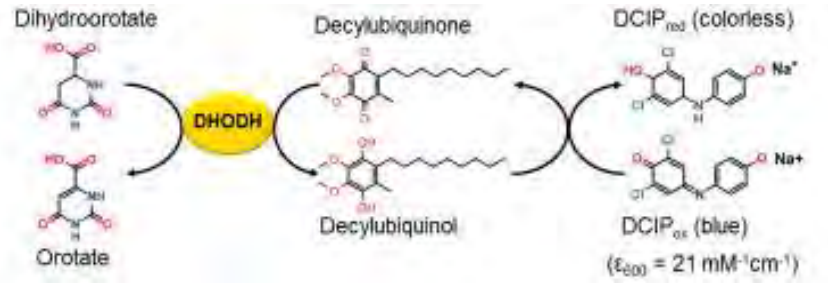
MQO: Malate:Quinone Oxidoreductase; DHODH: Dihydroorotate (DHO) dehydrogenase; G3PDH: Glycerol-3-Phosphate (G3P) Dehydrogenase; DHAP: DiHydroxyAcetone Phosphate; NDH2: Type II NADH Dehydrogenase; Q: Oxidized Quinone; QH_2: Reduced Quinone; Cyt c: Cytochrome c; SQOR: Sulfide:quinone oxidoreductase; EFTDH: Electron-transfer Flavoprotein Dehydrogenase; MDH: Malate dehydrogenase (NAD^+).

Assay system

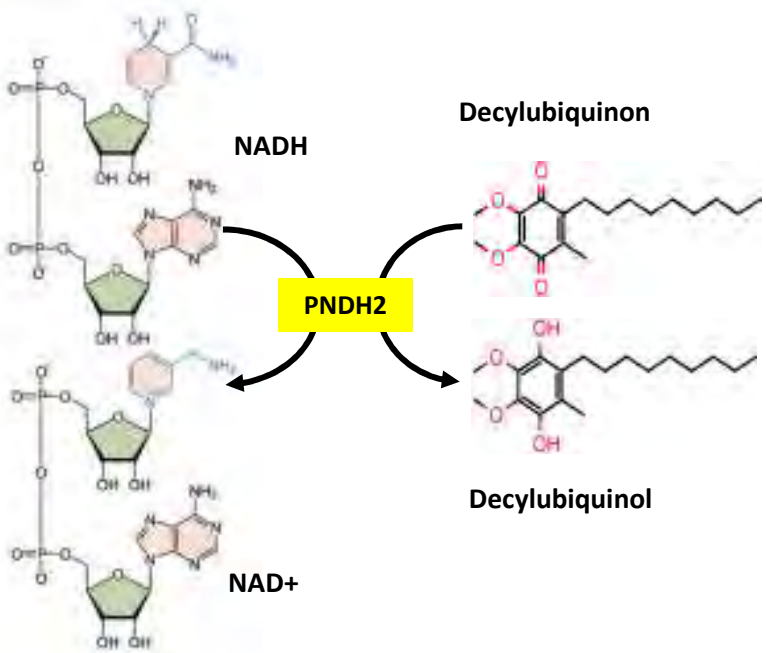
Target enzyme: *Pf*DHODH



Target enzyme: *Pf*MQO



Target enzyme: *Pf*NDH2

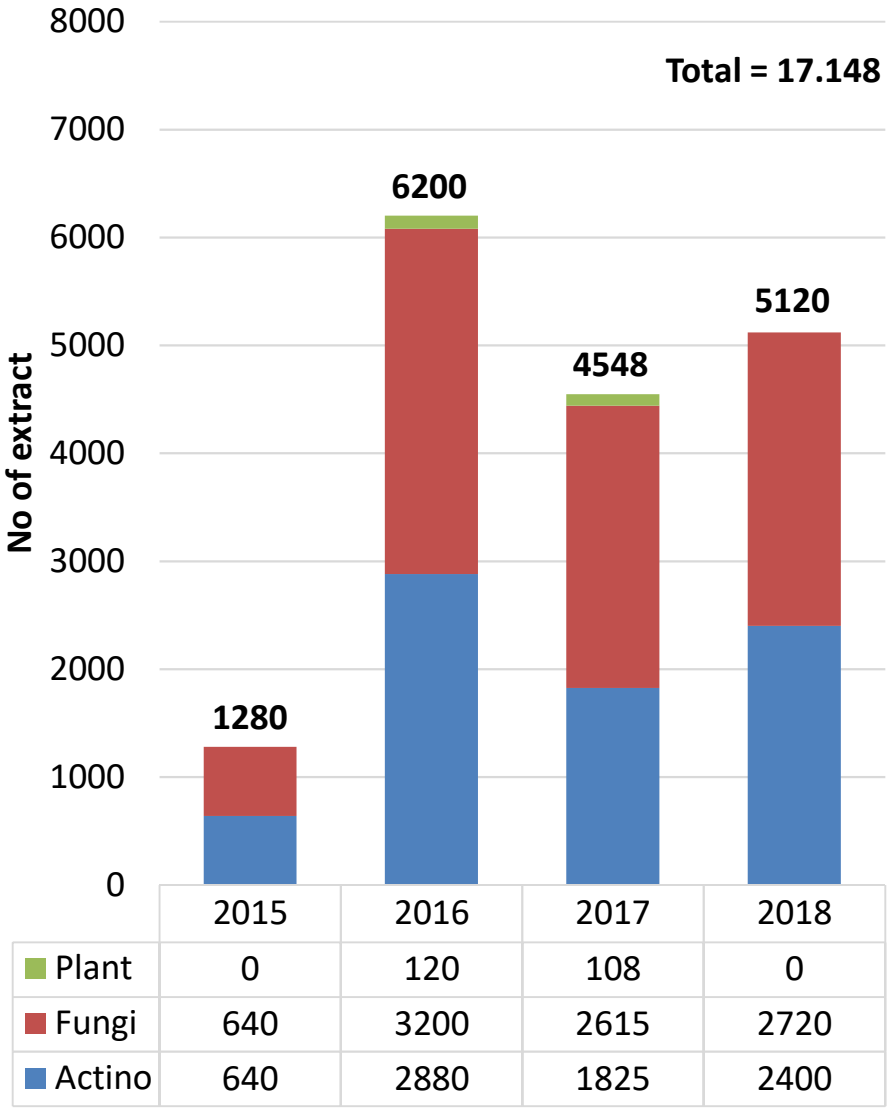


Progress 2018

Enzyme-based screening **Anti-malarial screening**

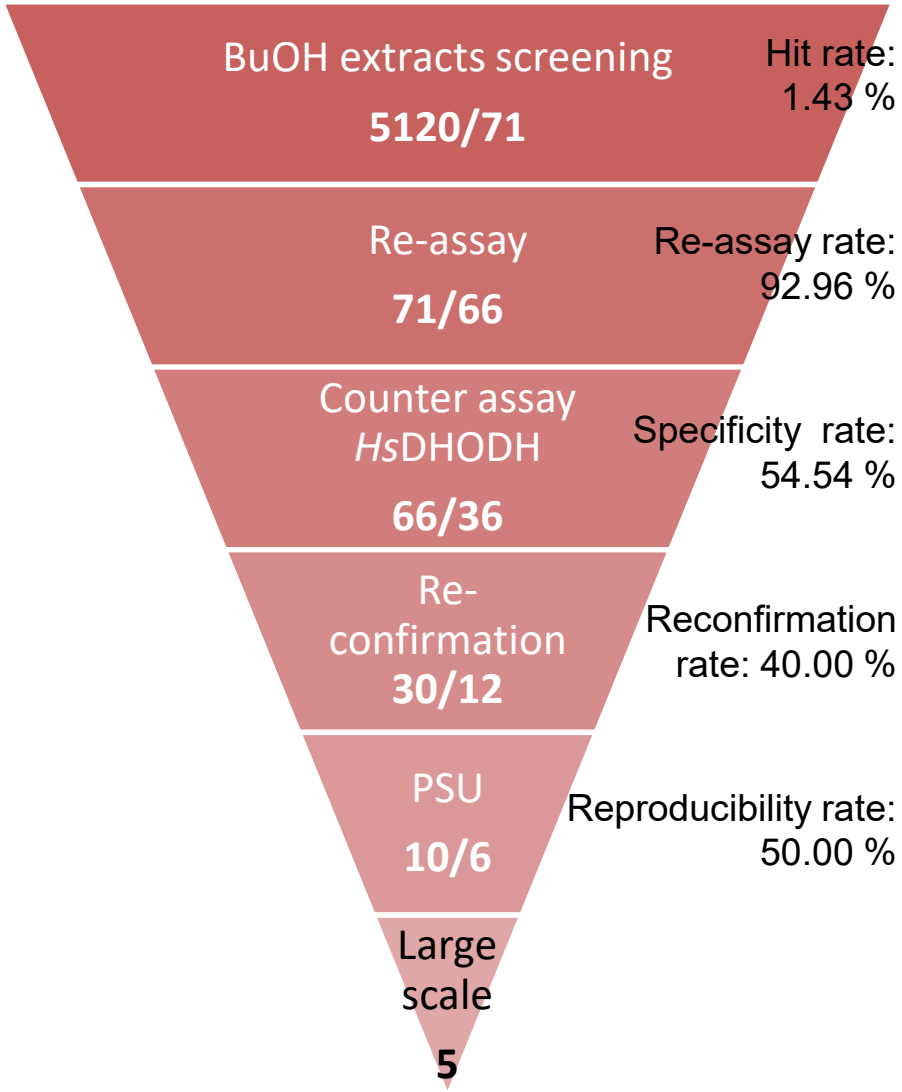
Result

*Pf*DHODH screening



	2015	2016	2017	2018
Plant	0	120	108	0
Fungi	640	3200	2615	2720
Actino	640	2880	1825	2400

Achievement in 2018

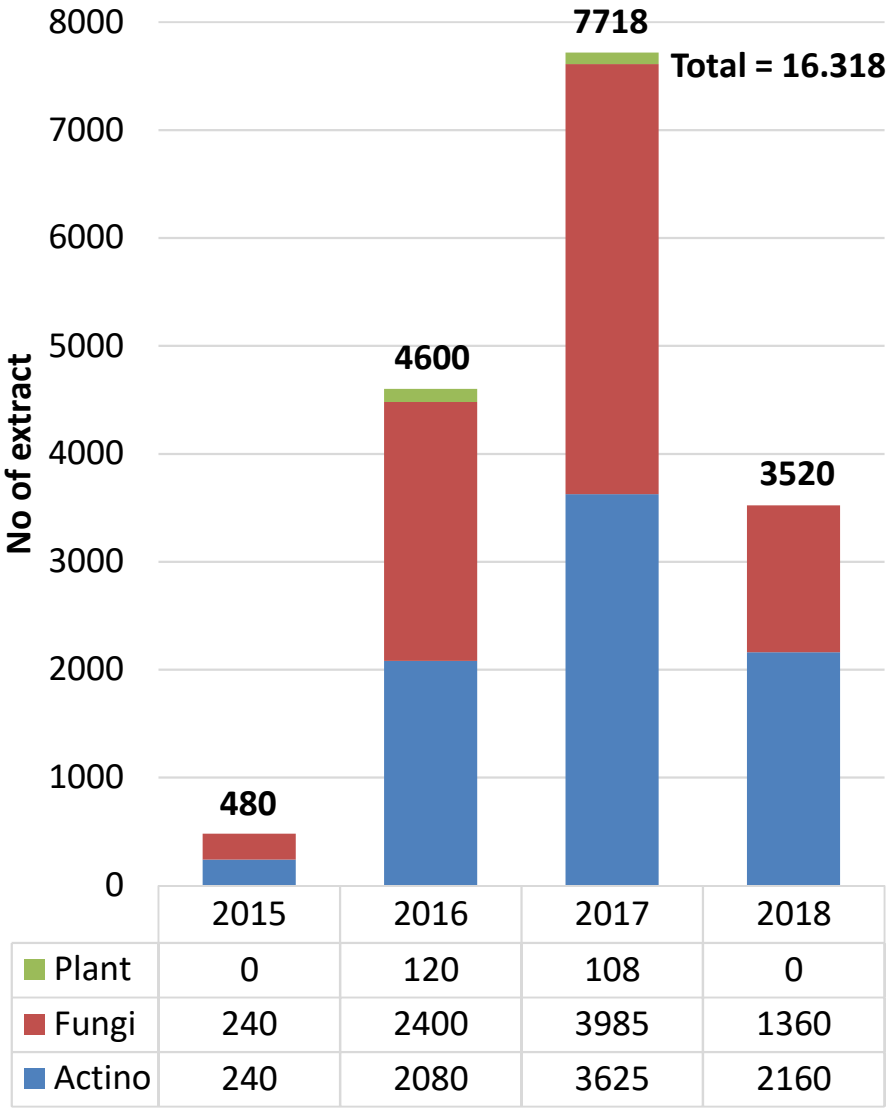


Progress 2018

Enzyme-based screening **Anti-malarial screening**

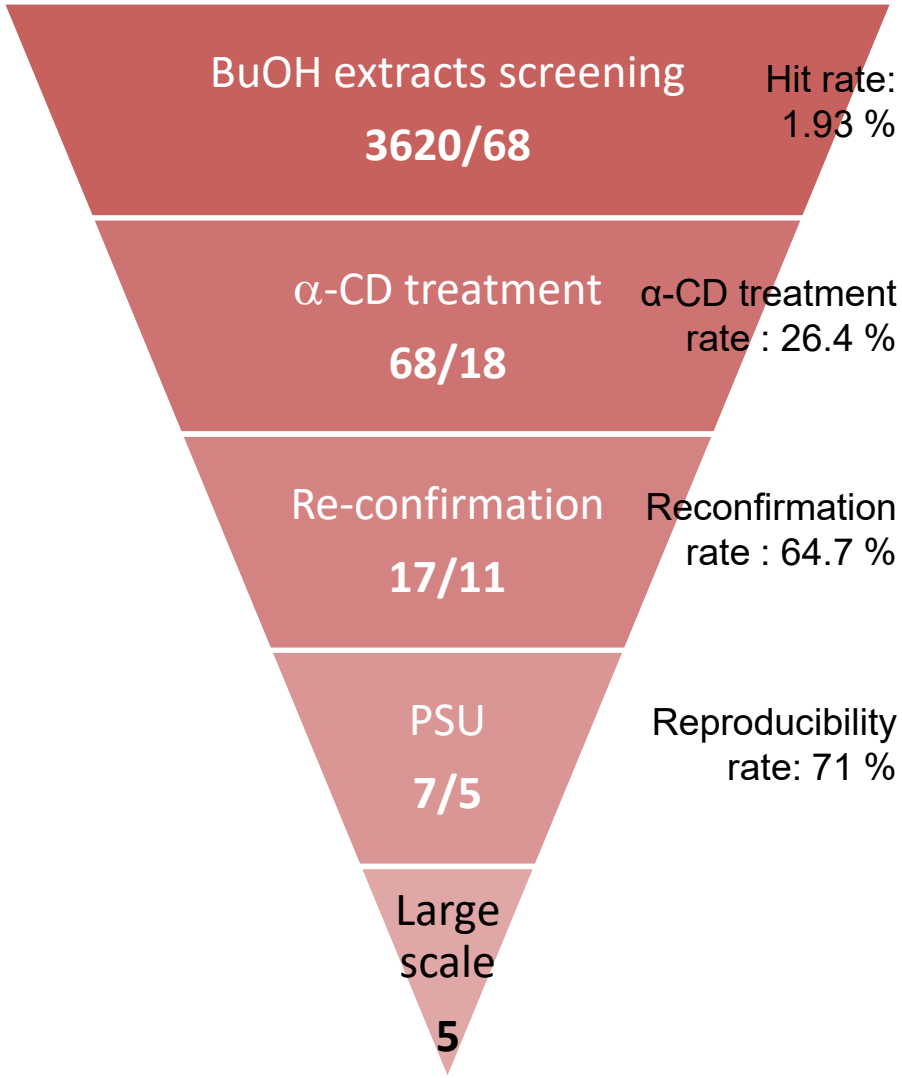
Result

*Pf*MQO screening



	2015	2016	2017	2018
Plant	0	120	108	0
Fungi	240	2400	3985	1360
Actino	240	2080	3625	2160

Achievement in 2018

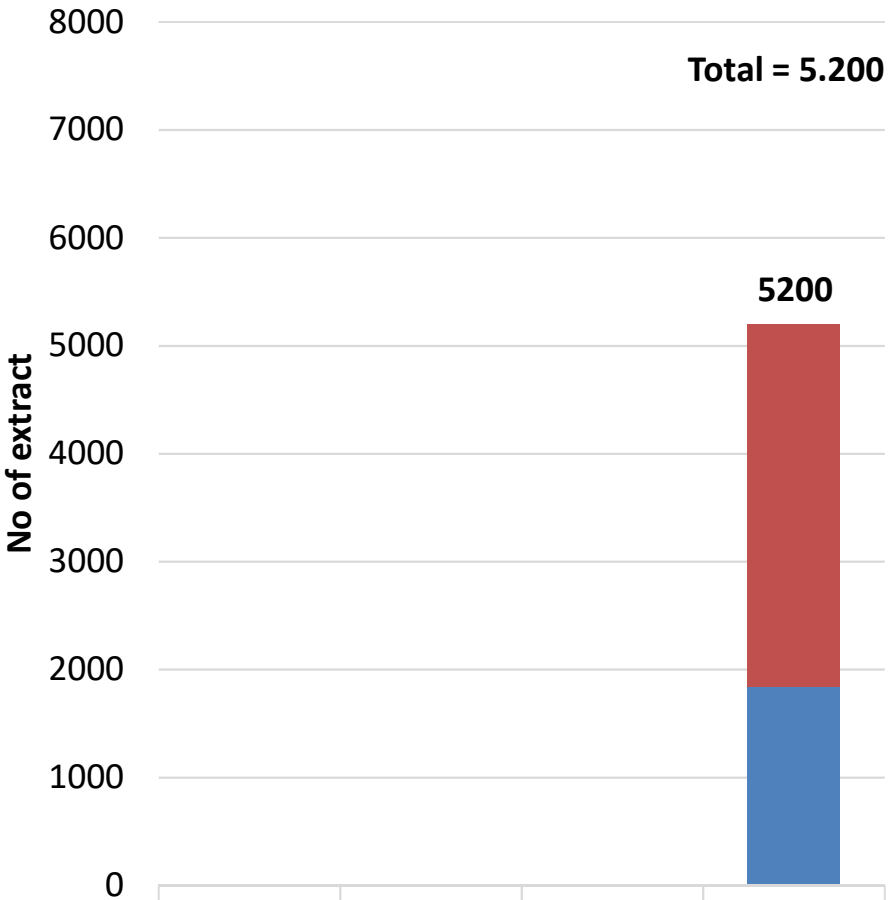


Progress 2018

Enzyme-based screening | **Anti-malarial screening**

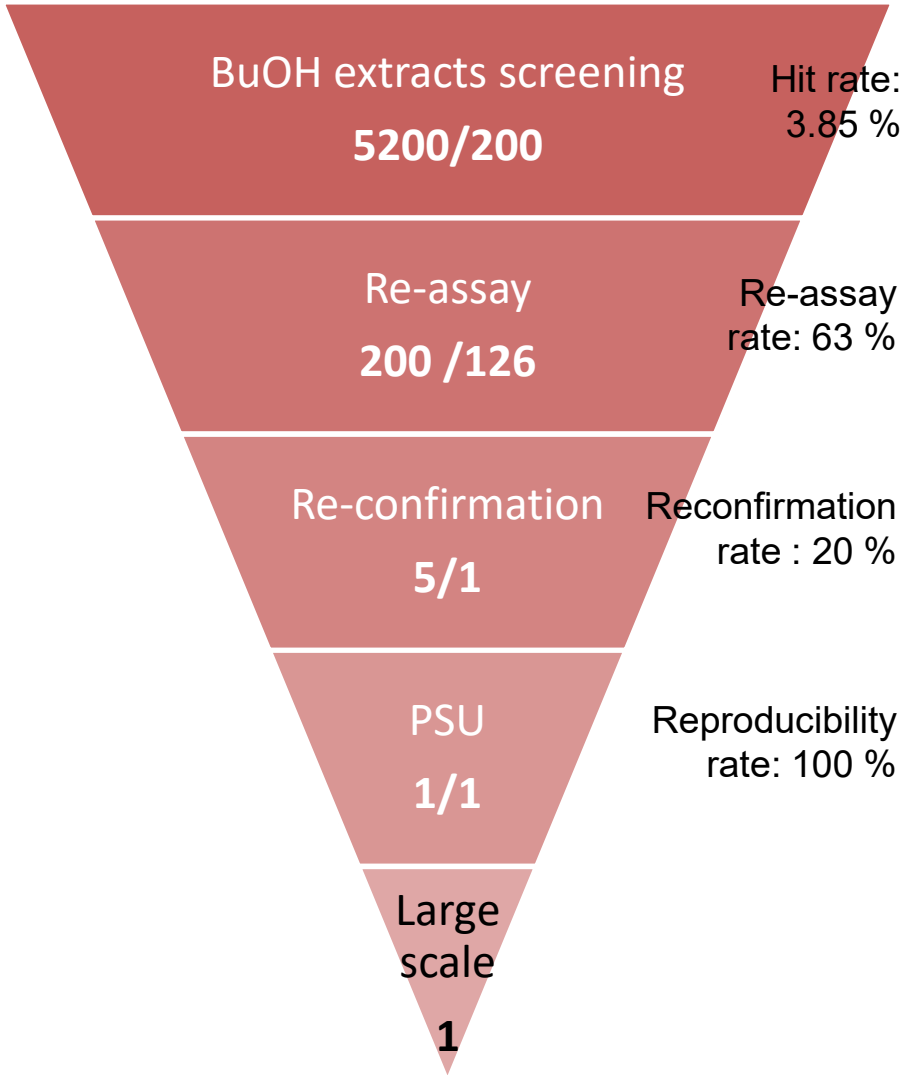
Result

*Pf*NDH2 screening



	2015	2016	2017	2018
Plant	0	0	0	0
Fungi	0	0	0	3360
Actino	0	0	0	1840

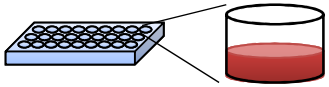
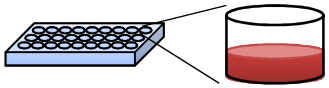
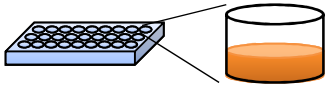
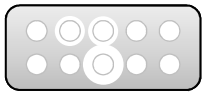
Achievement in 2018



Progress 2018

Cell-based screening **Anti-malarial screening**

Objective: To search extract with inhibitory activity to proliferation of malaria parasite cell

	Objective	Method	Assay Method
First screening	Searching for active microbial extracts	*Pf* 3D7 cell + Microbial extract 	LDH assay
Re-assay	Confirming activity of active extracts	*Pf* 3D7 cell + Active microbial extract 	LDH assay
Toxicity assay	Searching non-toxic active extracts	*Hs* DLD1 cell + Active microbial extract 	WST-8 assay
Dereplication	Searching active extract with non-frequent hit	Gram (+) bacteria + Active non-toxic microbial extract 	Halo-forming assay

Hit

Progress 2018

Cell-based screening **Anti-malarial screening**

Result

	Condition	Number of extract	Number of active extract	Screening rate
First screening	Initial parasitemia=0.3% (ring-form tropozoit) Hematochrit=3% Media=RBC (O+)+Albumax+RPMI Control=Atovaquone, DMSO (max 1%) Extract amount=2.500x dil. (final) Threshold=100%	10.160	713	7%
Re-assay	(Same as first screening)	713	463	65%
Toxicity assay	Initial cell number=$2.5x10^4$ (exponential phase) Media=DMEM+FBS Control=No cell, DMSO (max 1%) Extract amount=25x dil. (final) Selectivity=100x Threshold=50%	463	188	25%
Dereplication	Target=*Bacillus subtilis* ATCC 6633 Media=Nutrient agar Control=Chloramphenicol, DMSO Thershold=no halo (visual observation)	188	83	44%
Hit				
Reconfirmation		35	28	80%
Pre-scale up		10	9	90%
Scale up		8	6	75%

Hit rate = 0.82%

Progress 2018

Cell-based screening

Anti-amebic screening

Objective: To search extract with inhibitory activity to proliferation of amebic parasite cell

Anti-amebic screening result will be reported by AU

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

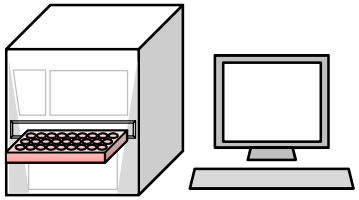
Progress 2018

Purification of active compound

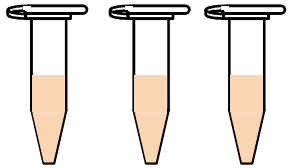
Objective: To obtain purified compound with antimalarial/antiamebic activity

General purification workflow

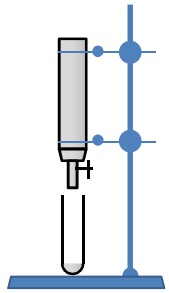
Large scale extract



Activity check
(butanol extract)



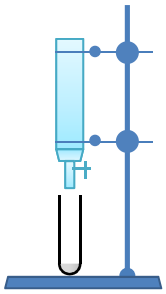
Pre-extraction Test
(PET)
to evaluate properties (stability, polarity) of active extract



Main purification
(activity guided)

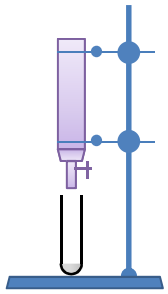
Typical main purification workflow

Extraction with
suitable solvent
(based on PET result)



Partition with
silica column

Partition with
preparative TLC
(silica) or
preparative
HPLC (ODS)



Partition with
size exclusion
(LH-20) column

Purified
compound

Progress 2018

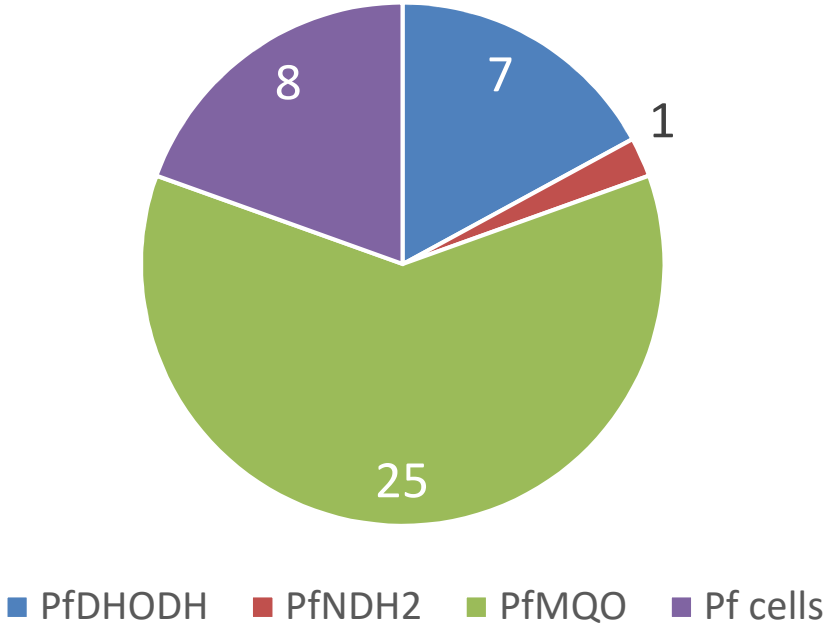
Purification of active compound

Anti-malarial active compound

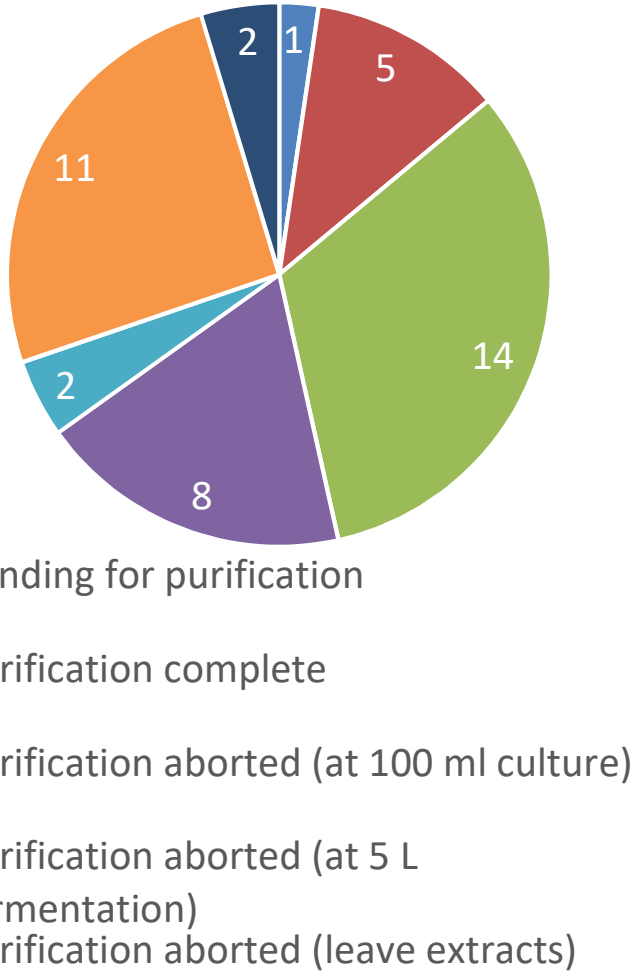
Purified Active Compunds

Total purified extracts = 41

Number of extract for purification in 2018 based on their activity



Resume of extract status for purification in 2018

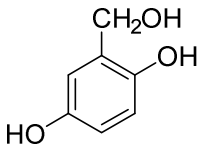
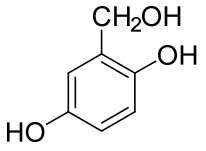
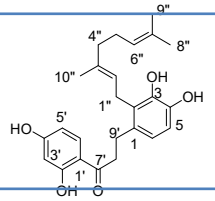
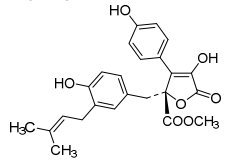
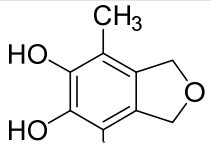
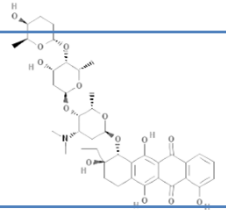


Progress 2018

Purification of active compound

Anti-malarial active compound

Structure Elucidated Active Compounds

Extract Code	Isolate Code	Source	Sampling Point	Isolation Method	Isolate Name	Compound Name	Structure	Activity
F15.1158	BioMCC-f.T.7495	Soil	Ambon	Wet method	*Aspergillus assiutensis* (99% similarity)	2,5 dihydroxy benzoil alcohol		*Pf*DHODH
F15.3082	BioMCC-f.T.5350	Soil	Pangan-daran	Wet method	*Aspergillus sp.* (morphology)	2,5 dihydroxy benzoil alcohol		*Pf*DHODH
Bread fruit (leave)	-	Plant	Tangsel	-	*Artocarpus altilis*	3,4 2',4'-tetrahydroxy-2-geranylchalcone		*Pf*MQO
F15.2274	BioMCC-f.T.1757	Soil	Flores	Lithium chloride method	*Aspergillus sp.* (morphology)	Butyrolactone-I		*Pf*DHODH
F15.2438	BioMCC-f.T.4328	Soil	Jepara	Wet method	*Aspergillus neoflavipes* (99% similarity)	1,3 dihydro- 7 ethyl-4,5,6-isobenzophurantriol		*Pf*MQO (false positive compound)
A21.1497	BioMCC-a.T.3335	Soil	Madura	Acid treatment method	*Streptomyces sp.* (morphology)	Cosmomycin		*P.falciparum*

Progress 2018

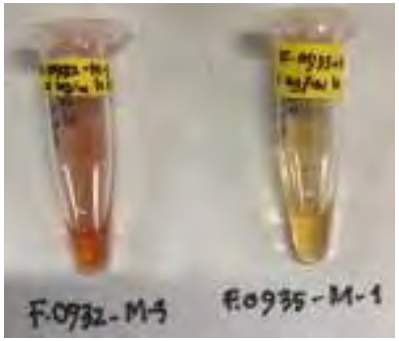
Purification of active compound | **Anti-amebic active compound**

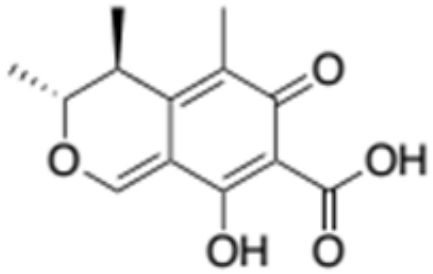
Extract code: F.0935

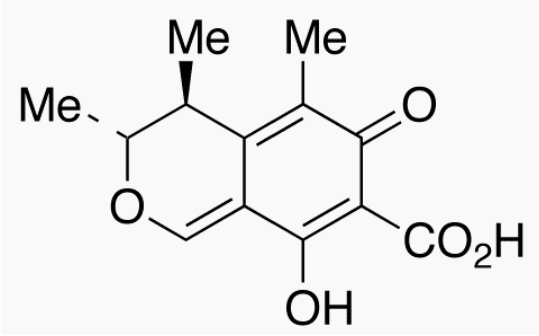
Isolate name	: *Penicillium citrinum*	Isolation time	: May 2, 2005
Isolate code	: BioMCC-f.mo.043	Sampling point	: Banjarmasin
Isolation source	: Marine organism	Bioactivity	: *E.histolytica* cell growth
Isolation method	: …	DNA analysis result	: 100% similarity *P.citrinum*

IC_{50} determination of identified compounds and its standard against *E. histolytica* cell

compounds	µg/ml	µM
F.0932-M-3	3.9 ± 0.2	15.6 ± 0.8
F.0935-M-1	8.1 ± 0.4	32.3 ± 1.6
Citrinin standard (Toronto, C523500)	40.8 ± 2.1	163.1 ± 8.4







F.0932-M-3; F.0935-M-1 *Citrinin standard (Toronto, C523500)*

* Purification was conducted at The University of Tokyo

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

Progress 2018

Training

Training in Japan

BPPT

No	Nama	Title	Period	Venue
1	Danang Waluyo	Determination of target for drug discovery	Mar 1-23, 2018	The University of Tokyo
2	Eka Siska	Purification of active compound	Sep 3-29, 2018	Kitasato University
3	Evita Chrisnayanti	Purification of active compound	Sep 24 – Oct 20, 2018	Kitasato University
4	Avi Nurul Oktaviani	Identification and characterization of Actinomycetes	Sep 3 – Dec 22, 2018	Kitasato University
5	Kristiningrum	Identification and characterization of Fungi	Oct 31 – Nov 29 , 2018	Kitasato University
6	Danang Waluyo	Determination of target for drug discovery	Nov 12 – Dec 7, 2018	The University of Tokyo

AU

No	Nama	Title	Period	Venue
1	Dr. Myrna Adianti	Cell toxicity assay and new enzyme assays for antiamebic compound discovery	Jan 8-29, 2018	The University of Tokyo
2	Ms. Hilkatul Ilmi	Cell toxicity assay and new enzyme assays for anti-Malaria discovery	Nov 4 – Dec 1, 2018	Nagasaki University
3	Ms. Lidya Tumewu	Structure elucidation of active compound	Sep 2-30, 2018	The University of Tokyo

Progress 2018

Training

On-site Training

No	Name	Institution	Topic	Period
1	Prof. Tomoyoshi NOZAKI	Univeristy of Tokyo	Progress Monitoring	25 Januari - 6 Feb 2018 6 - 15 Maret 2018 8 - 16 Mei 2018 27 Juni – 4 Juli 2018 9 – 13 September 2018 27 Nov – 7 Des 2018
2	Dr. Azuma WATANABE	MicroBioFarm Japan	Isolation, Purification and Structure Analysis of Chemical Compounds	30 Jan – 3 Feb 2018
3	Prof. Kazuro SHIOMI	Kitasato University	Isolation, Purification, and Structure Analysis of Chemical Compounds	28 Jan – 3 Feb 2018
4	Dr. Kazuyuki DOBASHI	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	21 Jan – 2 Feb 2018 19 April – 16 Mei 2018 25 Juli – 17 Agustus 2018 21 Nov - 13 Desember 2018
5	Dr. Mihoko MORI	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	24 Jan – 10 Februari 2018 7 – 18 Mei 2018 22 Agustus – 6 Sept 2018
6	Dr. Ken Daniel INAOKA	Nagasaki University	Malaria (Investigation and Analysis)	28 Jan - 3 Februari 2018 2 Juli – 13 Juli 2018
7	Dr. Takaya SAKURA	Nagasaki University	Malaria (Investigation and Analysis)	28 Jan – 3 Feb 2018 7 – 18 Mei 2018 2 – 13 Juli 2018
8	Dr.Michio YAMASHITA	University of Tokyo	Isolation, Purification, and Structure Analysis of Medical Compounds	28 Jan - 24 Feb 2018 24 Juni – 21 Juli 2018
9	Dr. Katsuhiko ANDO	Kitasato University	Collection and Isolation of Microbial Resources	7 - 18 Mei 2018 26 Agust – 7 Sept 2018
10	Dr. Toru OKUDA	Kitasato University	Isolation, Purification and Structure Analysis of Chemical Compounds	14 – 18 Mei 2018 27 – 31 Agustus 2018
11	Dr. Toshiyuki TOKIWA	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	28 – 31 Agustus 2018

Progress 2018

Training

Impact

Microbial isolation and identification

- Increased capability of morphology-based identification
  →Diversity of newly isolated microbes were increased
- Increased capability of identification of interesting microbe
  →Identification of new microbial species were performed

Extract production

- Increased reproducibility of active extract production
  →Number of extracts those lost their activities were reduced
- Increased capability on managing microbial extract
  →Request-based extract production management system was established

Screening system

- Increased capability on development of target for drug screening
  →A new screening system was proposed and developed (anti TB)

Purification and elucidation of active compound

- Increased capability on active compound purification
  →Number of purified and structure-elucidated active compounds were increased

Progress 2018

Technical meeting

Objective: To evaluate and monitor progress of the project

Scientific meeting

- 5 times (Feb 1, Mar 12, Jul 2, Oct 3, Nov 28)
- Agenda: Progress report and problem solving
- Supervised by Project Advisor

Weekly meeting

- Once a week (every Thursday)
- Agenda: Progress report of each team
- Supervised by Project Co-manager

Annual meeting

- Twice (Feb 14, Dec 20)
- Agenda: Evaluation and planning the project
- Supervised by Project manager and co-manager

Progress 2018

Networking

Airlangga University

- July 5th, 2018
- Technical discussion on progress of anti-amebic screening

LIPI

- Oct 31th, 2018
- Technical discussion on microbial preservation and sharing of microbial isolates for screening

Gadjah Mada University

- Nov 2nd, 2018
- Initiation of collaboration on development of anti-cancer agents

Progress 2018

Networking

Obihiro University of Agriculture and Veterinary Medicine

- Collaboration on development of anti-toxoplasmolysis agents by utilizing Indonesian bio-resources
- MTA was signed on Aug 25th, 2017
- More than 3800 microbial extracts were screened by end of 2018
- Currently, reconfirmation extracts are being produced

The University of Tokyo

- Collaboration on development of anti-tuberculosis agents by utilizing Indonesian bio-resources (together with Airlangga University, funded by TB Alliance USA)
- MTA was signed on July, 2018
- More than 3500 microbial extracts were shared for first screening
- Currently, screening are being performed in AU

Brawijaya University

- Providing training on *in vitro* anti-malarial assay (LDH assay)
- Training was conducted on Sep 4-7, 2018 at BTC-BPPT
- Attended by 2 trainees

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

Progress 2018

Budget and Expenses

BC for SLeCAMA project 2018

- Budget = Rp. 418.444.000

Insinas MoRTHE 2018

- Budget = Rp. 175.000.000

→ **Total = 593.444.000**

Description	Expenses (Rp.)	Note
Chemical & laboratory supplies	197.962.400	Incl. gases and liquid gases
Salary	204.174.080	Salary for not permanent BC member
Travel	43.675.100	Field trip, visit AU&LIPI
Equipment	137.162.000	AC, Printer
TOTAL	**582.973.580**	

Planning 2019

Planning 2019

Project Planning

1. Microbial isolation and identification
   →Isolation of microbial strain (from Bawean Island)
   →identification/taxonomy studies of isolated microbes

2. Extract production
   →Improving extract production management system
   →Improving reproducibility of microbial active extract

3. Screening
   →Continuing screening of extracts (plants, microbes

4. Purification
   →Establishment of new dereplication method

5. Efficacy test
   →Testing active compound in animal model (to be done under collaborative research with Brawijaya University)

Planning 2019

Activity Planning

1. Field trip for microbial sampling
   →Time: April 23-26, 2019 (tentative)
   →Venue: Bawean Island

2. International symposium
   →Time: Mid October, 2019
   →Venue: Jakarta (tentative)

3. Publication
   - Scientific journal: submission of at least 2 papers into scientific journal
   - Conference: participating in Asian Mycological Congress (Oct 1-4, 2019, Mie, Japan)

Networking Planning

Brawijaya University: Efficacy test of anti-malarial active compound
Gadjah Mada University: Screening of microbial extracts with specific anti-cancer activity
Obihiro Univ. of Agric.Vet.Med: Purification of anti-toxoplasmolysis agents

Planning 2019

Budget Arrangement

- BPPT allocated budget for FY 2019 as much as **Rp. 699.998.000**

- 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. 317.000.000**

Description	BPPT Budget (Rp.)	Note
Salaries	184.320.000	Salary for not permanent BC member
Reagents and consumables	218.800.000	Incl. gases and liquid gases
Travel	135.417.000	Transportation (airfare, sea, ground), accomodation, daily allowance
Equipment	75.000.000	Laboratory bench, etc.
Meeting	86.461.000	JCC Meeting, International symposium
TOTAL	**699.998.000**	

Target Review (2018)

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 2018
Blue: partially achieved 2018

Thank You

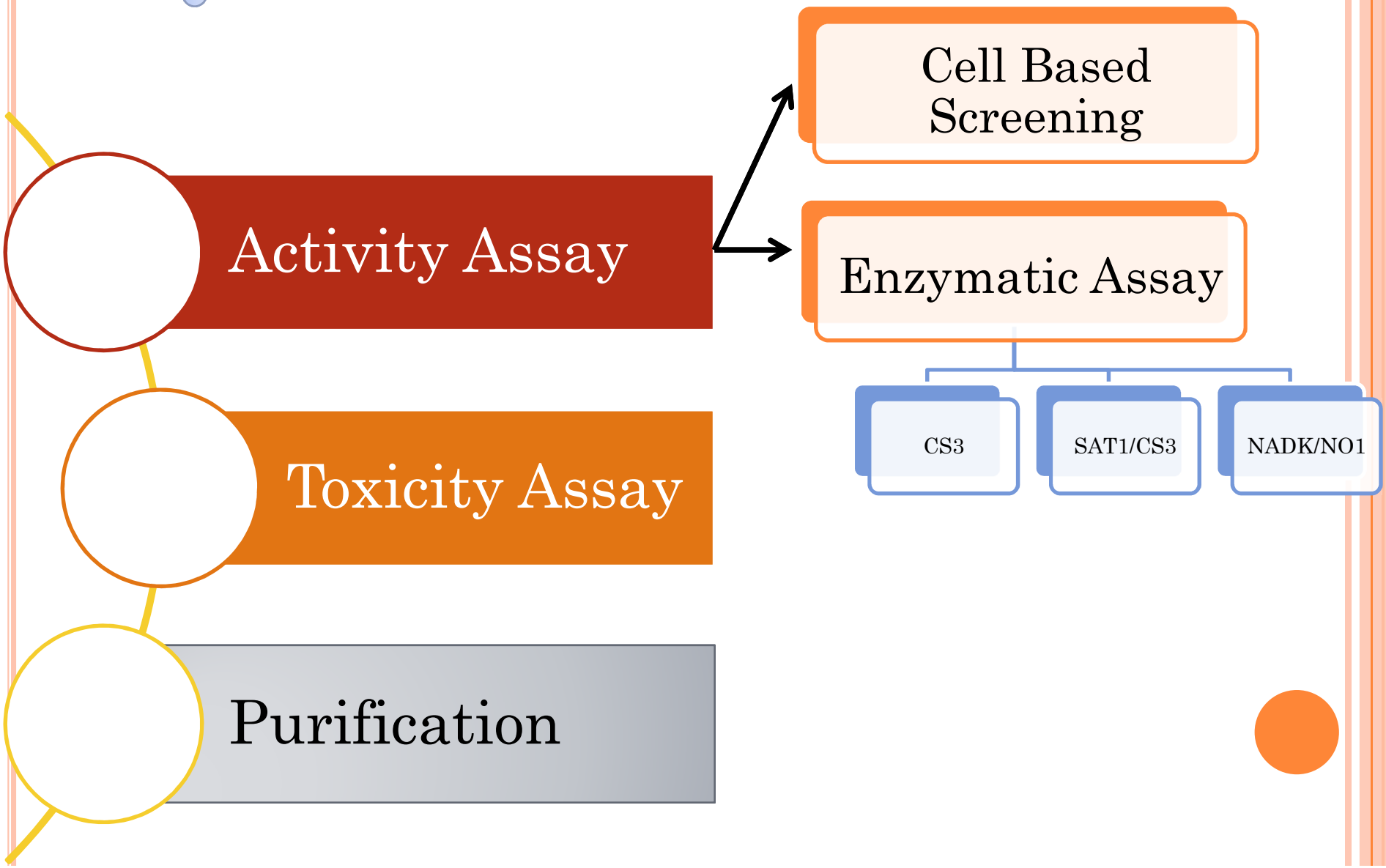
SATREPS **SLeCAMA Project**

Activities Report of ITD-UNAIR

"Project for Searching Lead Compounds of anti-Malarial and Anti-Amebic Agent by Utilizing Diversity of Indonesian Bio-Resources"

January 29, 2019

MAIN WORK at ITD-UNIVERSITAS AIRLANGGA



Extract from BPPT

Cell Based Screening

2016-2018 → 12380 → 1 active non toxic → On progress purification

Enzymatic Assay (CS3)

2015-2017 → 5400 → 1 active → On progress purification

Extract from BPPT

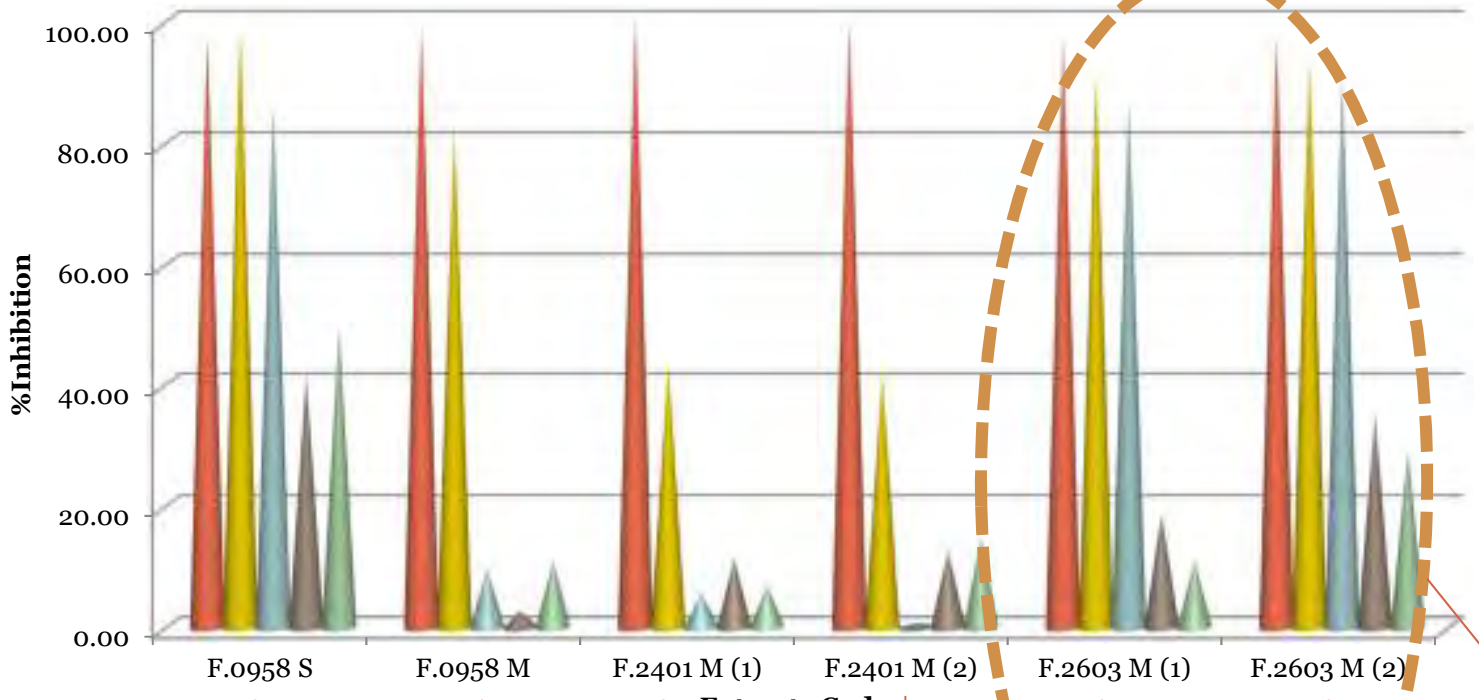
Enzymatic Assay
(CS3/SAT1 Coupled Assay)

2016-2018 → 6600 → 2 active → Large Scale Requested

Enzymatic Assay
(NADK/NO1 Coupled Assay)

2018 → 7260 → 50 hits

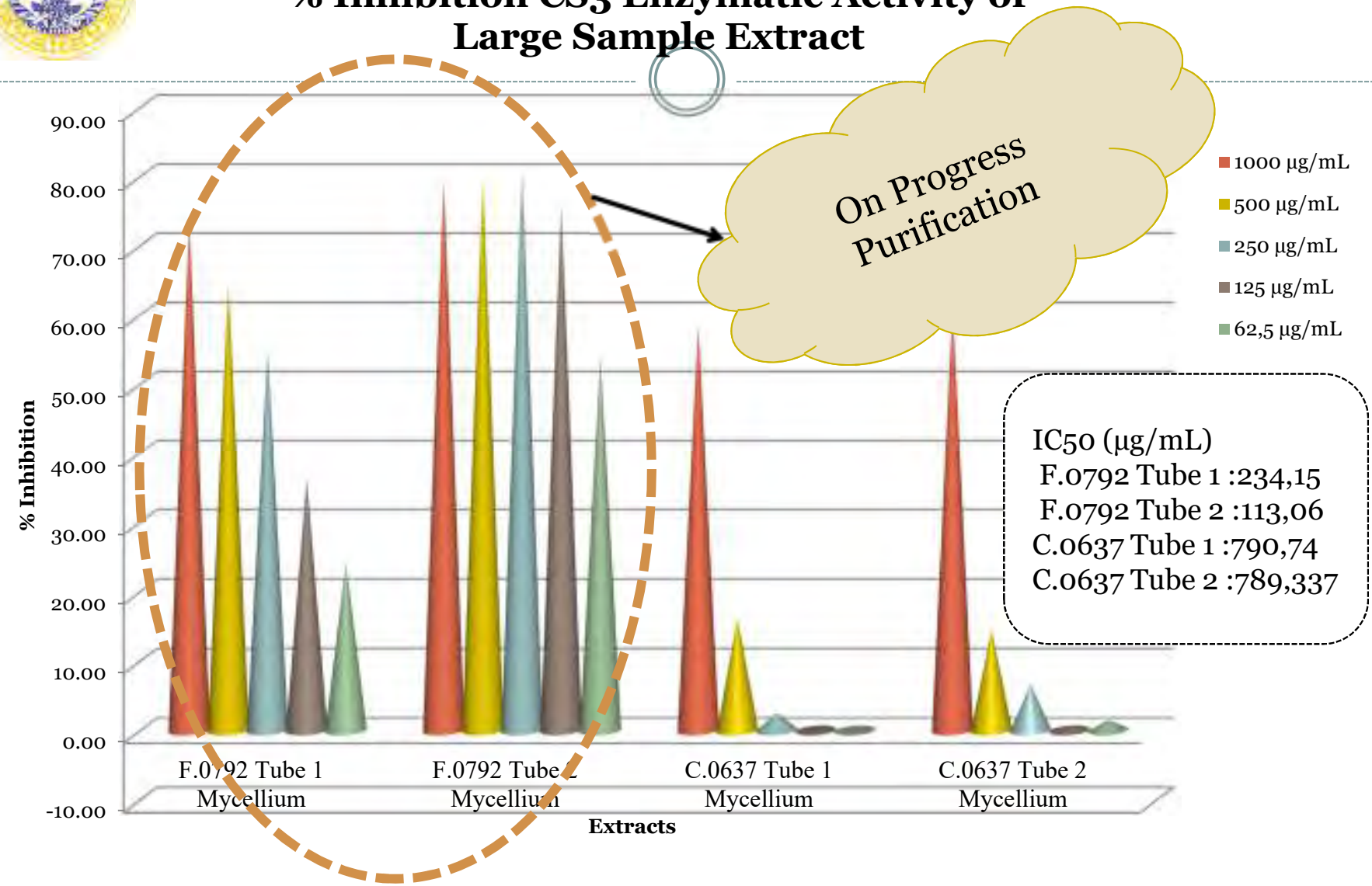
Activity and Toxicity Assay (Huh7it) Large Scale Extracts for Cell Based



	F.0958 S	F.0958 M	F.2401 M (1)	F.2401 M (2)	F.2603 M (1)	F.2603 M (2)
CC_{50} µg/mL	46.75	>1000	>1000	>1000	>1000	>1000
IC_{50} µg/mL	1.2	34	252	662	2.77	0.88

On Progress Purification

% Inhibition CS3 Enzymatic Activity of Large Sample Extract



IC50 (µg/mL)
F.0792 Tube 1 :234,15
F.0792 Tube 2 :113,06
C.0637 Tube 1 :790,74
C.0637 Tube 2 :789,337

TRAINING/TECHNOLOGY TRANSFER in 2018

Myrna Adianti, Ph.D

January 2-29, 2018 at University of Tokyo

Cell Toxicity Assay and New Enzyme Assay for Antiamebic Compound Discovery

Lidya Tumewu, M.Farm, Apt

September 2-29, 2018 at The University of Tokyo

Structure Elucidation of Compounds (Purification of F.0935.S as Antiamoeba)

Hilkatul Ilmi, M.Si

Nov 4 –Dec 2 2018 at Nagasaki University

Cell Toxicity Assay and New Enzyme Assay for Antimalarial Discovery

Instrument 2018

Victor Nivo Plate Reader

BOD Monitor

Ultrasonic Cleaner

THANK YOU

Cell Based Assay Report

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2016-207	5120	182	122	39	7	4	-	Requested LS
2018	7260	137	13	5	4	4	2	1 active non toxic

Enzymatic Assay (CS3) Report

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2016-207	5120	60	22	10	4	1	1	On progress purification

Enzymatic Assay (CS3/SAT1 Coupled Assay)

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2016-2017	2220	41	10	1	-	-	-	Not Growth
2018	4380	26	5	3	2	2	-	Request LS

Enzymatic Assay (NADK/NO1 Coupled Assay)

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2018	7260	50	-					Request Reconfirm

Toxicity MTT Assay (Huh7it)

Year	Hit Primary Screening	Hit Reconfirm	Hit PSU	Hit Large
2018	356	3	4	2

Extract Primary from BPPT

- Extracts (2016-2017):
  - 64 deep well-plate (5120 dry extract) ⟶ 182 hits cell based and 60 hits CS3 enzymatic assay (Total 242 hits)
- Extracts (January 2018)
  - 56 deep well-plate (4380 dry extract) ⟶ 112 hits cell based, 26 hits CS3/SAT1 Coupled Assay and 41 hits NADK/NO1 Coupled Assay (Total 179 hits)
- Extracts (May 2018)
  - 36 deep well-plate (2880 dry extract) ⟶ 25 hits non toxic cell based and 9 hits NADK/NO1 Coupled Assay (Total 34 hits)

Extract Reconfirmation from BPPT

- Extracts (2017):
  - 122 extracts for cell based and 27 extracts for enzymatic assay (CS3) (Total 149 extracts) ⟶ 25 hits active non toxic for cell based and 17 hits CS3 (Total 42 hits)
- Extracts (2018)
  - 13 extracts for Cell based assay ⟶ 5 active non toxic
  - 5 extracts for Enzymatic assay (SAT1/CS3 Coupled assay) ⟶ 3 hits active
  - 5 extracts for Enzymatic assay (CS3) ⟶ 1 hits active

  (Total received 23 extracts and get total 13 hits active)

Extract PSU from BPPT

- Extracts (September 2018):
  - 13 extracts for Cell based assay → 4 active non toxic
  - 4 extracts for Enzymatic assay (CS3) → 1 active
- Extracts (November 2018)
  - 12 extracts for Cell based assay → 8 active
  - 11 extracts for Enzymatic assay (CS3) → 2 active
  - 2 extracts for Enzymatic assay (SAT1/CS3 Coupled Assay) → 2 active
- Extract (December 2018)
  - 4 extracts for Cell based assay → 3 active non toxic

(Total 46 received and get 20 hits)

Extract Large Scale from BPPT

- Extracts (October 23, 2017):
  - 12 dry extracts for Enzymatic assay (CS3) ⟶ 1 active (on progress purification)
- Extracts (October 29, 2018):
  - 1 extracts (F.0958) for Cell based assay ⟶ Supernatant (IC50 1,2 µg/mL and CC50 46,75 µg/mL or toxic) and Mycellium (IC50 34 µg/mL and CC50 >1000 µg/mL or non toxic)
- Extracts (December 22, 2018):
  - 1 extracts (C.0637) for Enzymatic assay (CS3)
  - 2 extracts (F.2603 & F.2401) for Cell based assay ⟶ F.2603 active non toxic and F.2401 no activity non toxic

JCC fourth year

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

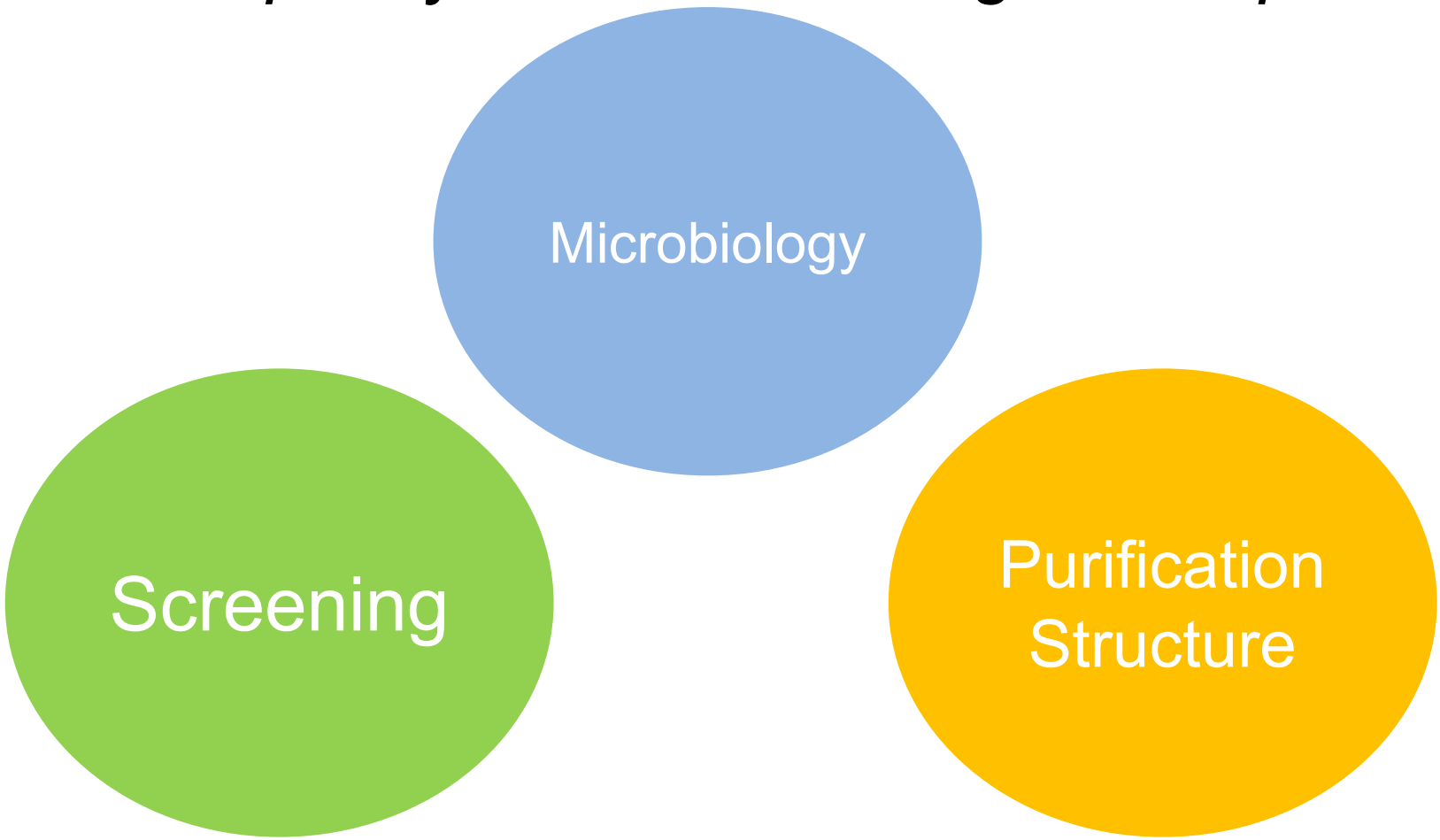
Achievements, needs, and solutions

Tomo NOZAKI
The University of Tokyo
Chief Advisor

Jakarta, January 29th, 2019

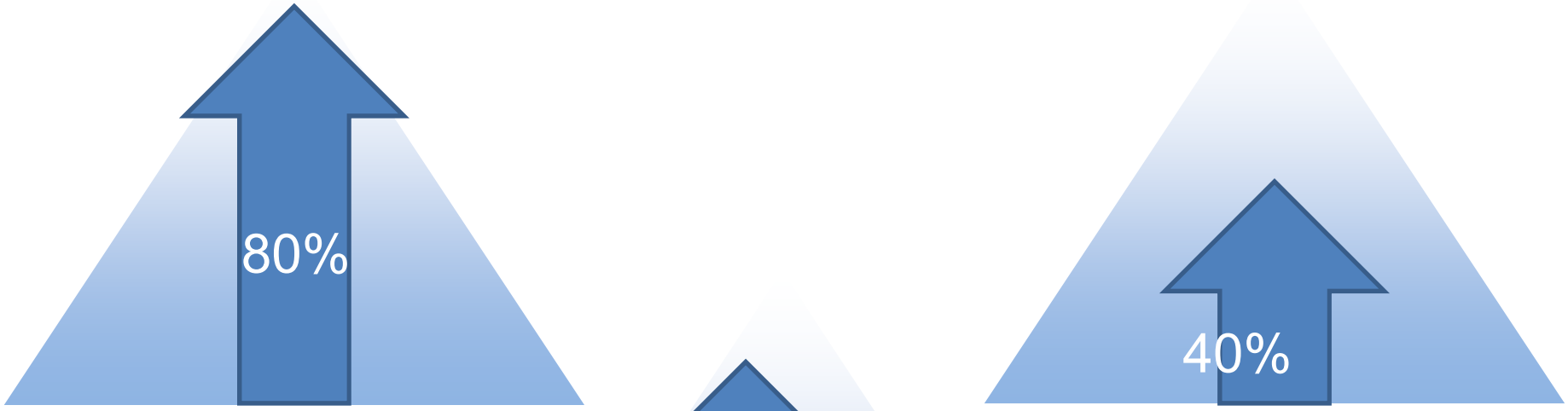
Goals of the project

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



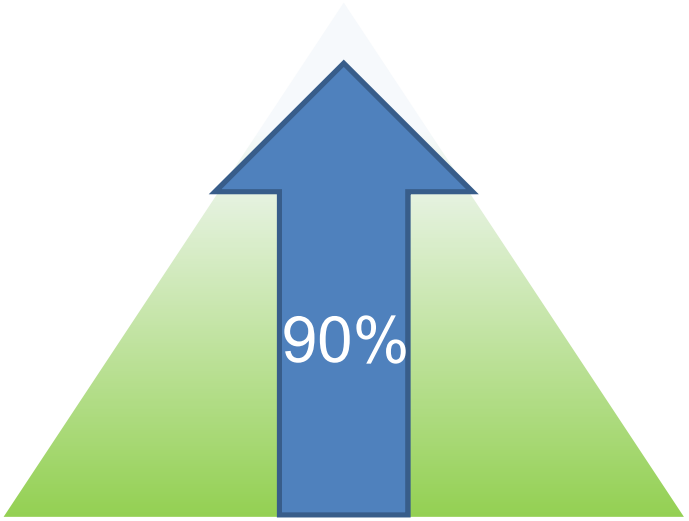
Microbiology
Sampling/Isol
ation/Culture

Microbiology
Classification
/Taxonomy

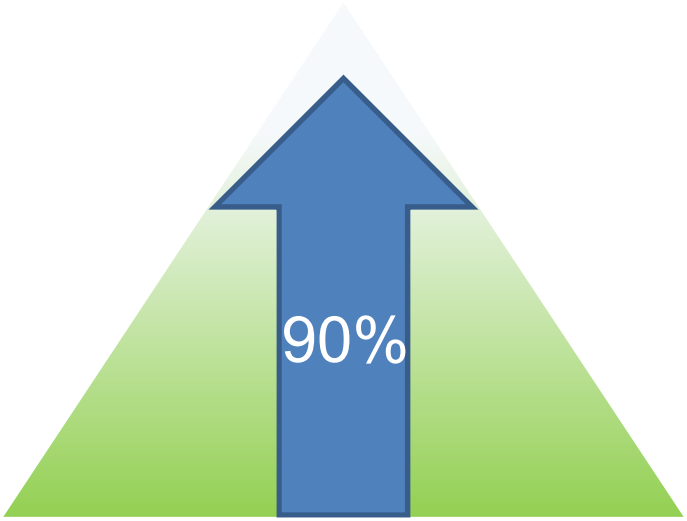
Microbiology
Extract production
Data management

Accomplishment of goals

Research Capacity Building – Screening



Screening
Enzyme-based assay

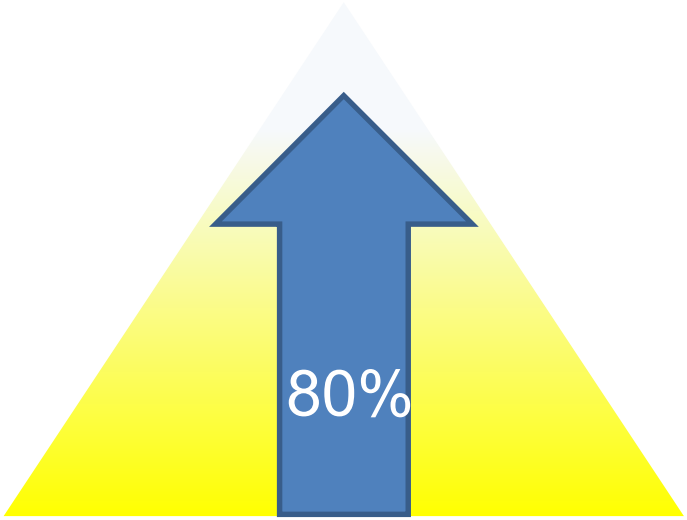


Screening
Cell-based (phenotypic) assay

Accomplishment of goals

Research Capacity Building

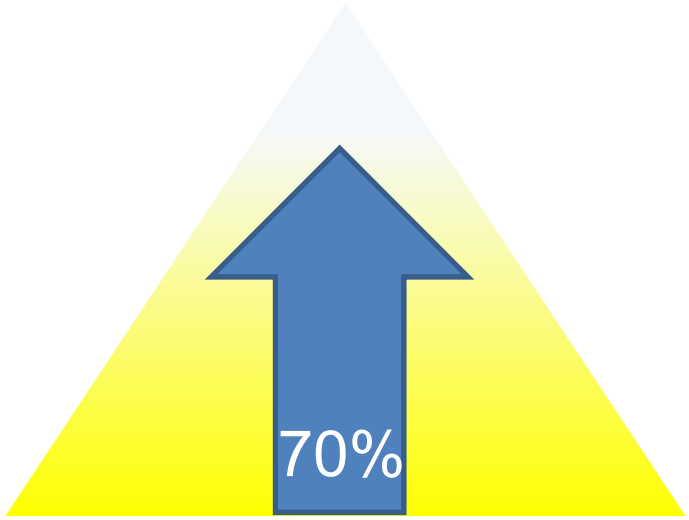
– Purification and structural elucidation



Purification

Liquid partition

Chromatography



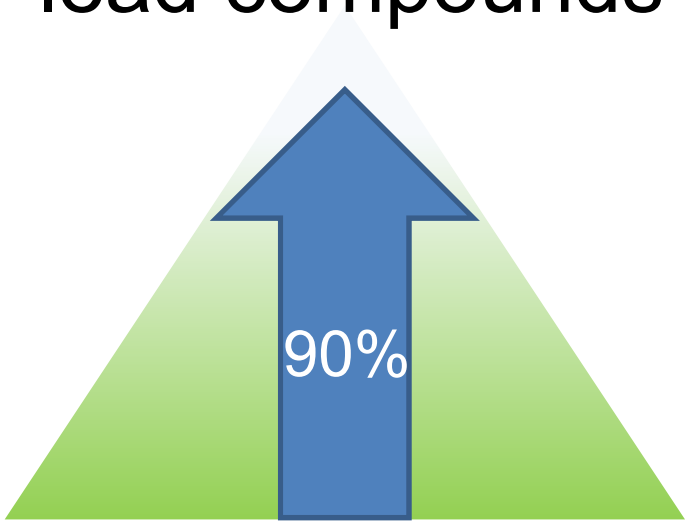
Structural elucidation

Mass spectrometry

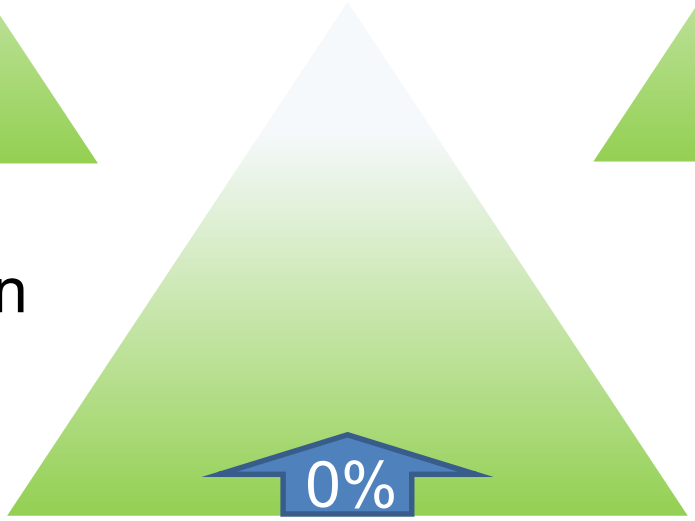
Nuclear magnetic resonance

Accomplishment of goals

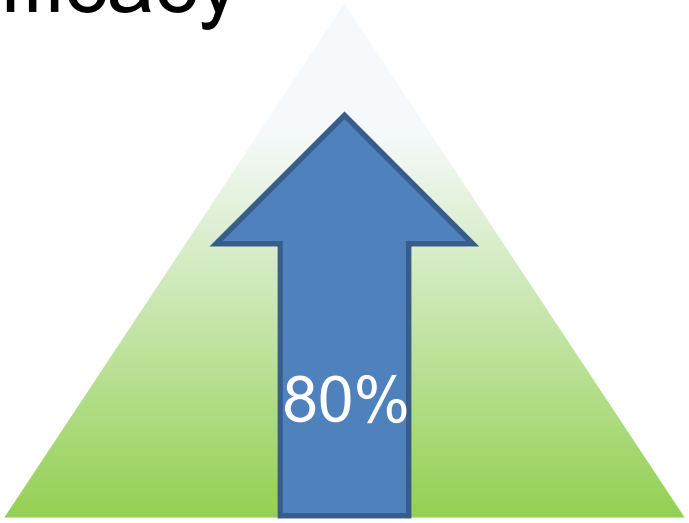
Identification of anti-malarial and anti-amebic lead compounds with in vivo efficacy



Antimalarial
Lead identification

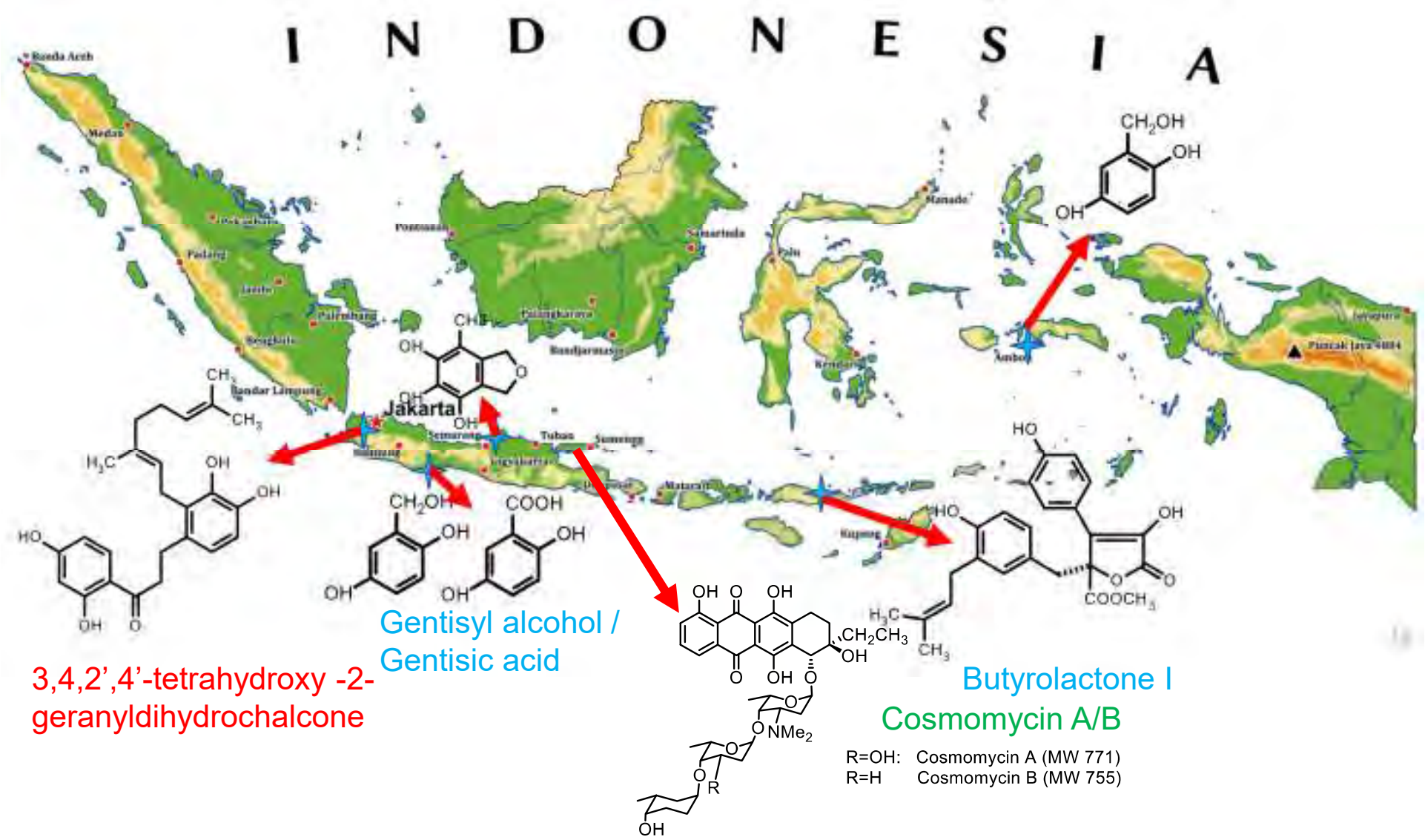


In vivo Efficacy confirmation



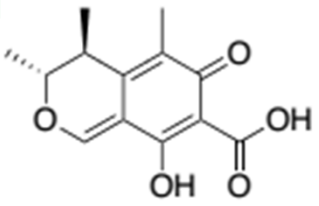
Anti-amebic
Lead identification

Highlights (2018) of Antimalarial discoveries: DHODH and MQO inhibitors and anti-proliferative compounds



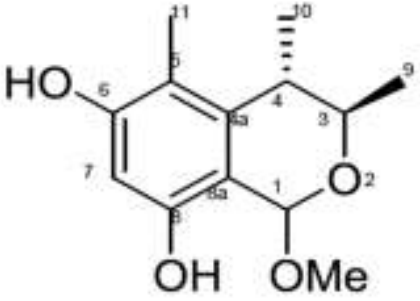
Highlights (2018) of Antiamebic discoveries: anti-proliferative compounds

Citrinin



Chemical Formula: $C_{13}H_{14}O$
Exact Mass: 250.08
Molecular Weight: 250.25

Decarboxylated citrinin



Problems / needs (Jan, 2018)

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

Suggested solutions to the needs (Jan 2018) and the current status (Jan 2019)

1. Characterization/archiving of microbial strains…..Enhance training for taxonomy…..Not satisfactory (particularly at molecular levels); Further improvement needed.
2. Exploitation of new targets and introduction of new screening platforms…New enzyme targets need to be selected and explored ….. Satisfactory; several target enzymes added.
3. Prioritization of identified hits for purification…Ranking of hits by selectivity index, counter-screening, taxonomy of isolates, preliminary extraction test….Partially satisfactory; Dereplication methods need to be developed.
4. Broadening of the bottleneck process(es) (purification/structure elucidation)…Inclusion of additional purification stations needed (Unair and UTokyo)….Satisfactory; New problems identified = A bottleneck was not restricted to large scale culture production; Schedule sharing started.

Inclusion of dereplication step during screening

De`rep`li`ca´tion

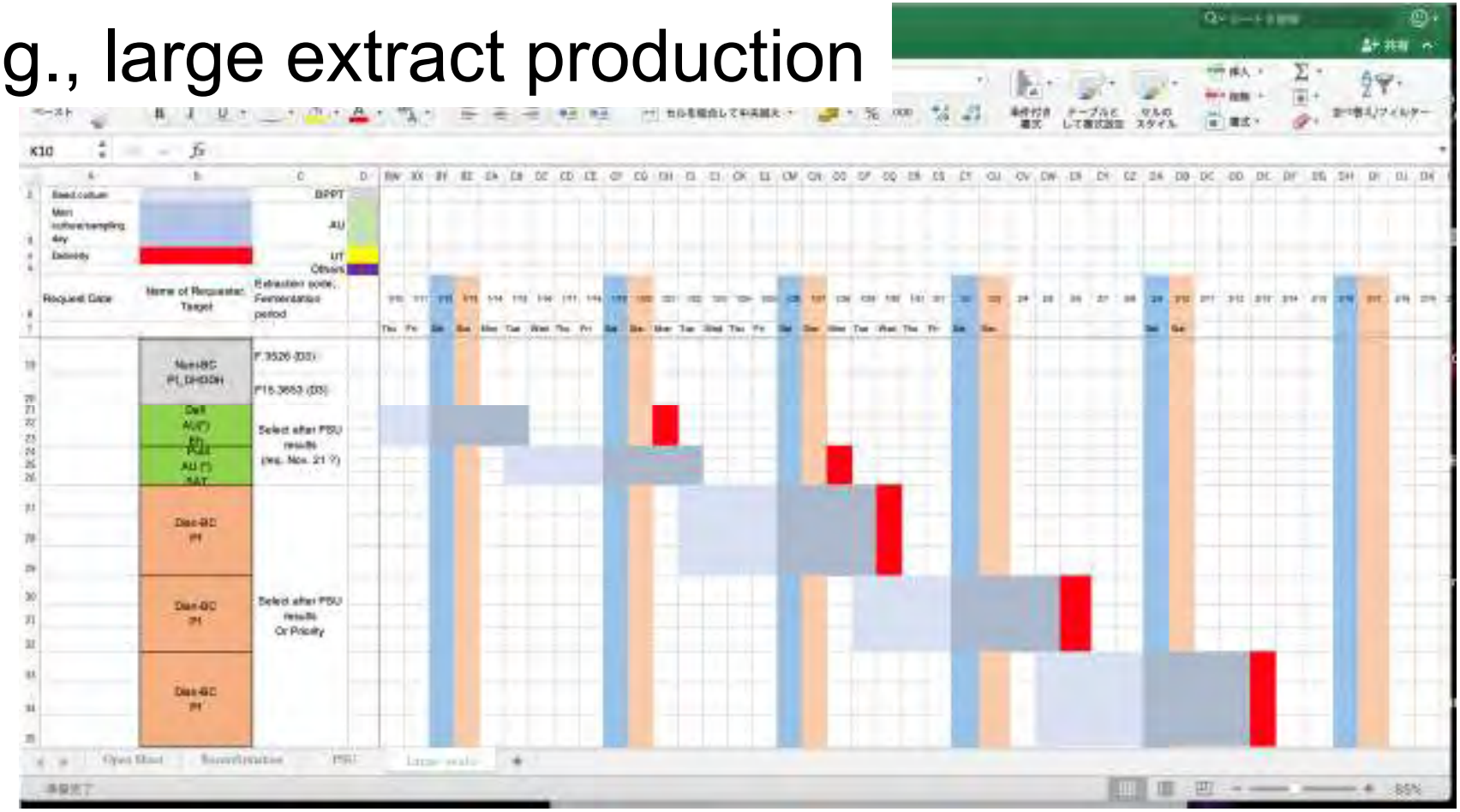
n. 1. *(Biochem.)* the process of testing samples of mixtures which are **active** in a screening process, so as to recognize and eliminate from consideration those active substances already studied; - a stage subsequent to the preliminary screening in the process of discovery of new pharmacologically active substances in mixtures of natural products; - also called counterscreening. See screening.

"Not to repeat discoveries of previously known compounds (including frequent hits)"

1. Use of other references (negative control organisms) (counter-screening)
2. TLC and PDA/HPLC profile-based identification (database?)
3. Preference to uncommon microbes

Schedule management and sharing

e.g., large extract production



1. The schedule is updated every Friday and shared on the last Friday of the month among all team members
2. Helps other teams plan ahead
3. Helps visualization of bottleneck processes

Suggested solutions to the needs (Jan 2018) and the current status (Jan 2019)

5. Cordination between BC/Airlangga/InaCC……Periodical mutual visits / joint meetings for data and method sharing; cross depositing of microbes…..Partially conducted
6. Establishment and development of a drug develop consortium (networking)……Utilization of next JCC meeting or International Symposium …..2nd International Symposium held in October
7. Broadening of disease areas….toward other infectious diseases (e.g., TB/HIV/Helicobacter/Hepatitis/Dengue) and non-communicable diseases (e.g., cancers/obesity/hypertension….) …..Patially conducted and further planned
8. Sustainable development of the capacity…..Continuous funding >5 years, continuous oversea collaboration…..New application to sustain the activity will be filed this year.

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

- 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

Achievements in capacity building in 2018

Training in Japan

- 4 Long-term (3-5 years) trainees (incl. other funding sources)
- 9 short-term (1-3 months) trainees
  - Microbe characterization
  - Purification

Training in Indonesia

- 29 dispatches of 11 Japanese experts (1-8 weeks)

Plan for capacity building in 2019

Training in Japan

- 7 Long-term (3-5 years) trainees (incl. other funding sources) (two more after 2020)
- 5-6 short-term (1-2 months) trainees
  - Microbe characterization
  - Purification

Training in Indonesia

- 20 dispatch of Japanese experts (1-8 weeks)
- International symposium

In summary…..

We had so many difficulties,….but

We have been doing great!

Let us achieve what we aimed at!

BPPT
BADAN PENGKAJIAN DAN PENERAPAN TEKNOLOGI

JICA
Japan International Cooperation Agency

The 1st JCC Meeting

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

Progress 2015 and Planning 2016

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

SATREPS

SLeCAMA Project

TARWADI

Project Co-manager

VIP Meeting Room, BPPT, Jakarta
February 2nd, 2016

Content

1. Target Review
2. Progress 2015
    a. Field exploration
    b. Microbes Isolation
    c. Extract preparation, Screening and Purification
    d. Training
    e. Reorganization in BPPT
    f. Room setup
    g. Equipment Setup Progress
3. Planning 2016
    a. Budget arrangement
    b. Training
4. Future Agenda

Target Review

Project purpose/Outputs	INDICATORS	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 2017) 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 2017) 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

Field Exploration

Overview

Date : 27-30 July 2015
Venue : **Ambon and Saparua Island, Maluku**
PIC : Danang, Kristiningrum

Schedule

27 Jul: Arrive in Ambon, visit Research Center for Deep Sea, LIPI
28 Jul: Move to Saparua Island, take samples
29 Jul: Move to Ambon, take samples
30 Jul: Depart from Ambon to Jakarta

Result

1. Number of samples (total=90)
   - Soils = 41
   - Termites = 11
   - Medicinal plants = 29
   - Plant litters = 9

2. Number of places of sample origin (total=11)
   - Saparua Island = 5
   - Ambon Island = 6

Number of isolated microbes (as 16 Nov 2015, total=427)

- Actinomyces = 157
- Fungi = 270

Extract Preparation, Screening and Purification

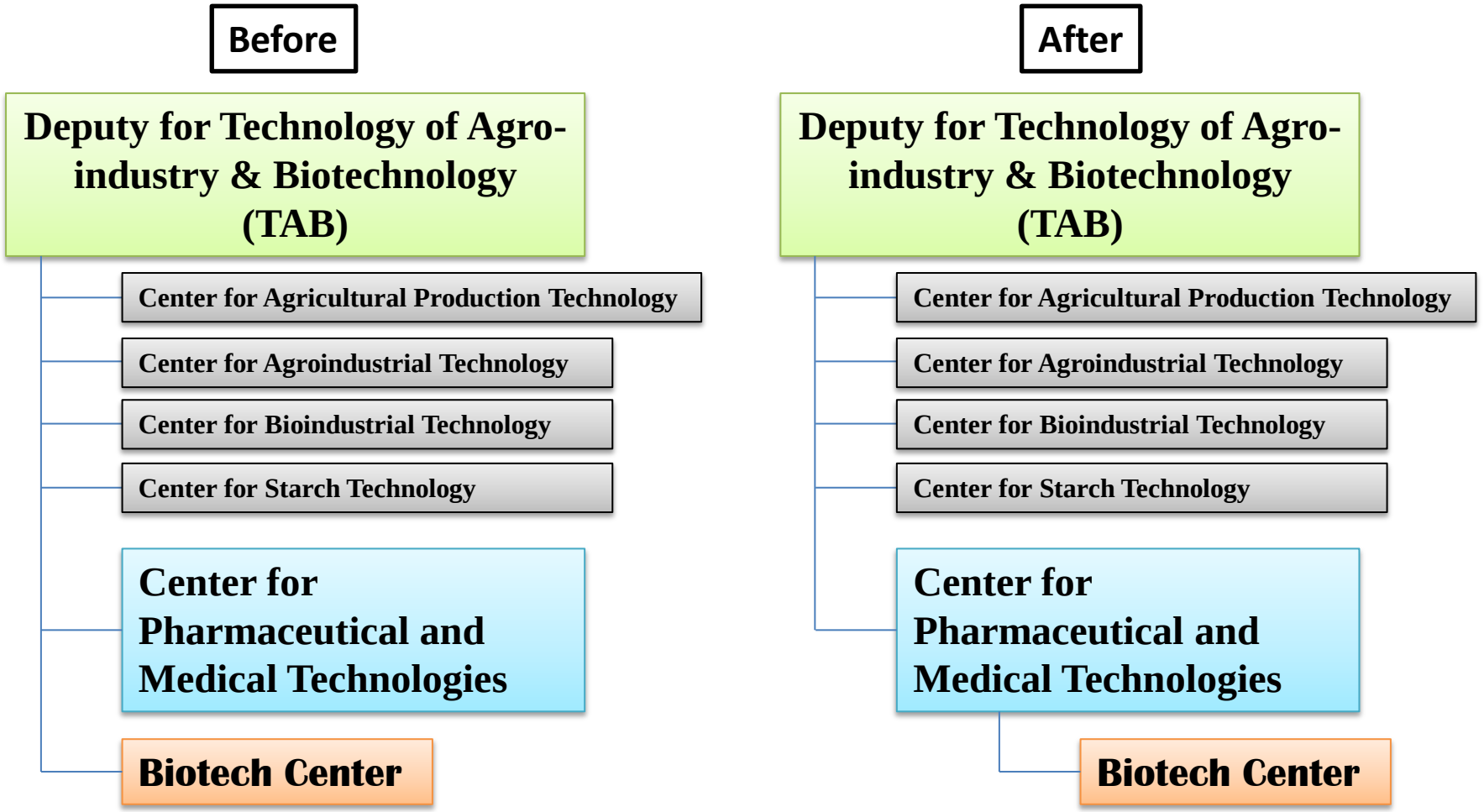
Group	No	Description	Total	Notes
Microbial isolation and extract preparation	1	Number of newly isolated microbes	560	Actinomycetes=159 Fungi=401
	2	Number of identified microbes	6681	Cummulative number Actinomycetes=3181 Fungi=3500 Total number of identified microbes in 2015=0
	3	Number of old isolated microbe to be revived from freezer	1698	Actinomycetes=867 Fungi=831
	4	Number of old isolated microbe revived from freezer	1302	Revival rate = 76.7% (Actinomycetes=84% (730), Fungi=69% (572))
	5	Number of extract produced for screening	800	Actinomycetes=200 Fungi=200 Type of medium=2
	6	Number of extract produced for purification	3	Fungi=3
Enzyme- and cell-based screening	7	Number of extract screened for antimalaria (enzyme-based)	1440	Old extract=640 (used for screening Dec 2014 and may 2015-Sep 2015) New extract=800 (used for screening May 2015 & Sep 2015)
	8	Number of extract screened for antiamebiasis (enzyme-based)	5200	Using old extract, used for screening May 2015
	9	Number of extract screened for antimalaria (cell-based)	320	Using new extract, used for screening May 2015
	10	Number of extract screened for antiamebiasis (cell-based)	320	Using new extract, used for screening May 2015
Purification and characterization	11	Number of purified extract	3	Antimalaria=3
	12	Number of identified active compound	2	Antimalaria=2

Training in Japan

Name	Home Institution	Title of Training	Duration of Training	Training Venue
Ms. Myrna Adianti Subianto	Airlangga University	Amebiasis Enzyme Assay	11-May-2015 ~ 10-Jul-2015	National Institute of Infectious Diseases
Ms. Ratna Wahyuni Zainuri	Airlangga University	Amebiasis Enzyme Assay	11-May-2015 ~ 10-Jul-2015	National Institute of Infectious Diseases
Ms. Amila Pramisandi	BPPT	Purification of Antiprotozoa Antibiotics	11-May-2015 ~ 10-Jun-2015	Kitasato University
Ms. Amila Pramisandi	BPPT	Purification of Antiprotozoa Antibiotics	17-Jun-2015 ~ 16-Jul-2015	Kitasato University
Ms. Endah Dwi Hartuti	BPPT	Malaria Enzyme Assay	11-May-2015 ~ 10-Jun-2015	University of Tokyo
Ms. Siska Andrina Kusumastuti	BPPT	Malaria Enzyme Assay	11-May-2015 ~ 10-Jul-2015	University of Tokyo
Ms. Astutiati Nurhasanah	BPPT	Malaria Enzyme Assay	11-May-2015 ~ 10-Jul-2015	University of Tokyo
Ms. Erwahyuni Endang Prabandari	BPPT	Cultivation and *Plasmadium faciparum* and Production, Purification and Assays of Plasmodial Enzymes	25-Sep-2015 ~ 23-Oct-2015	University of Tokyo
Ms. Astutiati Nurhasanah	BPPT	Cultivation of *Entamoeba histolytica* and Production, purification, and Assays of Amebic Enzymes	25-Sep-2015 ~ 23-Oct-2015	National Institute of Infectious Diseases
Ms. Dwi Peni Kartikasari	Airlangga University	Cultivation of *Entamoeba histolytica* and Production, purification, and Assays of Amebic Enzymes	28-Sep-2015 ~ 26-Nov-2015	National Institute of Infectious Diseases
Ms. Anis Herliyati Mahsunah	BPPT	Enzyme- and Cell-based Assays and Purification of target enzyme inhibitors	25-Sep-2015 ~ 23-Oct-2015	Kitasato University

8 persons from Indonesian institutes were dispatched to Japan for training

Reorganization in BPPT



Biotech Center becomes an implementation unit that focus on technological service
→SLeCAMA Project will be done in Center for Pharmaceutical and Medical Technologies
→Though, project site will be remained in Biotech Center

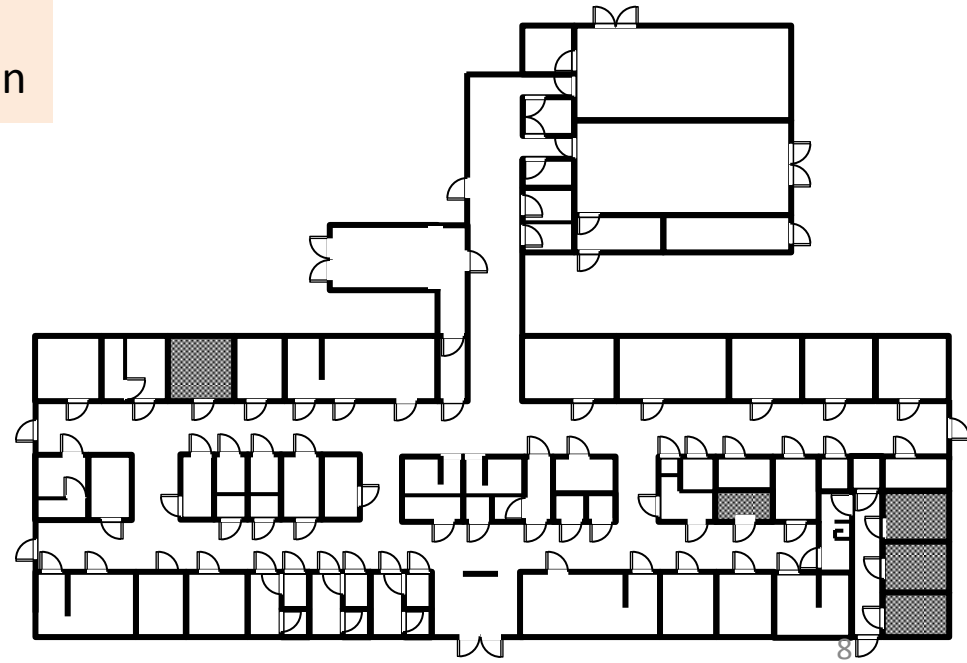
Progress on Room Setup and Equipment Installation

Room Setup

- *Experimental room*
  - Renovation date: March 2015
  - Renovated content:
    - Ceiling replacement
    - Wall and floor cleaning
    - Room clearance (removal of unused fermenter)
    - Light replacement
    - Air conditioner replacement
    - Exhaust reparation
    - Wall electric outlet installation
- *Cold room*
  - Renovation date: September 2015
  - Renovated content:
    - Room, desk, rack clearance (cleaning)
    - Wall and floor cleaning
    - Temperature check (approx. 6 deg C)

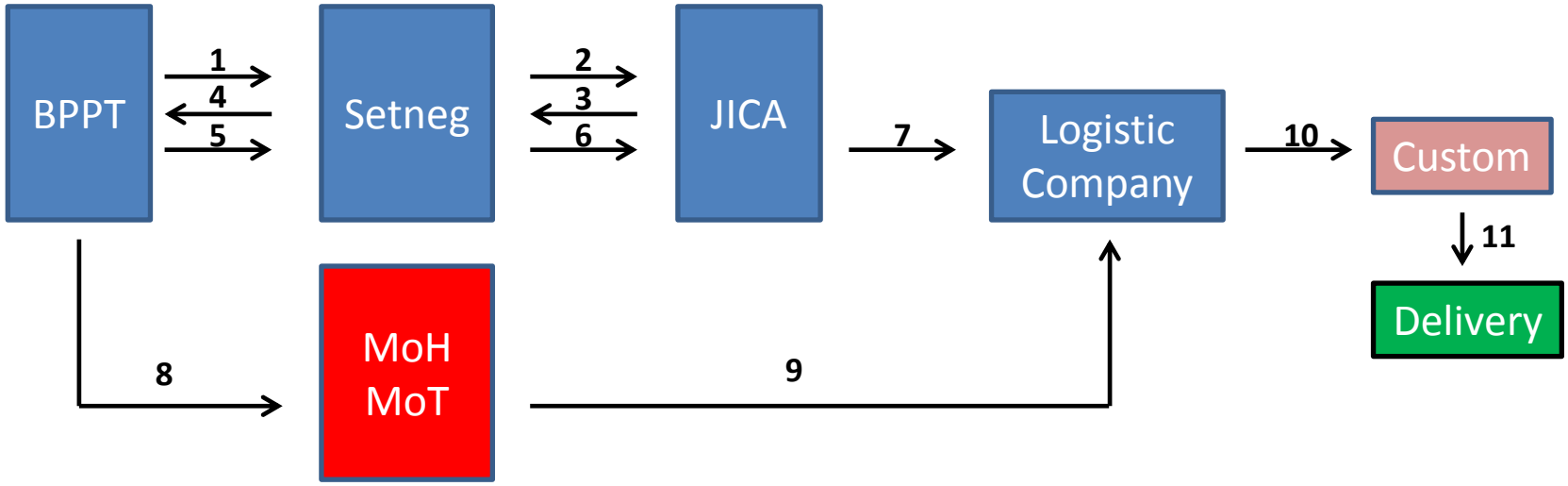
Before

After



Equipment Setup Progress

➢ *Equipment request flow*



1. BPPT sends *form A4* (equipment request) to JICA through Setneg
2. SETNEG receives *form A4*, then officially request to JICA on behalf of Indonesian gov't.
3. JICA replies by sending a request to obtain tax exemption letter to Setneg
4. SETNEG sends a confirmation letter to BPPT
5. BPPT confirms the letter to SETNEG
6. SETNEG issues tax exemption letter for the requested equipment and send to JICA
7. JICA sends the letter to logistic company
8. BPPT applies import permit for specific equipment to MoH and MoT
9. BPPT sends import permit to logistic company
10. Logistic company requests tax exemption to custom by attaching the letter from Setneg and import permit
11. Once the custom approved the request, equipment can be delivered

- Currently, process 9 is undergone by MoT (import permit from MoH is already issued)
- The equipment is predicted to be delivered on March 2016

List of Equipment to be Installed

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	Screening Room Cell Based
UV-Vis Spectrophotometer	JASCO	Screening Room Enzym Based
Ultrasonic Crusher(DIGITAL)	Branson	Genetic Room
96-well Microtiter Plate Reader	Molecular Device	Screening Room Enzym Based
Ultracentrifuge	HITACHI	Screening Room Enzym Based
Rotor for Ultracentrifuge	HITACHI	Screening Room Enzym Based
HPLC (PDA Detector) (2)	Shimadzu	Purification Room
Incubator	ASTEC	Screening Room Cell Based
HPLC-Column (2 sets)	SHISEIDO	Purification Room
Incubator	ASTEC	Screening Room Cell Based
Flask Plate for Rotary Shaker	IWASHIYA BIO SCIENCE	Cultivation Room
High Speed Refrigerated Centrifuge	TOMY	Purification Room
Rotor	TOMY	Screening Room Cell Based
High Speed Refrigerated Centrifuge	TOMY	Screening Room Cell Based or Enzym Based
Resin and Gel for Chromatography		Purification Room
Electric Pipette 12 channel (3 sets)	Mettler Toledo	Screening Room Cell Based
Multichannel Pipette (8)	Nichiryo	Purification Room
Ergonomic pipette (10)	Nichiryo	Purification Room
Glass column		Purification Room
Ultrasonic Cleaner	AS ONE	Purification Room
Liquid Nitrogen Tank 30L	CEBELL	Screening Room Enzym Based
Biomedical Freezer (513Lt)	Nihon Freezer	Screening Room Cell Based or Purification Room
Glasswares		Purification Room
Analytical Balances	Shimadzu	Purification Room
Agarose Gel Electrophoresis	Atto	Genetic Room
Fraction Collector	BIO RAD	Cold Room
EGP Combo	BIO RAD	Cold Room

Budget Arrangement for 2016

- BPPT allocated budget for FY 2016 as much as Rp. 450.000.000
- Airlangga University allocated budged for FY 2016 as much as approx. Rp.450.000.000

Training Schedule (tentative)

Name	Training venue	Training title	Tentative time	Tentative length of stay
Tarwadi	NIID	Cell-based screening for anti-amebic activity	July 2016	1 month
VIP visit (2 persons)	(tentative)	VIP visit	August 2016	1 week
Anis H Mahsunah	Kitasato University	Structure elucidation of active compound	September 2016	1 month
Eka Siska	Kitasato University	Screening and purification of anti-malarial active compound	September 2016	1 month
Diana Dewi	Kitasato University	Scaling-up cultivation of prospective microbes	September 2016	1 month
Anis H Mahsunah	Kitasato University	Structure elucidation of active compound	November 2016	1 month
Nurlaila	Kitasato University	Assay and purification of anti-amebic active compound	November 2016	1 month
Danang Waluyo	NIID	Cell-based screening for anti-amebic activity	November 2016	1.5 month
Erwahyuni E Prabandari	NIID	Enzyme-based screening for anti-amebic activity	November 2016	1.5 month

Future Agenda

- **Field exploration**
  → Will be held in May 2016 in Biak, Papua

- **Regular managerial meeting in BPPT**
  → Will be held monthly

- **Regular coordination meeting with local counterparts**
  → Will be held quarterly

- **Implementation Arrangement**
  → Will be signed soon (currently is under reviewed by legal division of BPPT)

- **Work Breakdown Structure (WBS)**
  → Will be defined soon by involving partner's institution

THANK YOU

BADAN PENGKAJIAN DAN PENERAPAN TEKNOLOGI

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

Project Overview

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

SATREPS SLeCAMA Project

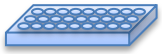
TARWADI
Project Co-manager

VIP Meeting Room, BPPT, Jakarta
February 2nd, 2016

Contents

Background

Objective and Output



Scope

Project Roadmap

Schedule

Progress 2015

Closing

Indonesia as a Mega-Bio-diversities Country

Countries with Mega-Bio-diversity

Sumber: Wikipedia

Indonesia is one of “mega-bio-diversity” country

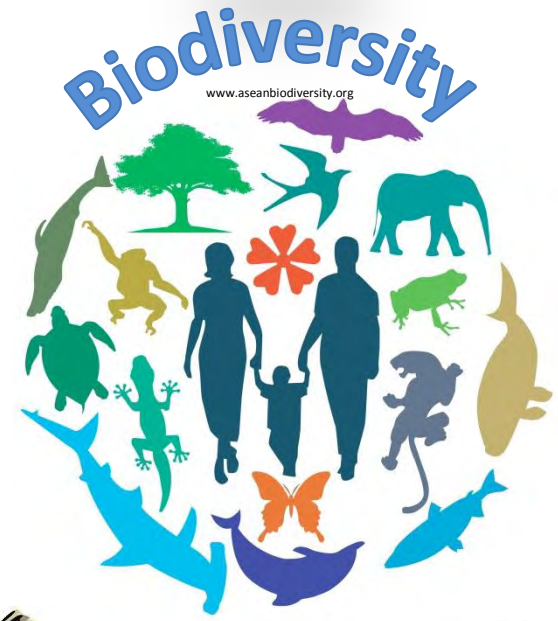
→ The importance of management and utilization of biological resources for human welfare according to Convention on Bio-resources (Nagoya Protocol 2011)

Biodiversity for Human Welfare

Support food security

Reducing the risk of disaster

As a sourse of health research



Important for adaptation of climate change

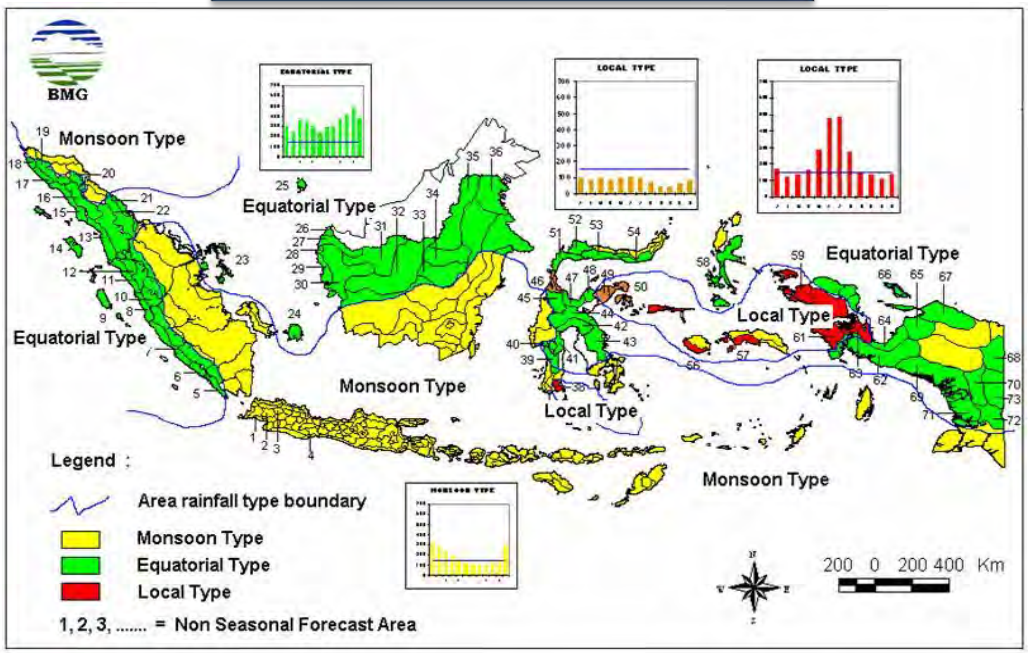
As a source of traditional and modern medicine

Has social, cultural and spiritual significance in society

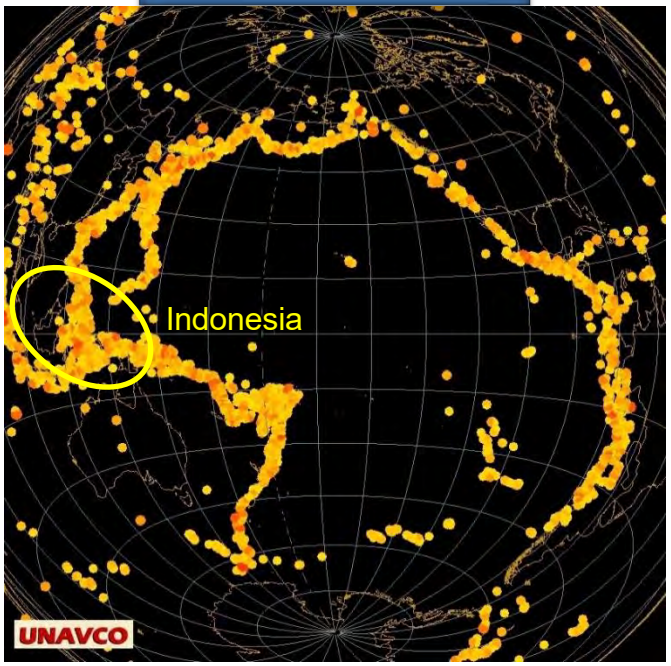
Important in the regulation and management of infectious diseases

Indonesia: Unique Place for Plants and Microorganism

Type of rainfall in Indonesia



"World Fire Ring"



The diversity of rainfall type, tropical climate, volcanic soil, geographic location are indicated that Indonesian bio-resources has high diversity

→Exploration and utilization of bio-resources become very important

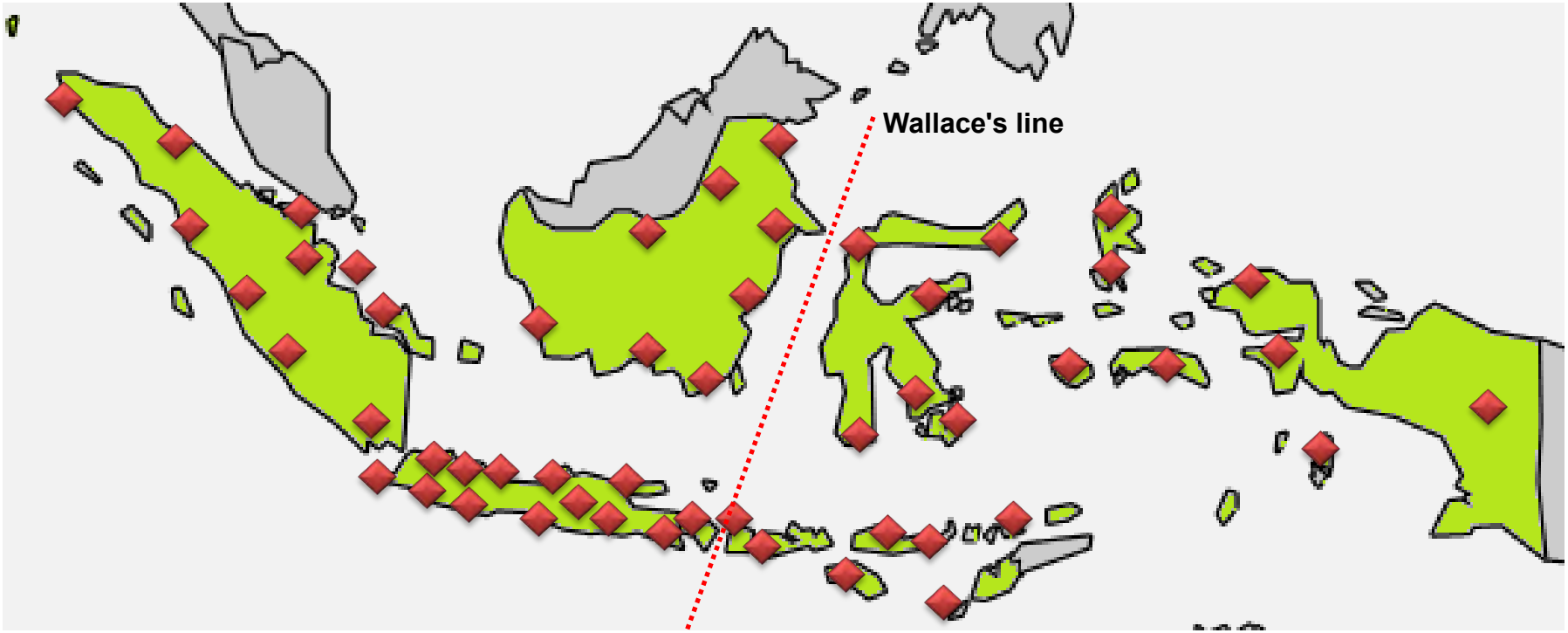
Exploration of Microorganism in Indonesia

Kind of sample : **Soil, insects, plants, marine life**

Target : **Fungi and Actinomycetes**

Location of sampling : **21 islands, 62 locations**

Location of sampling



Collection of Indonesian Microorganism at BPPT

- Isolation of Microbe (total: **23653** isolates)
  - Fungi 11383 isolates
    - Insects 1423 isolates
    - Plants 2279 isolates
    - Soil 7272 isolates
    - Marine sources 357 isolates
    - Litter 52 isolates
  - Actinomycetes 12270 isolates
    - From Insect 1389 isolates
    - From Plants 1313 isolates
    - From Soil 8332 isolates
    - From Marine sources 232 isolates
    - From Litter 1004 isolates
- Genetic analysis (total: **6681** isolates, 28.2% from total collection)
  - Fungi (28rDNA) 3181 isolates (with similarity >99%)
  - Actino (16rDNA) 3500 isolates (with similarity >98%)

Collaboration

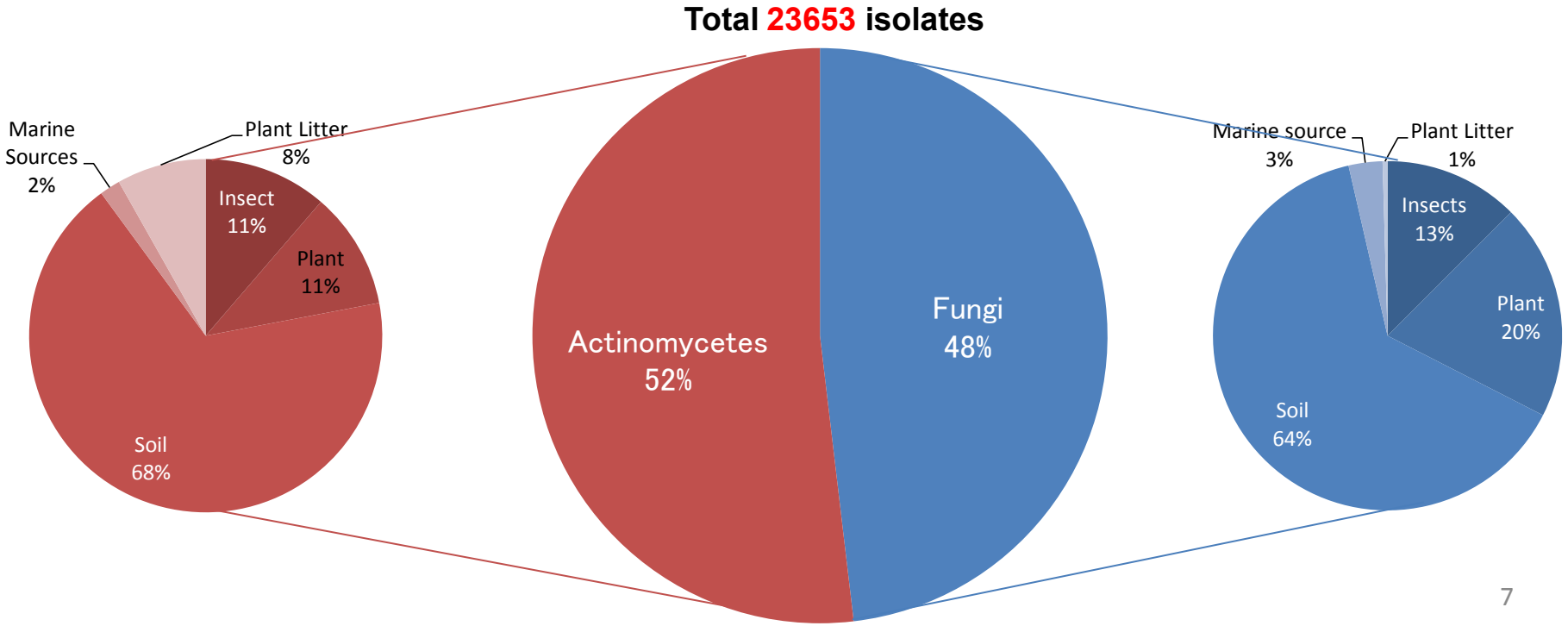
BADAN PENGKAJIAN DAN PENERAPAN TEKNOLOGI

MicroBiopharm Japan

(2004-2015)

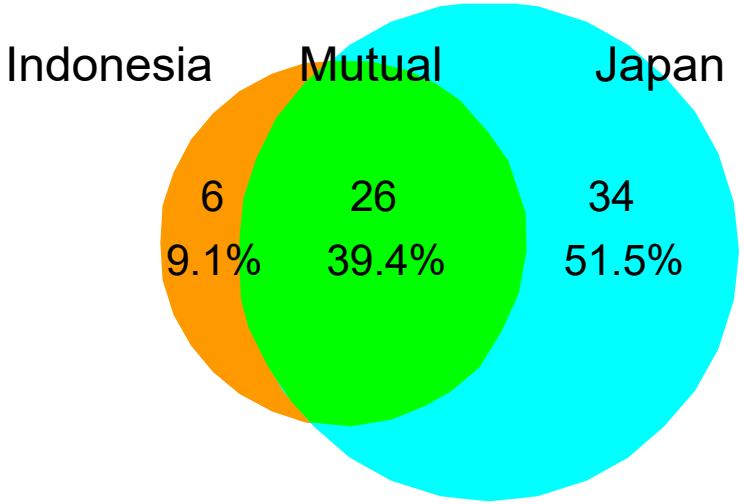
→the biggest microorganism collection in Indonesia

(fungi and actinomycetes)

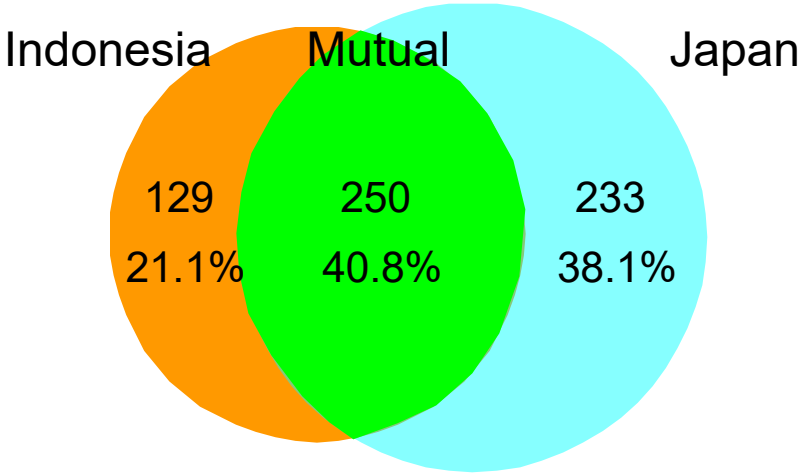


Diversity of Indonesian Actinomycetes

	Indonesia	Both	Japan	Total
Genus	6 9.1%	26 39.4%	34 51.5%	66
Species	129 21.1%	250 40.8%	233 38.1%	612
Strain	1,999		3,389	



Genus



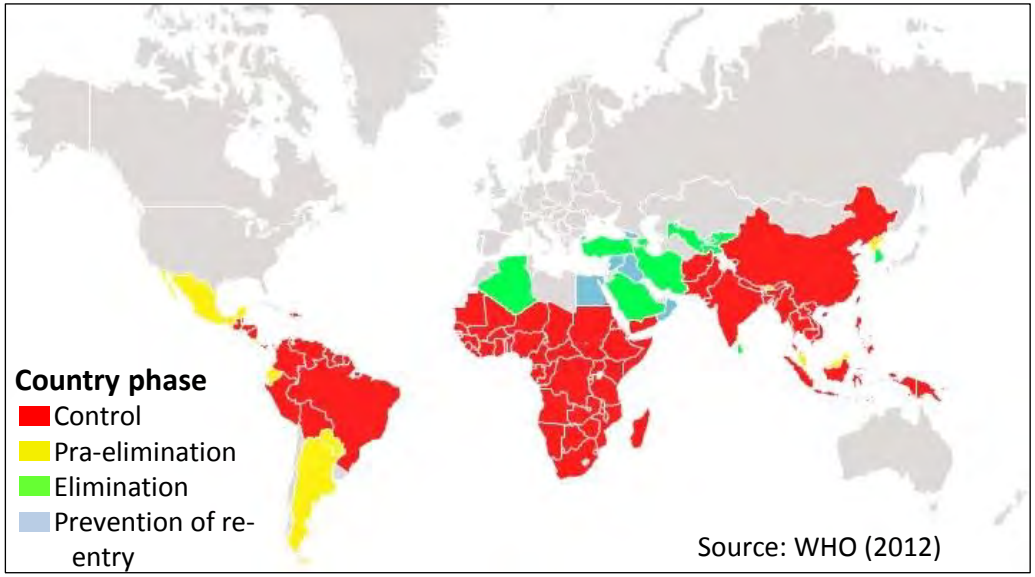
Species

Sugimoto et.al. (JSBBA, 2011)

More than 21% isolated species are found in Indonesia only
→Diversity of Indonesian Actinomycetes are different from Japan

Malaria

Classification of Malarial Countries

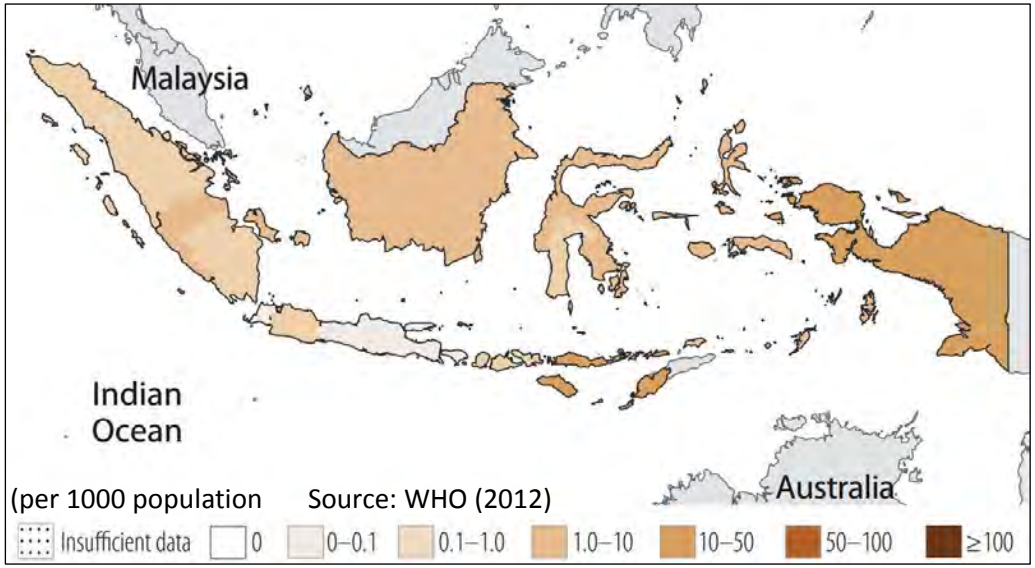


Status of Malaria Global

- Endemic area: 99 countries and 5 regions (2012), mostly at the tropical countries
- 3.3 billions population are at risk for infected malaria (2011)
- Area with high risk: Sub-Saharan Africa (80% cases and 90% death)
- Main patient: children <under 5 year old> and pregnant women

(Source: World Malaria Report, WHO, 2012)

Distribution of the confirmed malaria cases in Indonesia



New Cases of Malaria in Indonesia

- New Cases in Indonesia=22.9 pmp (per million population)
- New cases outside Java-Bali Island=45.2 pmp
- New cases at Java-Bali Island=7,6 pmp
- The highest cases: Papua (261.5 pmp), West Papua (253.4 pmp)

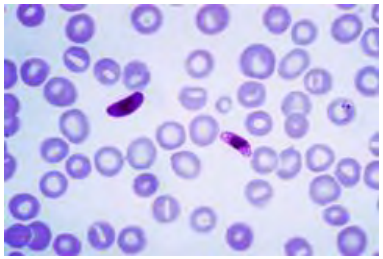
(Source: Riskesdas (Basic Health Research) , MoH, 2010)

Malaria remains as a threat in Indonesia

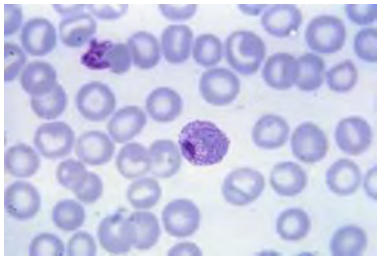
Malaria

Malaria is caused by parasites of the genus Plasmodium, infects humans through the bite of an infected female mosquito (> 30 Anopheles species)

- Species of main Plasmodium in Indonesia: *P. falciparum* (86.4%), *P. vivax* (6.9%)
- Species of main Anopheles in Indonesia: *A. sundaicus, balabacensis, maculatus, farauti, subpictus*



P. falciparum



P. vivax

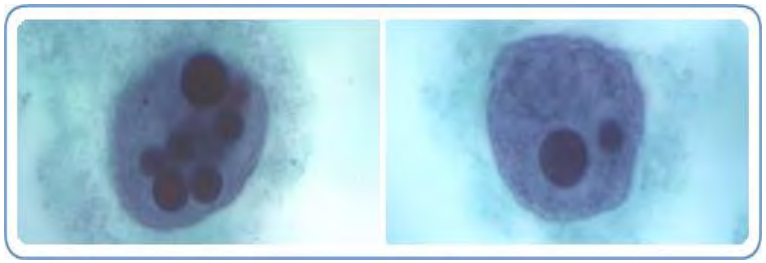
A. sundaicus

WHO recommended Malaria treatment is using ACTs (*artemisinin-based combination therapies*) for *P.falciparum* and chloroquine for *P.vivax*.

- *Artemisia annua*, the plant which produce artemisinin, general difficult to be cultivated in tropical area
- Only 33,7% malarial patients in Indonesia receiving treatment ACTs
- 15% malarial patients in Indonesia who are not received ACTs, they use traditional medicines
- The patient who resistant to artemisinins found in Cambodia, Vietnam, Thailand, and Myanmar

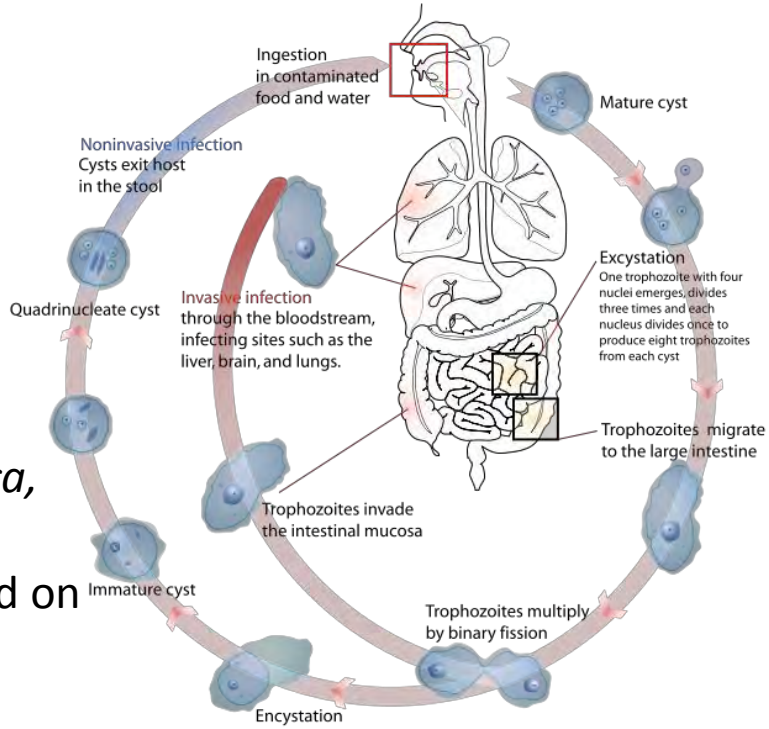
Development of new anti-malarial medicine is very important

Amebiasis



Entamoeba histolytica

(Source: Centers for Disease Control and Prevention)



Life cycle of *E.histolytica*

- Cause of disease: parasite *Entamoeba histolytica, suffered by* 40% of diarrhea patient
- Prevalence amebiasis in Indonesia: 1-14% based on fesses diagnostic, or 1.6-34% base on serology diagnostic in the bad sanitation
- Amebiasis global: CFR 1.9-9.1% (Aristibazal et.al., 1991), 40-50 million cases are die every year → the second killer parasite after plasmodium

- Drug : metronidazole, diiodohydroxyquinoline (iodoquinol), diloxanide, emetine, nitazoxanide, ornidazole, paromomycin, secnidazole, and tinidazole
  → Case resistant to these drugs has emerged

Development of new anti-amebic drug is very important

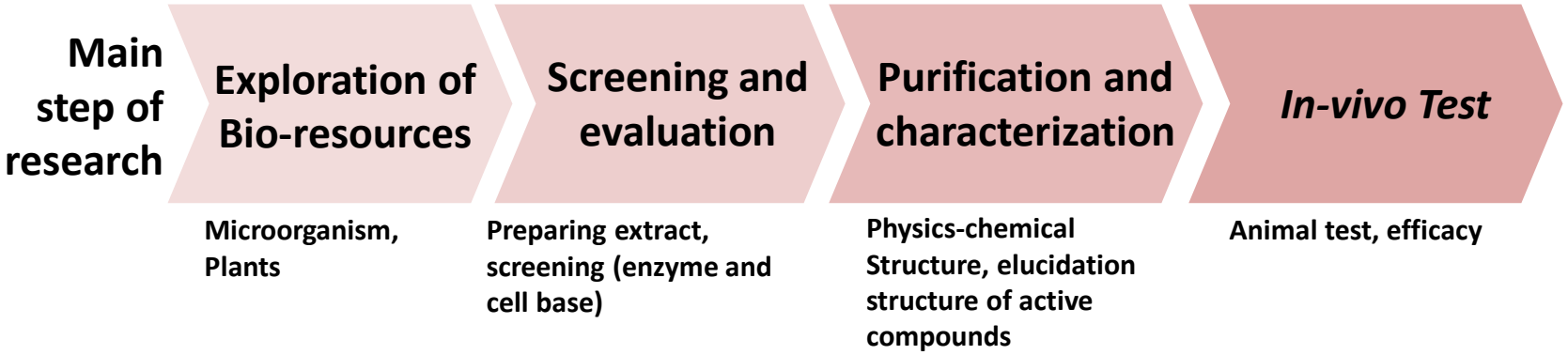
Objective

To conduct screening of **Indonesian Biological resources** (microorganism and plants) to develop **drug candidate lead compounds** for **anti-malarial** and **anti-amebic**

Output

- **Biological Resources Referral (BANK DATA)** of microbial and medicinal plants potential for the development of health products
- **EVALUATION SYSTEM** of Biological resources that have anti-amoebic and anti-malarial activity
- **PROTOTYPE OF DRUG CANDIDATES** for anti-malarial and anti-amoebic with high activity
- **Human Resources of BPPT** for developing active compounds drugs from Biological Resources microorganisms and plants

Scope of activity



Target: Drug candidate for
- Antimalaria
- Antiamoeba

Additional Activities:
1. Exploration of biological resources (microorganism, plant medicine)
2. Increased research skills through training by experts
3. Strengthening research collaboration and networking through seminars and workshops
4. Updates research facilities through installation of equipment

Institutions Involved in this Research Collaboration

JAPAN

Coordinator

筑波大学
University of Tsukuba

MEMBERS

東京大学
THE UNIVERSITY OF TOKYO

北里大学
KITASATO UNIVERSITY

MicroBiopharm Japan

INDONESIA

Coordinator

BPPT
BADAN PENGKAJIAN DAN PENERAPAN TEKNOLOGI

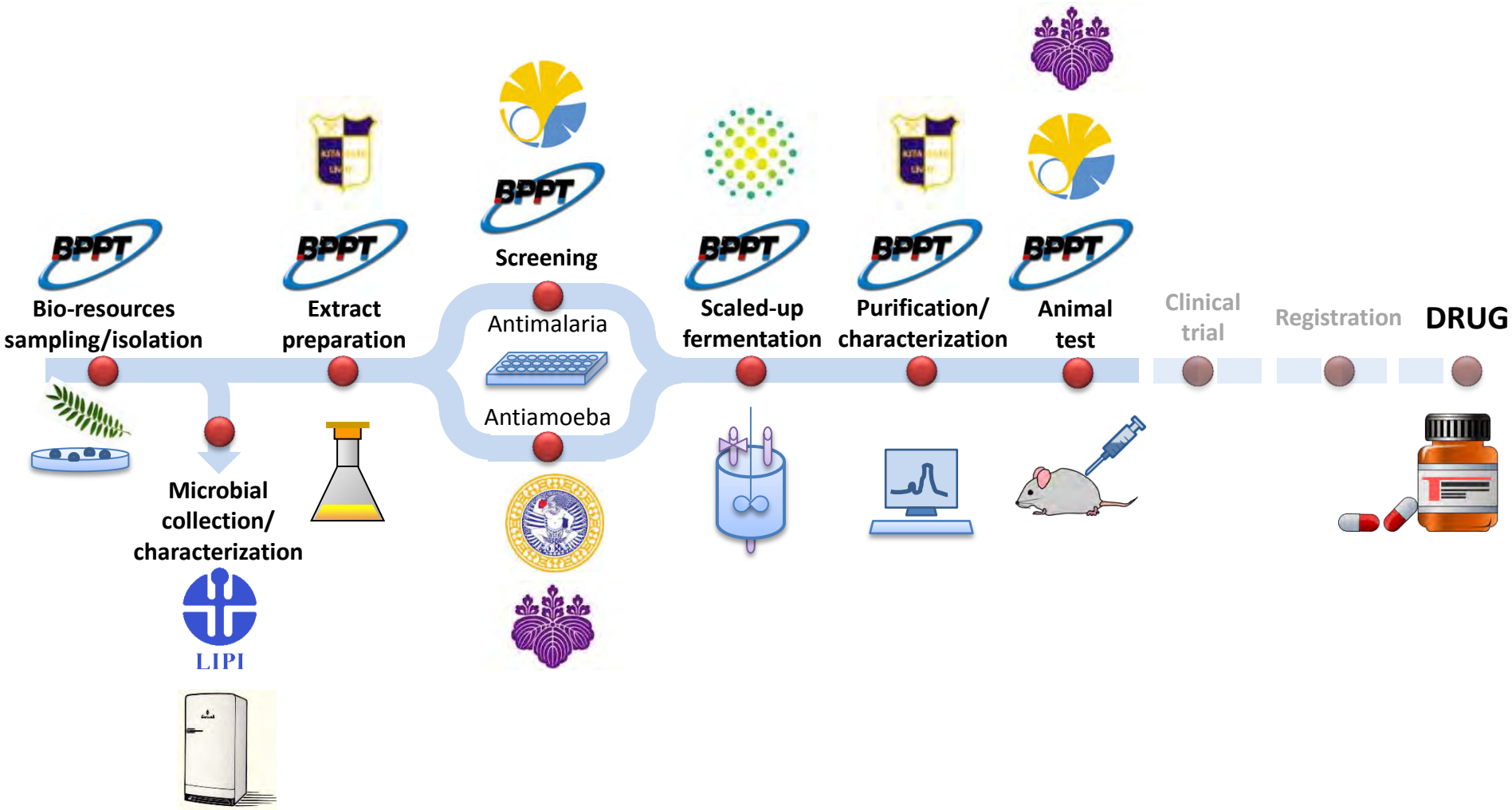
MEMBERS

Institute for Tropical Disease
Airlangga University

LIPI

Research Center for Biology
Indonesian Science Institute (LIPI)

Scope



SATREPS Project 5 yrs
(FY 2015-2019)

Plan of Operation

Activities	2015	2016	2017	2018	2019	Remarks
1. Preparing Facility and Equipment						
a. BSL-2 biocontainment	→					
b. Equipment for bioassay of anti-parasite	→					
c. Equipment for isolation of active compounds	→					
2. Exploration Biological Resources						
a. Screening and Isolation of microbial	→	→	→	→		
b. Screening and Isolation of medicinal plant	→	→	→	→		
3. Preparation of Extract and Screening						
a. Preparation of extract	→	→	→	→		
b. Bioassay	→	→	→	→		
4. Purification and characterization						
a. Isolation of active compounds		→	→	→		
b. Characterization/identification of active compounds			→	→	→	
5. Formulation of Pre-clinical trial				→	→	
6. Training, workshop and seminar						
a. Training on enzymatic assay	→					
b. Training on cultivation of parasite	→					
c. Training on isolation and purification of active compound		→	→			
d. Training on structure analysis	→		→		→	
e. Progress Report Meeting			→			
f. International Seminar on recent developments in anti-parasitic drug development						

Coordination with Partners

Technical coordination with Japanese collaborators for preparing SATREPS Project (July 2014)

Discussion for formulating *Minutes of Meeting between* BPPT-JICA-Japanese and Indonesian Collaborators at BPPT (Oct 2014)

Signing *Minutes of Meeting* by Deputy Chairperson TAB BPPT and team leader of JICA Survey Team (Oct 2014)

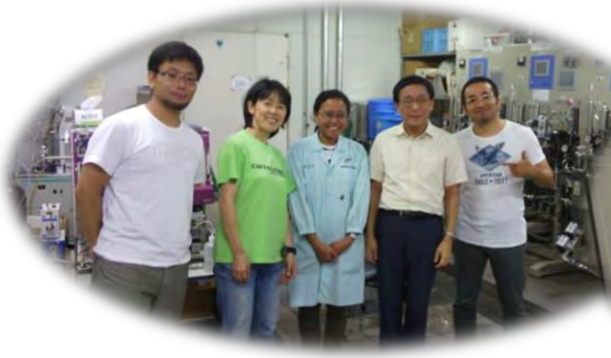
Meeting attendant from BPPT and all partners for formulating Minutes of Meeting (Oct 2014)

Renovation of BSL-2 Laboratory

Before renovation

After renovation

Thank You



"Update on Project Framework"

Searching Lead Compounds of Anti-Malarial and Anti-Amebic Agents by Utilizing Diversity of Indonesian Bio-resources

SLeCAMA Project

February 2nd 2016

Mitsuhiko IWASHITA
JICA Coordinator

Purpose

To get consensus among project members on the present status and plan of operations of SLeCAMA Project in order to realize smooth implementation during coming 2nd year of the project.

Contents

I. Alteration of project implementation structure (1/3)

Re-organization in BPPT 2016

Research function of Biotech Center (BTC-BPPT) was transferred into Center for Pharmaceutical and Medical Technologies(PTFM-BPPT)

- Project Manager : Director, PTFM
- Project Co-Manager: Program Head, PTFM

I. Alteration of project implementation structure (2/3)

Reviewing Project Co-Manager in LIPI

The reason why main implementing institute in LIPI as SLeCAMA Project must be Indonesian Culture Collection (InaCC), Research Center for Biology

- Project Co-Manager: Head, InaCC, LIPI

I. Alteration of project implementation structure (3/3)

Japanese Research Funding Agency

The function for supporting research relating infectious disease of JST (Japan Science and Technology Agency) was transferred to AMED (Japan Agency for Medical Research and Development), the role of AMED is manly to assess the project from scientific view points.

- An observer of JCC: AMED instead of JST

II. Sum Up of #1 JCC Meeting

A. Progress Review

1. More than 500 microbes were isolated from collected samples in Maluku
2. Approximately 800 extracts have been prepared for screening
3. Approximately 5,000 extracts have been screened for both antimalarial and anti amebic activities
4. Two (2) compound with anti-malarial activities have been purified and structurally elucidated

II. Sum Up of #1 JCC Meeting

A. Progress Review

5. Japanese Researchers

Twelve (12) turns of short term Japanese researchers and a long term JICA expert were dispatched to SLeCAMA Project.

6. Trainings in Japanese institutes

Total 11 short term trainings were implemented in NIID, KU and U.Tokyo since project start

II. Sum Up of #1 JCC Meeting

A. Progress Review

7. Allocation of budget

- BPPT and AU prepared budget for operational cost of SLeCAMA Project around 450 million rupiah each for 2016.
- Disbursement of UT supported by JICA in JFY2015 is around 11 billion rupiah

8. Coordination meetings among Indonesian institutes

- Members among AU, LIPI and BPPT agreed to have coordination meeting quarterly.

9. Preparation of required equipment

- 43 items of required equipment were procured in Japan, however due to the complicated process of importation in Indonesia, it have delayed to install in the laboratories.

II. Sum Up of #1 JCC Meeting

B. Tentative plan for project implementation in 2016

Base on the plan to have screening 5,000 extracts annually in PDM, the following activities were set up.

1. Microbes and extract preparation

Prepare > 5,000 extracts, and isolate >500 microbes

2. Enzyme-based Screening

Prepare target enzymes and screen >5,000 for inhibition activity

3. Cell-based Screening

Maintain parasite, Plasmodium falciparum 3D7 and Entamoeba histolytica HM-1:IMSS clone 6, and to maitain cell line DLD-1.

Screen 5,000 extracts for antimalarial activity,

4. Purification

Purify > 6 extracts (antimalarial: 3, antiamoeba :3)

II. Sum Up of #1 JCC Meeting

B. Tentative plan for project implementation in 2016

5. Regular Managerial Meeting

- Monthly meeting chaired by Project Manager with 4 working teams and Japanese experts

6. Coordination Meeting

- Quarterly coordination meeting among AU. LIPI and BPPT

7. Training in Japan in 2nd year

- Total 10 short term trainings are planned in NIID, KU and U.Tokyo in JFY 2016
- A researcher of ITD-AU will participate in Postgraduate degree course in T.U.

8. Field exploration in Biak

- A field exploration to Biak island is planned in 2016

II. Sum Up of #1 JCC Meeting

B. Tentative plan for project implementation in 2016

9. Japanese Researchers

- Around 13 Japanese researchers are planned to be dispatched to Indonesia

10. Laboratory equipment

- All equipment procured in Japan in 2015 should be installed in BPPT and AU urgently. To catch up the plan of implementation

11. Implementation Arrangement

- To clarify research cooperation scheme between Indonesia and Japan, BPPT and UT will sign on the “Implementation Arrangement” as soon as possible.

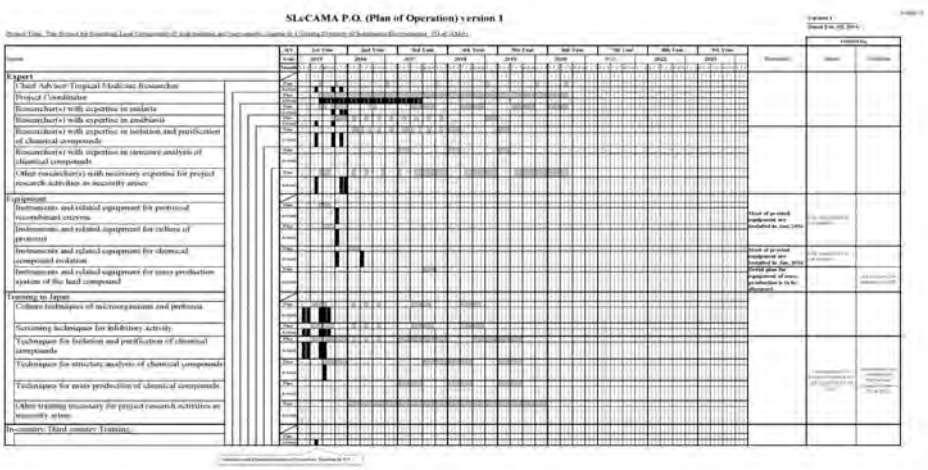
III. Others

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

III. Others

2. Plan of Operation (P.O.) version 1.



III. Others

3. Amendments to the Record of Discussions (R/D) signed on 17th February 2015

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

I. Alteration of project implementation structure (BPPT, LIPI and Japanese institute)
II. Sum up of #1 JCC Meeting
III. Others

Budget Limitation
Plan of Operation Version 1.
Amendments to Record of Discussion

↓

draft of Minutes of Meeting

SLeCAMA Project

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  C/O. Biotech Center-BPPT, #630 Building Puspiptek, Serpong
- jicaslecama@gmail.com
- 021-7560155

Science and Technology Research Partnership for Sustainable Development (SATREPS)
(AMED/JICA 2014-2020)

Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bio-resources

Tomo NOZAKI
University of Tsukuba

Kick-off Ceremony, BPPT, Jakarta, Feb 2, 2016

Background

Parasitic Diseases＝Global issues

Malaria

- ✓ 104 countries, 3.4 billion risk of infection
- ✓ 210 million infected and 627 thousands deaths annually
- ✓ The most important protozoan cause of mortality

✓ **No vaccine**
(RTS,S/AS01, GSK, phase 3)

- ✓ Artemisinin combined therapy (ACT) for *P. falciparum*

✓ **Drug resistance**

- ✓ Artemisinin-resistant *P. falciparum* in at least 4 Asian countries
  - Cambodia, Myanmar, Thailand, Vietnam
  - In 2010, 27% of cases were resistant to dihydroartemisinin/piperaquine in Cambodia
- ✓ Chloroquine-resistant *P. vivax* in 23 countries

Amebiasis

✓50 million infected and 100 thousand deaths annually

✓Deadly protozoan infection only after malaria

✓No vaccine

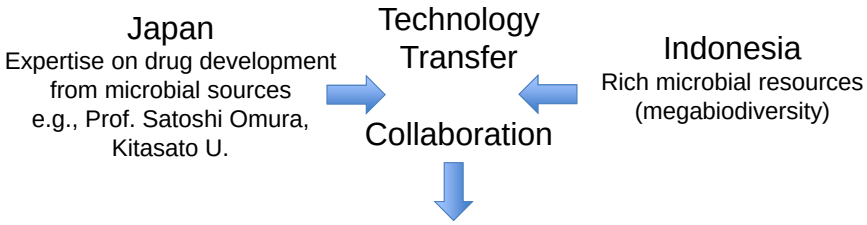
✓Metronidazole (MTZ) is the only available drug

✓MTZ is teratogenic/carcinogenic

✓Resistance reported for *Giardia, Trichomonas*, and *Helicobacter pylori*, which share anaerobic metabolism

2

Objective of the project



A new platform for drug development

Aims

- Screen Indonesian microbial extracts to identify active compounds that inhibits enzymes and growth of *P. falciparum* and *E. histolytica.*
- Purify the active compounds and determine their structures

Members/PIs+CoPIs

Japan

Univ Tokyo/Nagasaki
Inaoka
Screening of antimalarial compounds

U Tsukuba
Nozaki
Screening of antiamebic compounds

Kitasato U
Shiomi
Purification/Structure

MicroBiopharm Japan
Watanabe
Library/Archiving

Collaboration

Human development
Capacity building

Indonesia

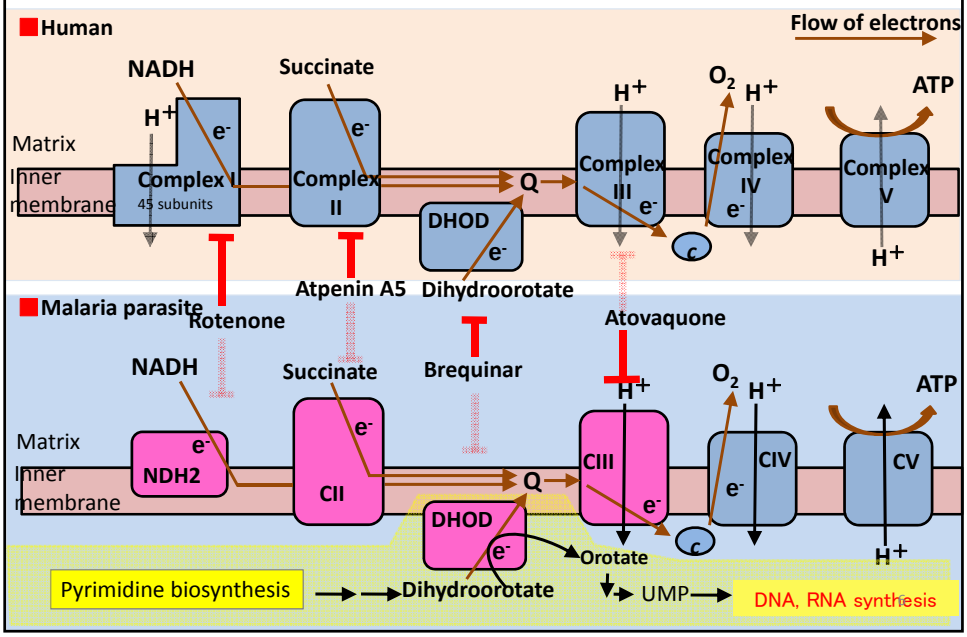
Center for Pharmaceutucal and Medical Technologies (PTFM)
Former Biotech Center, BPPT

Institute of Tropical Disease
Airlangga Univ

Indonesian Culture Collection (InaCC)-Biol Res Cntr, Indonesian Institute of Sciences, LIPI

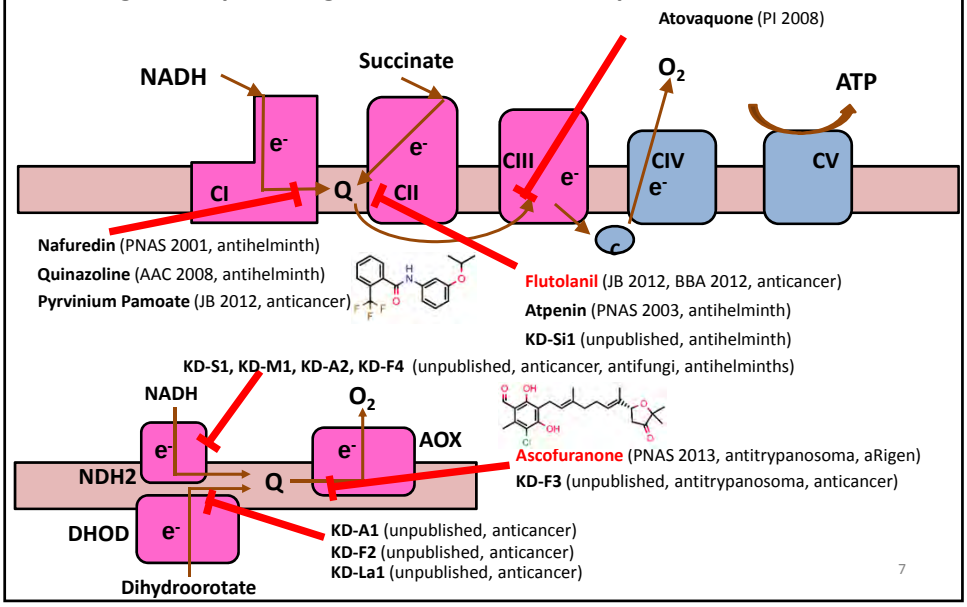
5

Drug target for antimalarials: Mitochondrial respiration



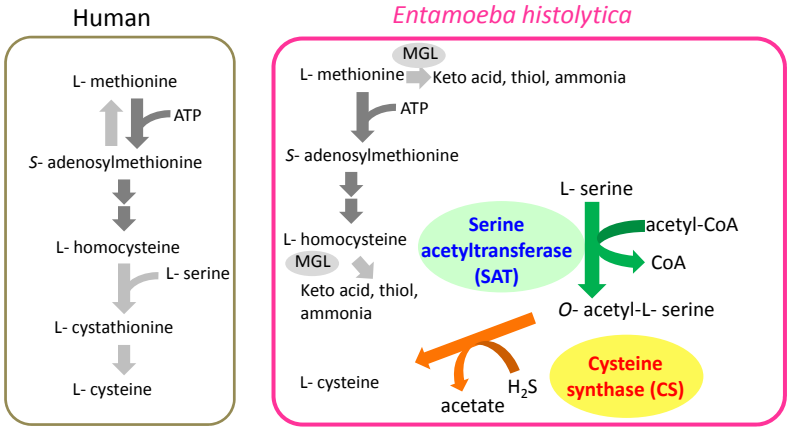
Accomplishment of Univ Tokyo

Drug development against mitochondrial respiration



7

Drug target for anti-amebiasis compounds: Cysteine biosynthesis



- ✓ 6,400 fungi and 5,900 actinomycetes screened
- ✓ 330 and190 extracts inhibited SAT and CS activity
- ✓ 5 compounds purified; structure determined

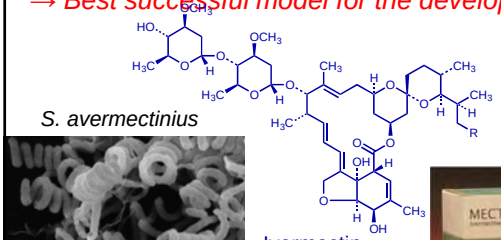
e.g., Nozaki J Biol Chem 1999; Ali and Nozaki Adv Parasitol 2005; Mori Front Microbiol 2015

8

Accomplishment of Kitasato University
Ivermectin

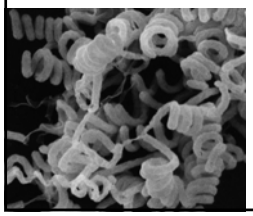
- Prof. Satoshi Omura was awarded for Nobel Prize in 2015
- Discovered and produced in collaboration of Merck and Kitasato
- Avermectin purified from *Streptomyces avermectinius*
- Agonist for glutamatergic chloride channel, absent in mammals
- No.1 sale in >20 years for anti-nematodes for animals
- Donation for elimination of oncocerciasis and lymphatic filariasis through WHO
- 220 million people treated in 2012

→ *Best successful model for the development of drugs for neglected diseases*



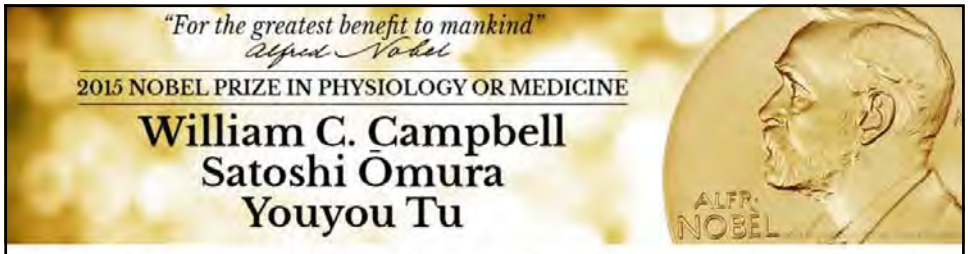
Ivermectin

S. avermectinius



Oncocerciasis

Lymphatic filariasis



The Nobel Prize in Physiology or Medicine 2015 was awarded with one half jointly to William C. Campbell and Satoshi Ōmura for their discoveries concerning a novel therapy against infections caused by roundworm parasites and the other half to Youyou Tu for her discoveries concerning a novel therapy against Malaria.

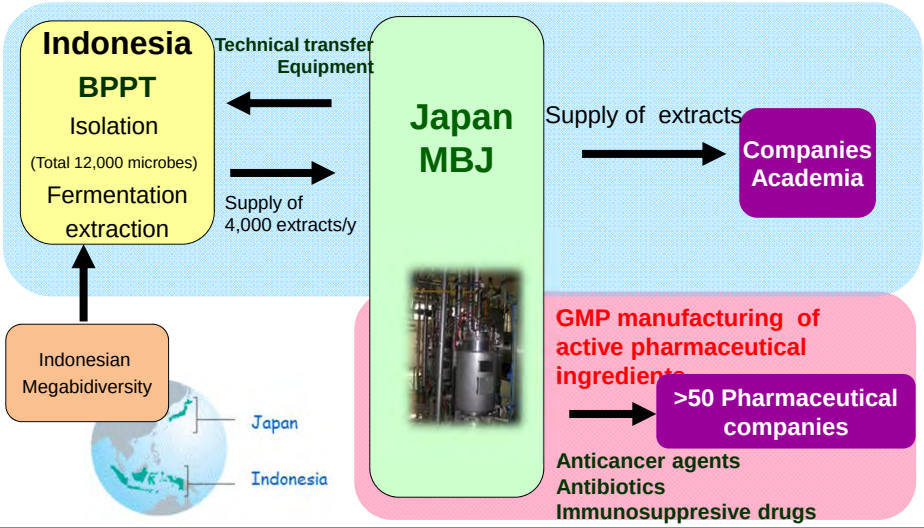
Ill. N. Elmehed. © Nobel Media AB 2015.
William C. Campbell

Ill. N. Elmehed. © Nobel Media AB 2015.
Satoshi Ōmura

Ill. N. Elmehed. © Nobel Media AB 2015.
Youyou Tu

http://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/index.html

MicroBiopharm Japan (MBJ)
Experience in Fermentation



Achievements of MicroBiopharm Japan (MBJ)
Utiliztion of Indonesian Bioresources

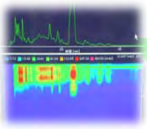
- ✓ Fermentation business for >70 years
- ✓ Upgrading to commercial scale (max.100KL tanks and downstream facilities)
- ✓ Compliance with cGMP (inspected by FDA/TGA)
- ✓ Strain improvement/Process optimization

12

Expected outcomes

Japan

Utilization of abundant Indonesian bioresources for future drug development



Extend to other diseases

Development of Science and Technology

Medicine-driven development of Japan

Indonesia

Establishment of knowledge and infrastructure needed for lead discovery against infectious diseases

Domestic production of drugs against Infectious diseases

Utilization of its own bioresources for biotechnology

Masterplan for Acceleration and Acceleration of Indonesia Economic Development 2011–2025 (MP3EI)

13

BPPT

JICA
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

1. **Target Review and Research Flowchart**

2. **Progress 2016**
   a. Field exploration
   b. Microbes Isolation and Extract Preparation
   c. Screening of Active Extract
   d. Purification of Active Compound
   e. Technical Support

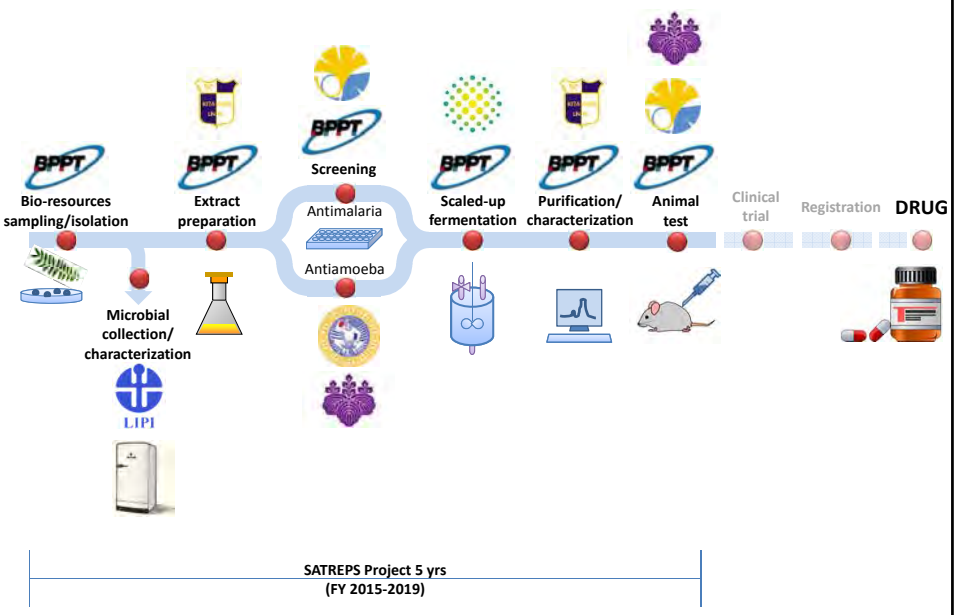
3. **Planning 2017**
   a. Research Activities
   b. Training and Technical Support
   c. Budget Arrangement
   d. Project Management

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

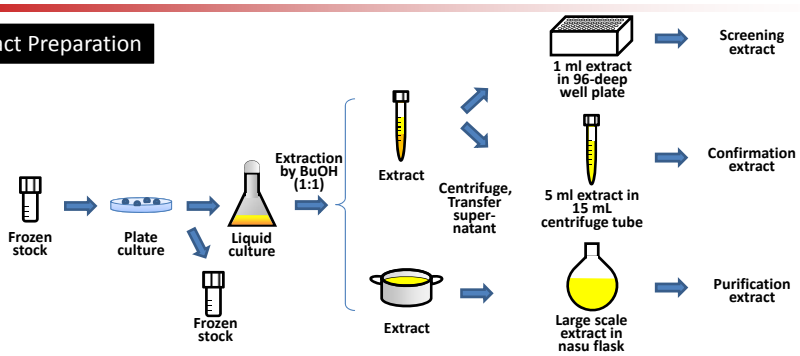
Type of Microbes	Old collection (<2015)	Ambon collection (2015)	Biak collection (2016)	Total
Actinomycetes	11,266	500	405	12,221
Fungi	12,335	401	478	13,214
Total	23,601	901	883	25,435

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

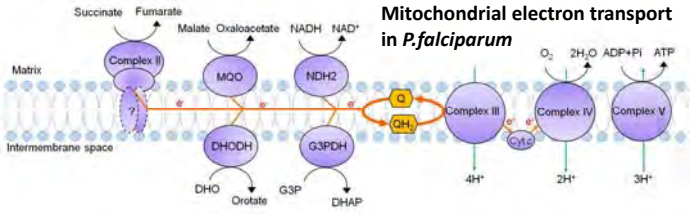
Data as per Dec 30th, 2016

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

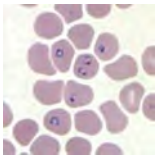


Mitochondrial electron transport in *P.falciparum*

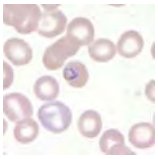
Cell-based screening

Screening target: extracts with inhibitory activity for proliferation of *Plasmodium falciparum*

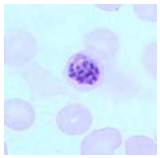
Life-stage of *Plasmodium falciparum*



Ring-form trophozoites



Trophozoites

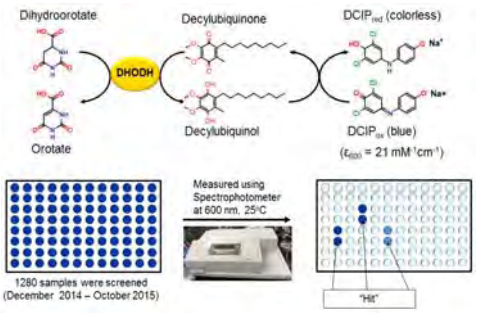


Schizonts

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

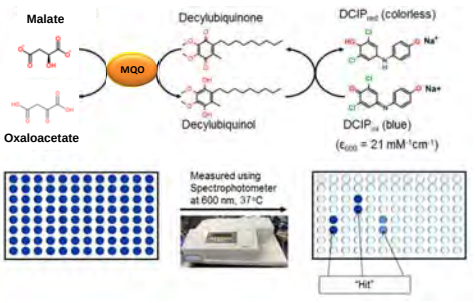
Total number screened extract = **12,519 extracts**

*) 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

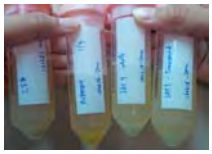
Method

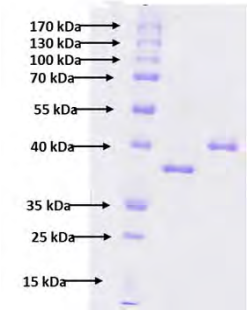
Enzyme	Producer	Cultivation method	Lysis	Purification
CS3	*E.Coli* BL21 (DE3) pET 15b	500 ml 2xYT (in 2L flask), 37°C, 200 rpm, induced by IPTG 200 uM at OD_{600}=0.6. Continue at 20^{0}C, 200 rpm, overnight	Sonication	Ni-NTA column
SAT1	*E.coli* BL21 (DE3) pET 15b	500 ml 2xYT (in 2L flask), 37°C, 200 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	-





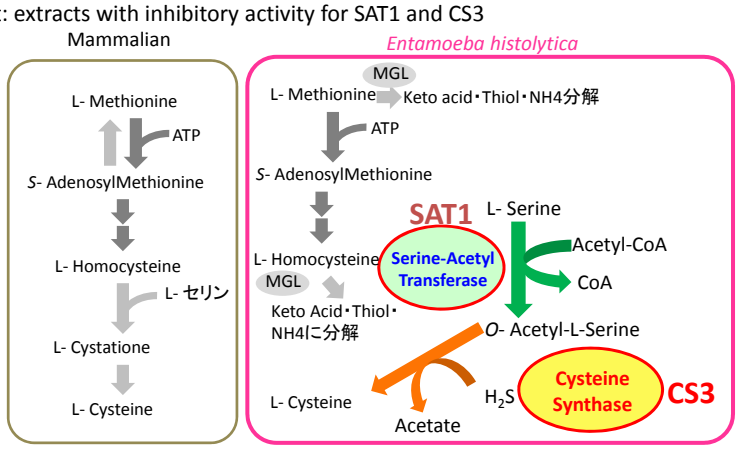
Remarks:
1 : Marker
2 : CS3
3 : SAT1

Progress 2016 Screening of Active Extract Anti-amoeba

Enzyme-based screening

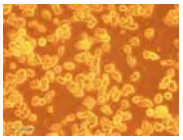
Screening target: extracts with inhibitory activity for SAT1 and CS3

Cysteine biosynthesis pathway



Cell-based screening

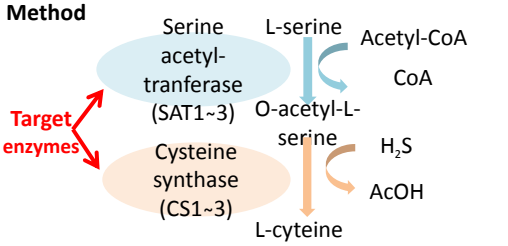
Screening target: extracts with inhibitory activity for proliferation of *Entamoeba histolytica*

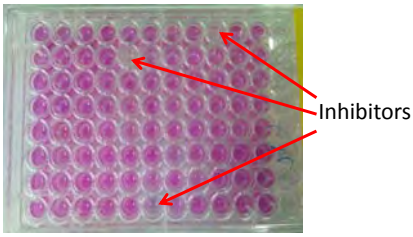


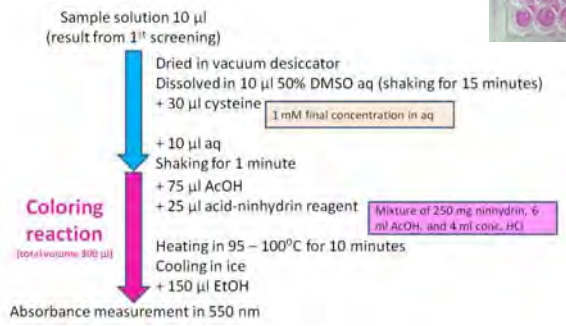
Progress 2016 — Screening of Active Extract — Anti-amoeba

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**

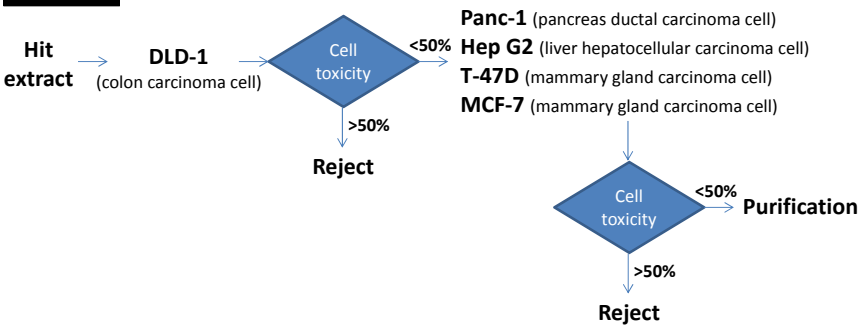
*) Recultured microbes were determined after dose-dependent test and toxicity test against MRC-5

Progress 2016

Screening of Active Extract

Toxicity Assay

Screening **(Cell toxicity test)**



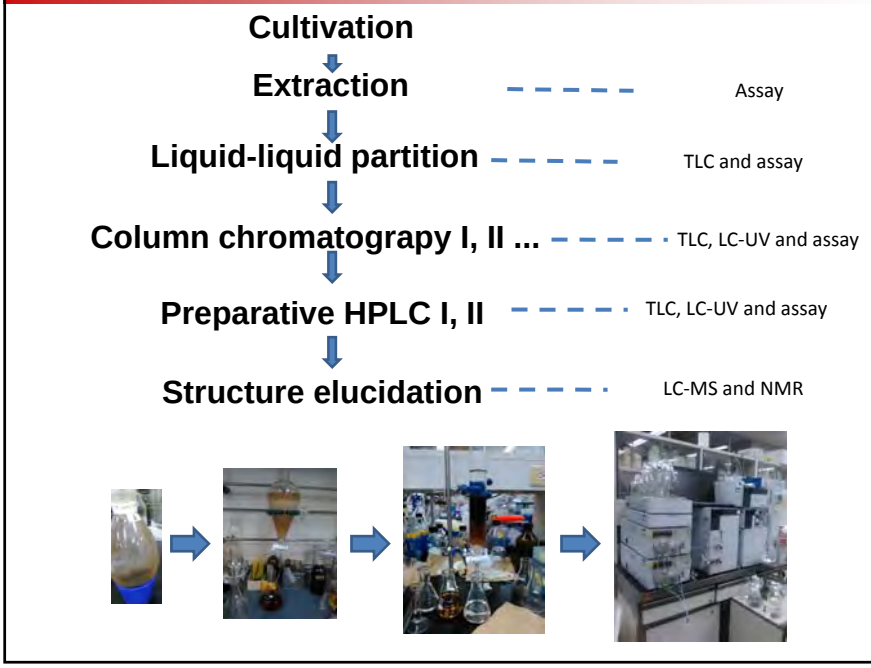
Activity	Number of extracts	Non-toxic agaist DLD-1	Non-toxic against others
Inhibitor of pfDHODH	38	28	24
Inhibitor of pfMQO	55	49	20
Inhibitor of proliferation of *E.histolytica*	32	-*)	4*)

Data as per Dec 30th, 2016

*) Toxicity test were done against MRC-5 cell (fetal lung fibroblast cell)

Progress 2016

Screening of Active Extract



Progress 2016 — Screening of Active Extract

Inhibitory activity	Sample No.	Extraction	Liquid-liquid partition	Open column	Prep. HPLC	LC-MS	NMR	Structure	Remark
Anti-amebic activity									
CS3	SU16-01	(5 L)	→	→	→				
	SU16-02	(5 L)	→	→	→				
	SU16-03	(5 L)	→	→	→	→			
	SU16-04	(5 L)	→	→					
Cell prolifera-tion	SU16-08	(5 L)	→						
	SU16-09	(5 L)	→						Activity was low, reculturing
	SU16-10	(5 L)	→						Activity was low, reculturing
	SU16-11	(5 L)	→						
Anti-malarial activity									
DHODH	SU15-1	(5 L)	→	→	→	→	→	→	Finished
	SU15-2	(5 L)	→	→	→	→	→	→	
	SU16-05	(5 L)	→						Activity was low, reculturing
	SU16-06	(5 L)	→	→	→				Recultured, being purified
	SU16-07	(5 L)	→	→	→	→	→	→	
	SU16-12	(5 L)	→	→					Activity was low, reculturing
	F1(1898A)	(100 mL)	→	→					
	F1(1898B)	(100 mL)	→	→					
	F1(997)	(100 mL)	→	→					
	F15(868)	(100 mL)	→	→					
	F1(2201)	(100 mL)	→	→					
MQO	11 F1	(100 mL)	→	→					
	11 F15	(100 mL)	→	→					
	28 F1	(100 mL)	→	→					
	29 F1	(100 mL)	→	→					
	42 F	(100 mL)	→	→					

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

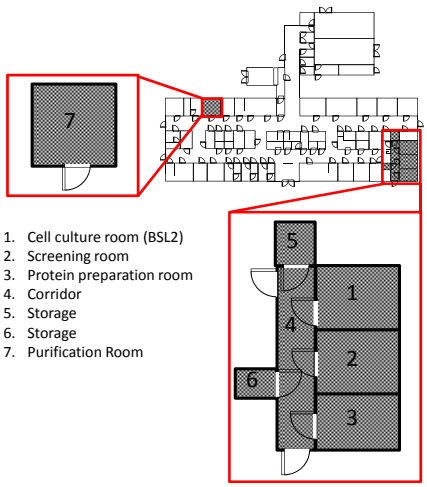
Equipment Installation

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)

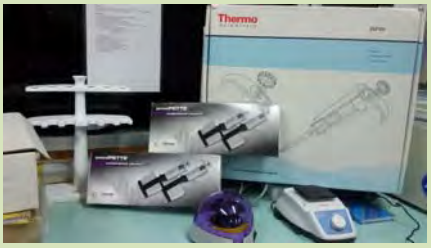
Lab. set up

- 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 ⅡA	AIRTECH JAPAN.LTD	BHC-1007ⅡA2	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

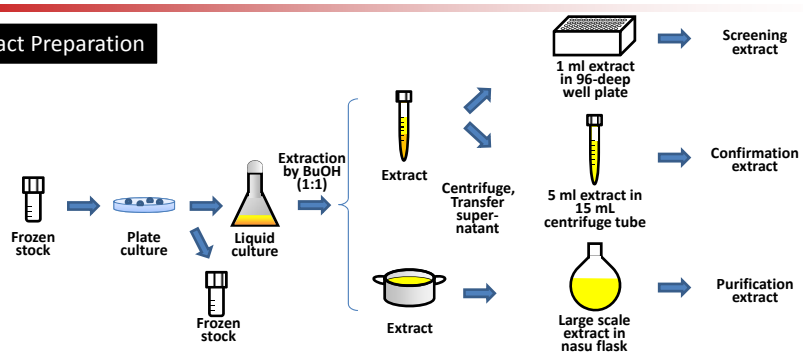
Data as per Dec 30th, 2016

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*)

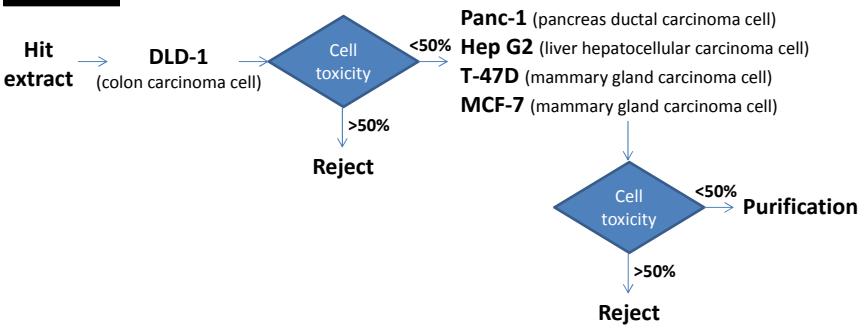
Data as per Dec 30th, 2016

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

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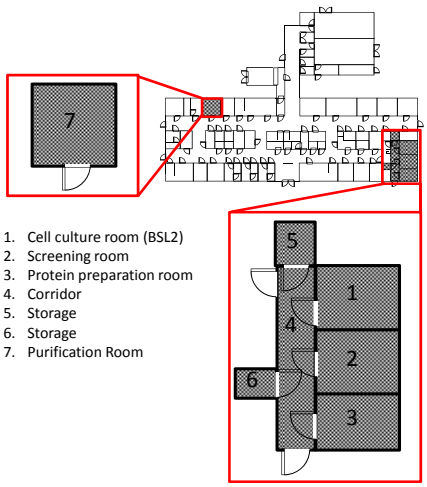
Progress 2016

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

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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]	[illegible]			

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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

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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

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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

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Tentative Budget Plan

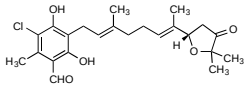
	Tentative Budget Allocation Design (Japanese Side supported by JICA)					
	Approximate data in Japanese Yen					
		2015-2016	2017	2018	2019	total
1	Dispatching Japanese Researchers	19,000,000	16,200,000	15,200,000	13,150,000	63,550,000
2	Acceptance of Indonesian Trainees	25,000,000	15,350,000	15,060,000	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

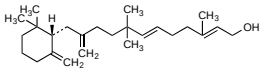
(1) Screening of Kitasato Natural Products Library

(215 compounds, final 50 μg/ml)

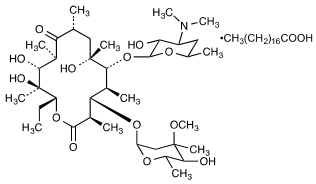
MQO inhibitors: 8 compounds



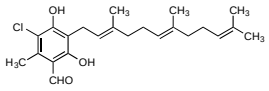
Ascofuranone



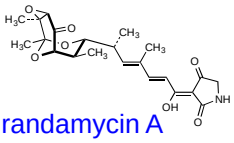
Diumycinol



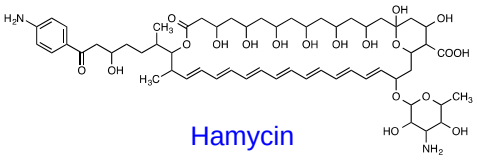
Erythromycin stearate



LLZ-1272a



Tirandamycin A



Hamycin

BA-17039-A (peptide, structure unknown)

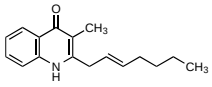
Virantmycin B (structure unknown)

By Mihoko MORI

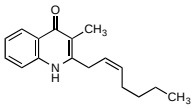
Accomplishment: Discovery of Malaria DHODH inhibitors

(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

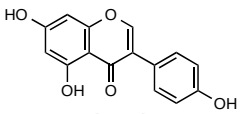
By Mihoko MORI

Problem during screening of Malaria MQO inhibitors

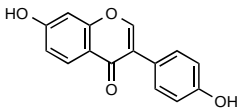
We found the medium contain soy bean meal showed strong PfMQO inhibitory activity.

Methanol extract of soy bean meal has an inhibitory activity.

Well-known bioactive component of soy bean meal is isoflavones



genistein
(no MQO inhibition)

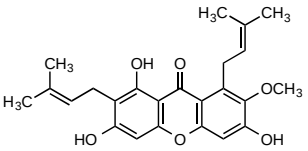


daidzein
(MQO inhibition is unknown)

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

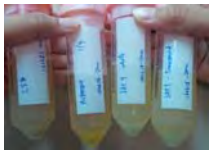
Enzyme preparation

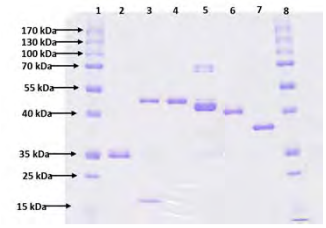
Method

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

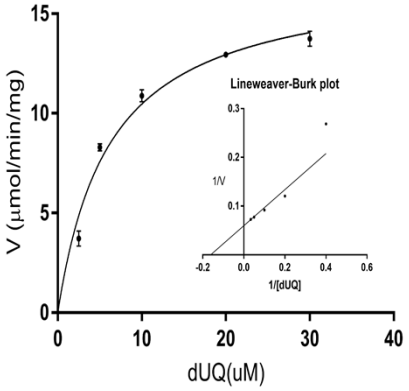


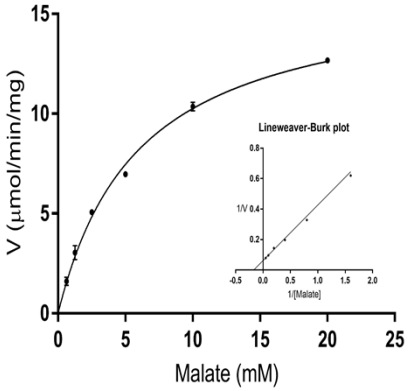


Remarks:
1,8 : Marker
2 : SUMO Protease
3 : PfDHODH + protease
4 : Cleaved PfDHODH
5 : HSDHODH
6 : CS3
7 : SAT1

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

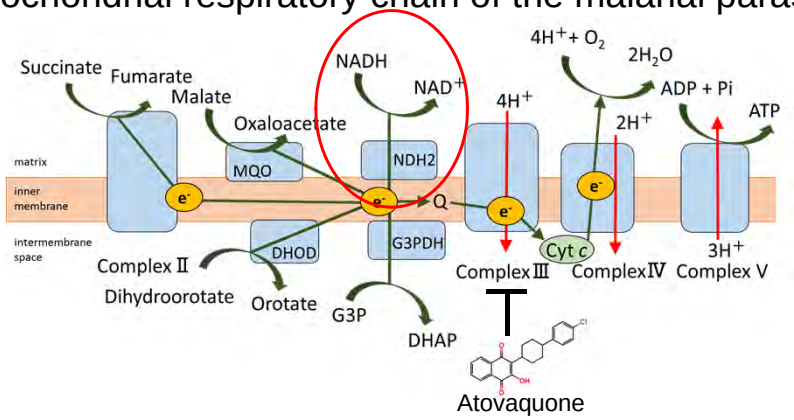
Data obtained from Malate-dependent dUQ reduction activity (dUQ e_{278} = 15 $mM^{-1}cm^{-1}$) and analyzed by GraphPad Prism 7. Assay buffer contained 1.0% ethanol.

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



- 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)

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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)

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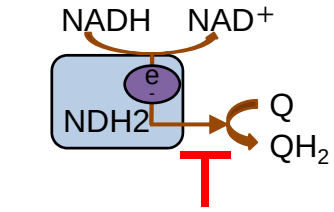
Search for new inhibitors



Respiratory chain inhibitors

Q-site inhibitor library (300 compounds)

1st Screening → Inhibition at 10 μM > 50%

Determination of IC_{50} values

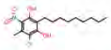
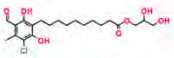
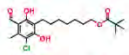
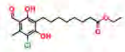
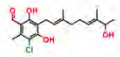
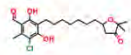
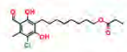
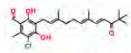
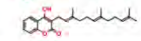
IC_{50} < 1 μM

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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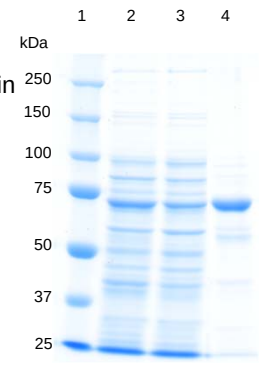
His6-SUMO-PfNDH2 purification

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

42

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

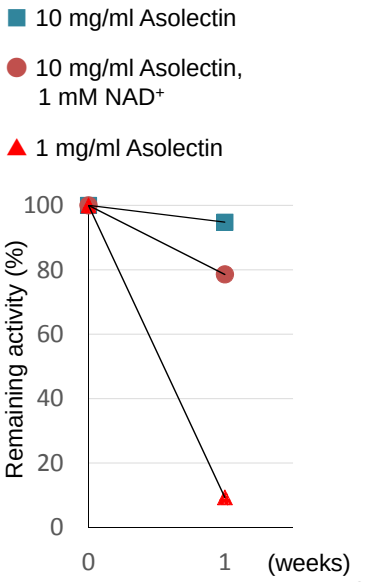
Wash

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.)

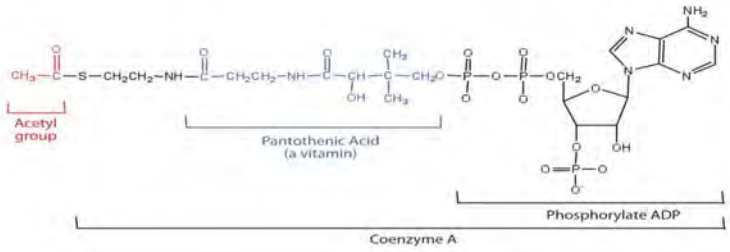
Now purification is progress in Kitasato Univ.

Coenzyme A biosynthesis as new drug target for anti-malaria and anti-amebiasis drugs

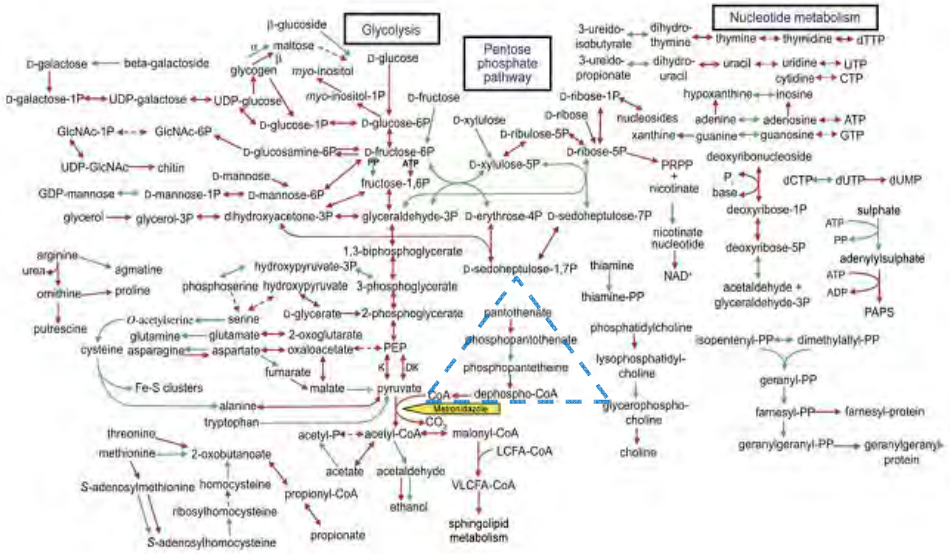
INTRODUCTION

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**

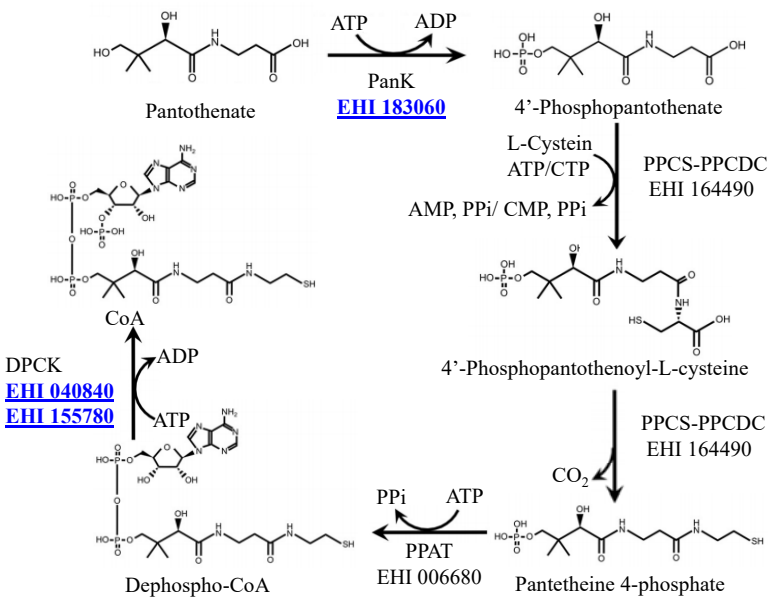


Predicted Metabolism in *E. histolytica*

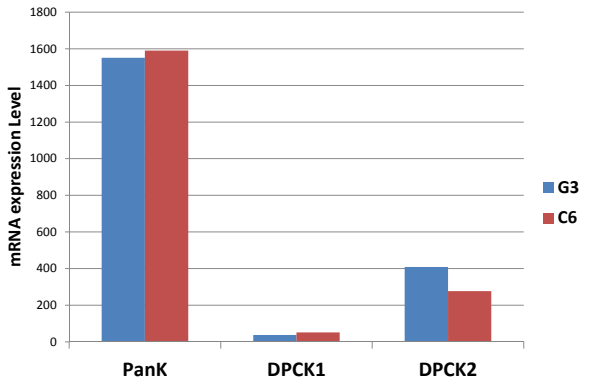


(Lotfus *et al*; 2005 in *Letter of Nature*)

Prediction of CoA biosynthesis *E. histolytica*



mRNA expression level of PanK, DPCK1 and DPCK2 from E. histolytica (Clone 6 and G3 strain)

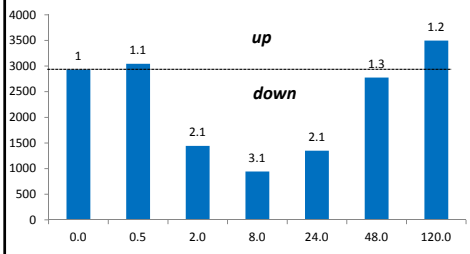


Comparative Individual gene Transcriptomic microarray in *E. Invadens*

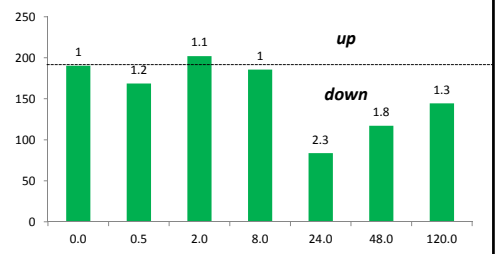
Up and down regulation in *E. invadens*

0 h = trophozoite stage
0.5 – 120 h = encystation stage

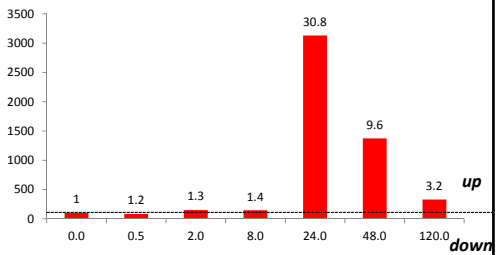
Pantothenate kinase (EIN_016770)



Dephospho CoA kinase 2 (EIN_256160)

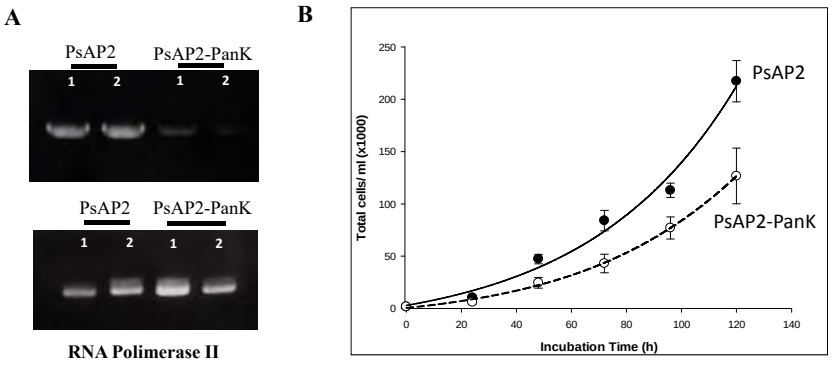


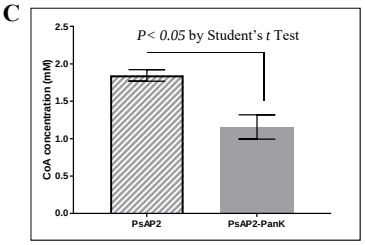
Dephospho CoA kinase 1 (EIN_147090)

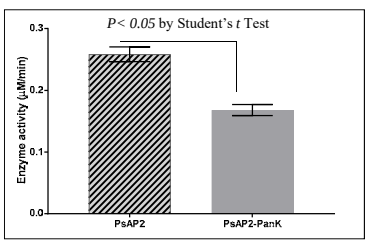


(Raw data sources : De Cadiz *et al*, 2013)

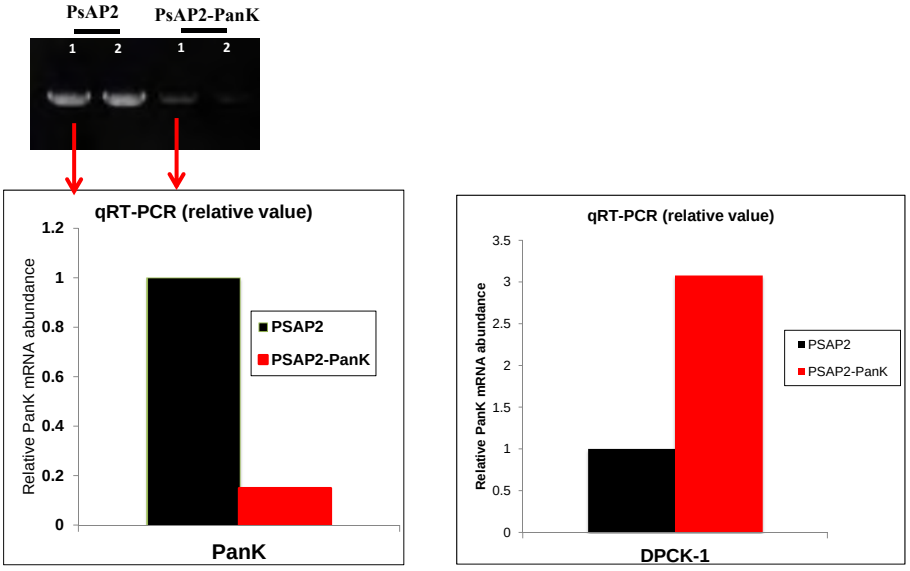
Analyses of genotypes and phenotypes of EhPanK gene silencing transforman strain







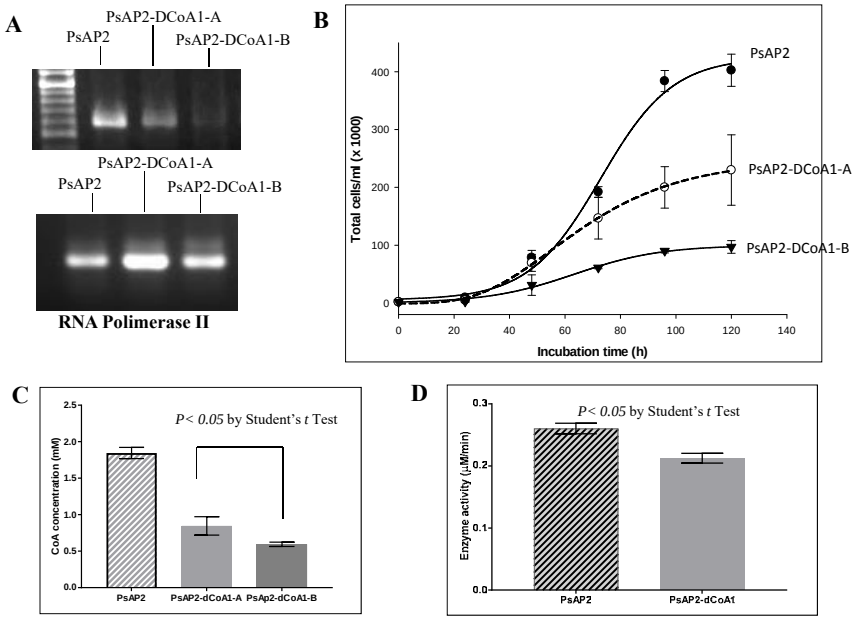
qPCR of PanK calculation



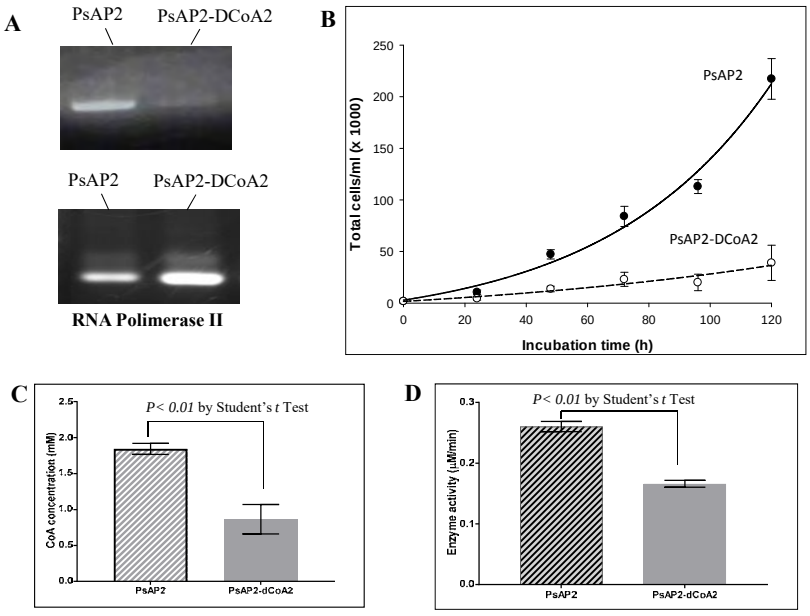
85% PanK gene was knocked down

DPCK-1 was increased 3x

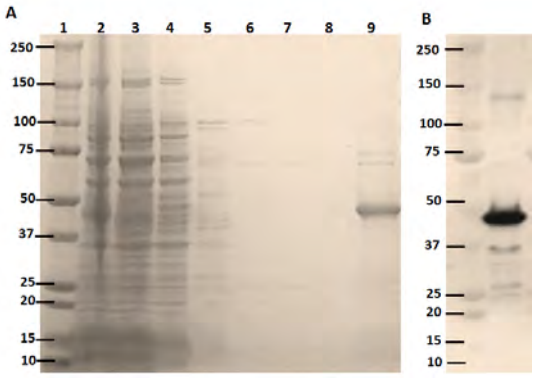
Analyses of genotypes and phenotypes of EhDPCK1 gene silencing transforman strain



Analyses of genotipes and phenotypes of EhDPCK2 gene silencing transforman strain

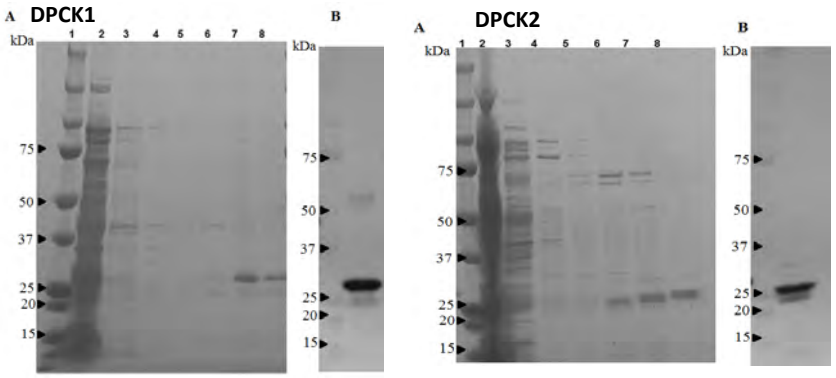


Expression and purification of recombinant EhPanK



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.

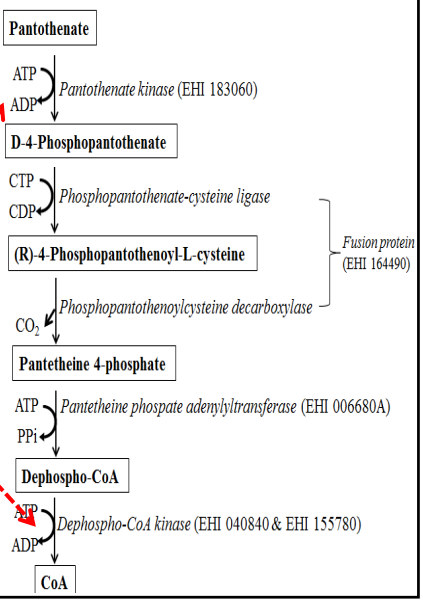
Enzymatic assay of natural compounds againts *E. histolytica*

Extract sources	Total
Fungi	560
Actinomycetes	840
Total	1,400

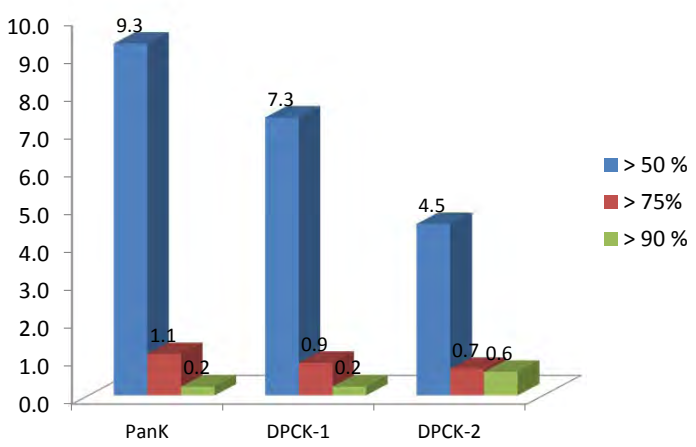
1. Pantothenate kinase (PanK)

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

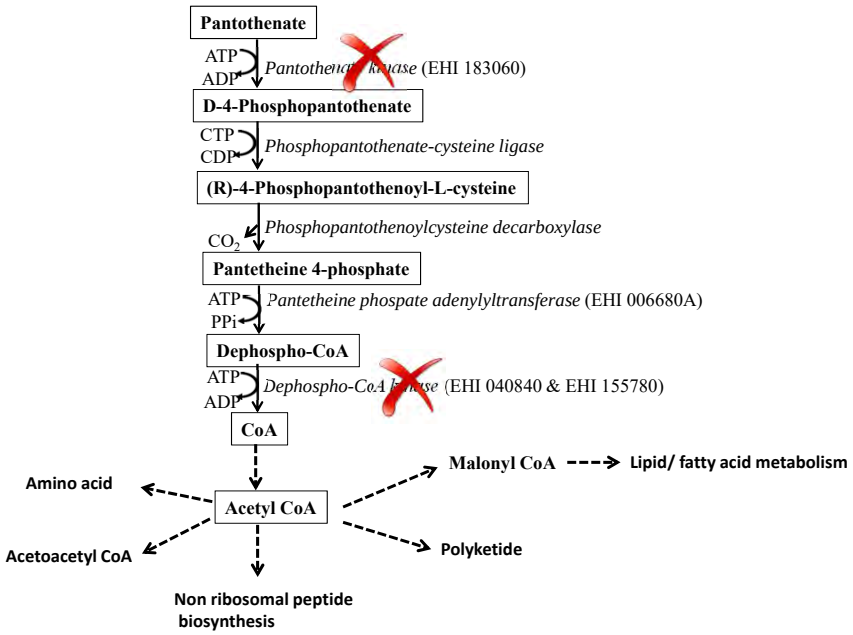
Criteria to determine :

- ✓ **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.

BPPT
BADAN PENGKAJIAN DAN PENERAPAN TEKNOLOGI

JICA
Japan International Cooperation Agency

LIPI
Indonesian Institue of Science

Airlangga University

東京大学
THE UNIVERSITY OF TOKYO

北里大学
KITASATO UNIVERSITY

長崎大学
NAGASAKI UNIVERSITY

MicroBiopharm Japan

AMED
Agency for Medical Research and Development

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

1. **Target Review and Research Flowchart**

2. **Progress 2017**
   a. Microbes Isolation and Extract Preparation
   b. Screening of Active Extract
   c. Purification of Active Compound
   d. Other Activities
   e. Budget Arrangement

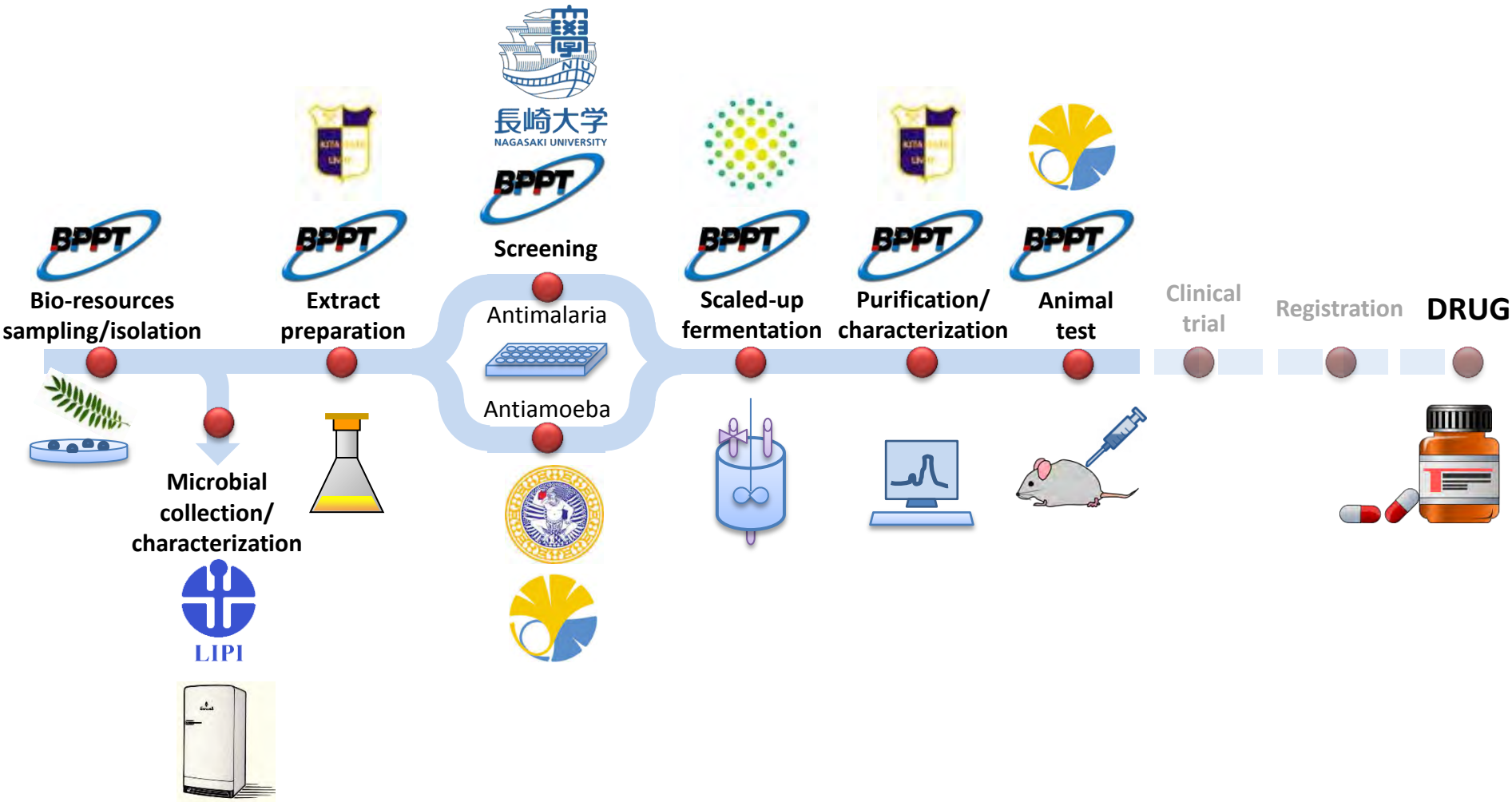
3. **Planning 2018**
   a. Research Activities
   b. Training
   c. Budget Arrangement
   d. Project Management

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

Research Flowchart



SATREPS Project 5 yrs
(FY 2015-2019)

Progress 2017

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

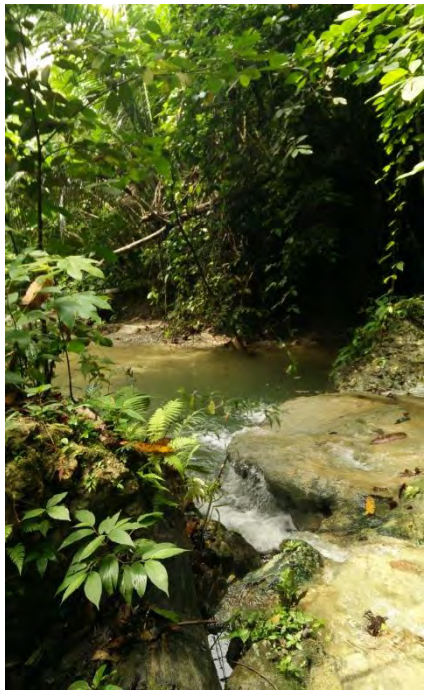
Other Activities

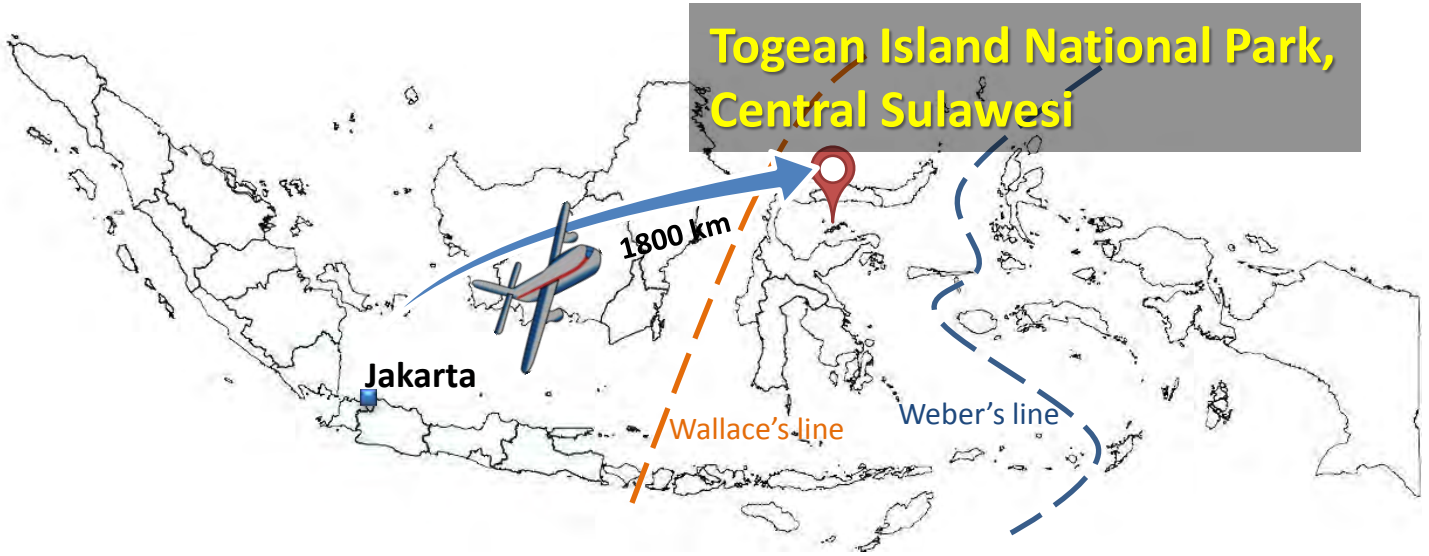
Budget Arrangement

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

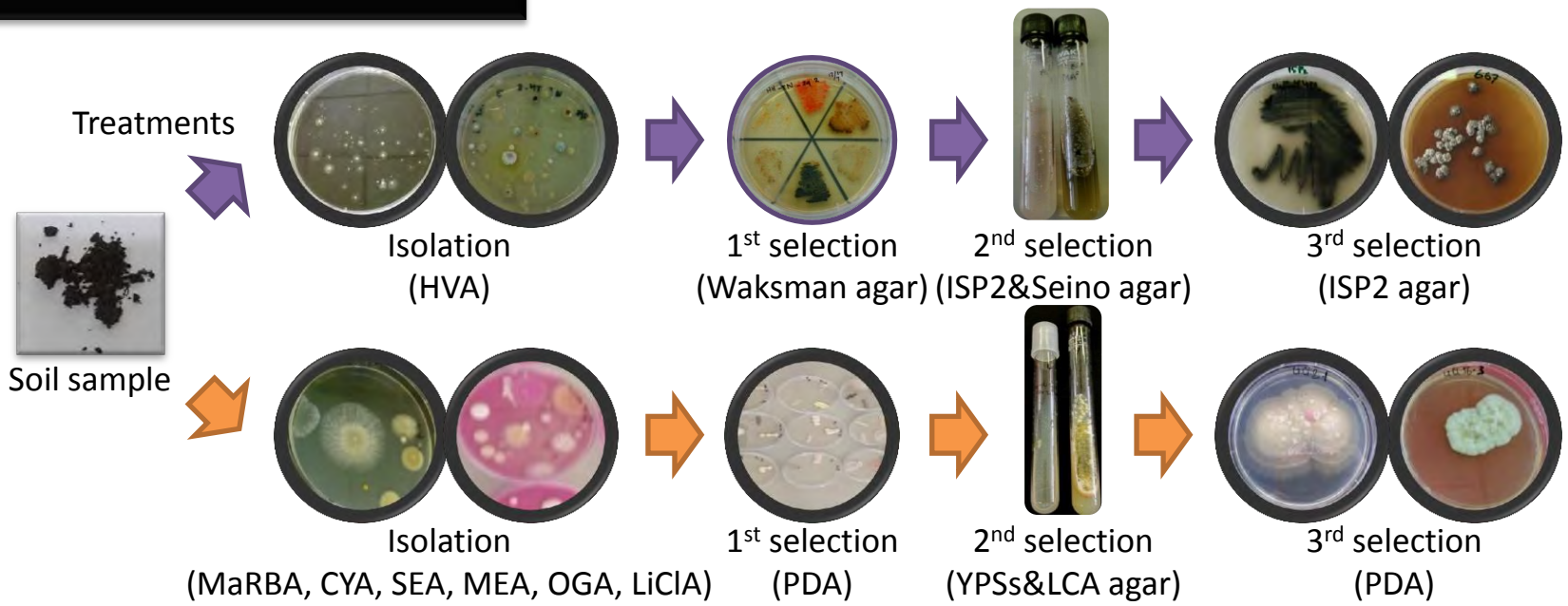
Type : Soil, plant litter, mushroom
Location : Terrestrial, shore side, river side
Total number : 92 samples

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**

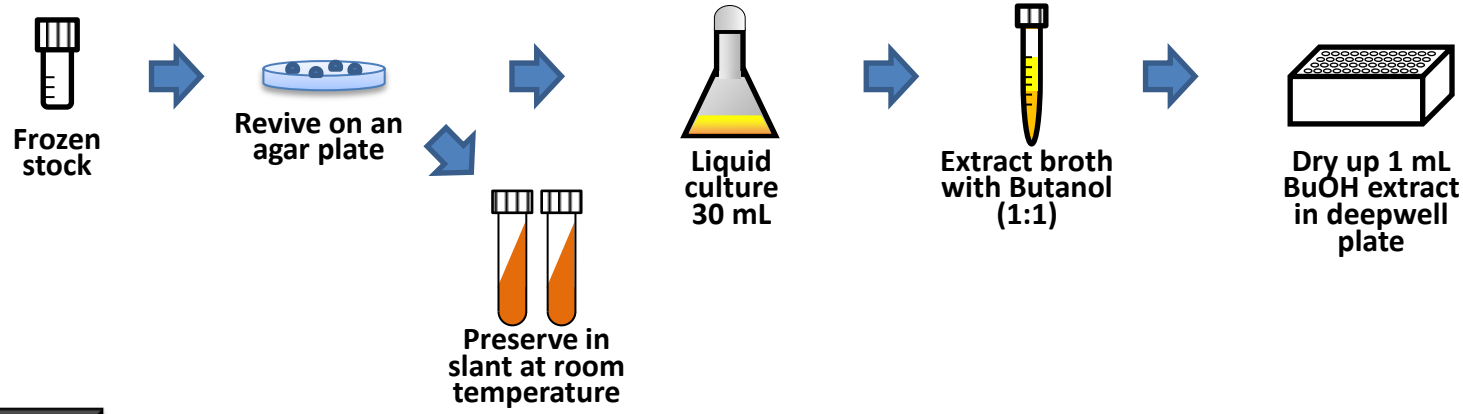
* Currently isolation is still on going

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**

Progress 2017

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

Progress 2017

Screening of Active Extract

Anti-malaria

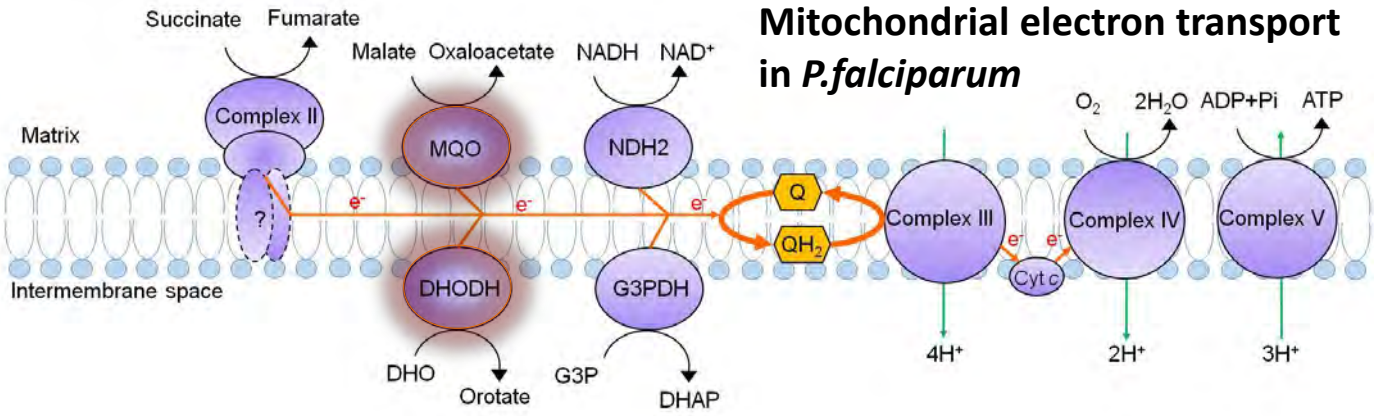
Objective: To search extract with antimalarial activity

Enzyme-based screening

Screening target: extracts with inhibitory activity for PfDHODH and PfMQO

DHODH : Dihidroorotate dehydrogenase
MQO : Malate-quinone oxidoreductase

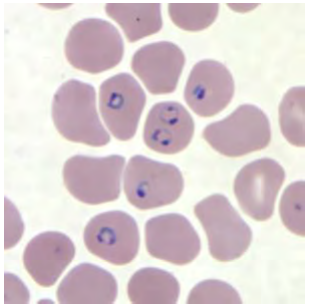
Mitochondrial electron transport in *P.falciparum*



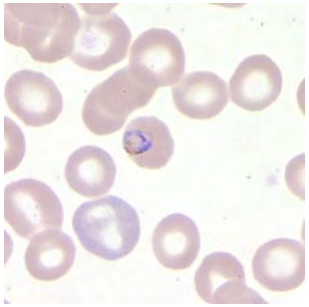
Cell-based screening

Screening target: extracts with inhibitory activity for proliferation of *Plasmodium falciparum* 3D7

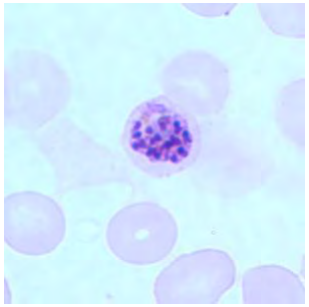
Life-stage of *Plasmodium falciparum*



Ring-form trophozoites



Trophozoites



Schizonts

Progress 2017

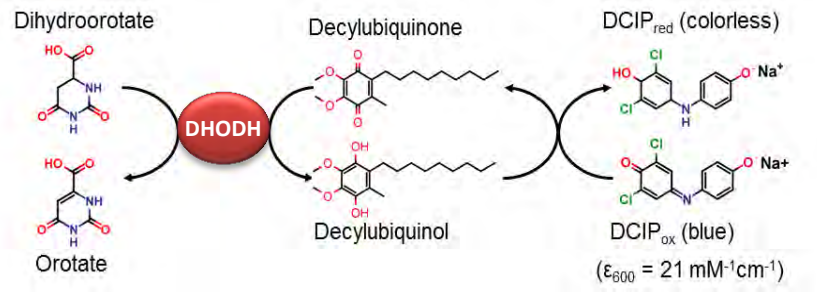
Screening of Active Extract

Anti-malaria

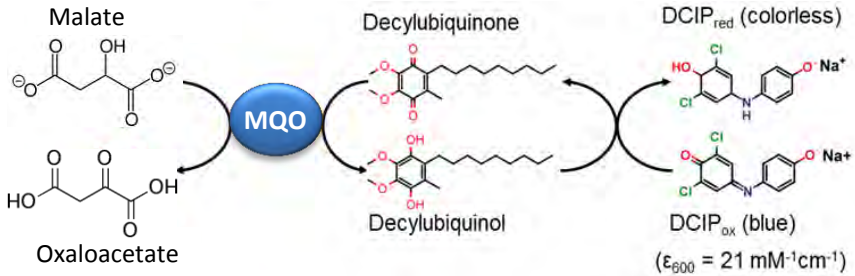
Enzyme-based Screening

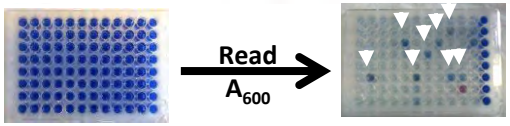
Objective: To search extract with inhibitory activity against malaria parasite specific target enzymes

Target enzyme: *Pf*DHODH



Target enzyme: *Pf*MQO





Start

End (Hit= inhibit>50%)

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**

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)

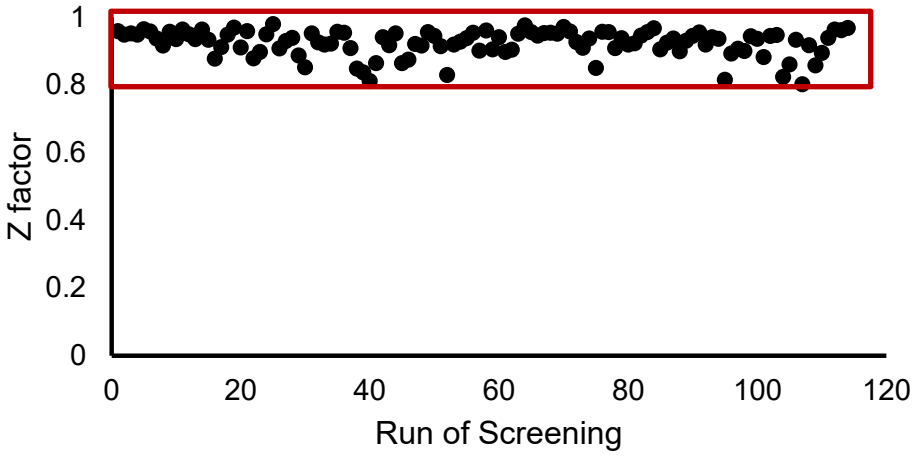
$$\mathbf{Z} = 1 - \frac{(3\sigma_s + 3\sigma_c)}{|\mu_s - \mu_c|}$$

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

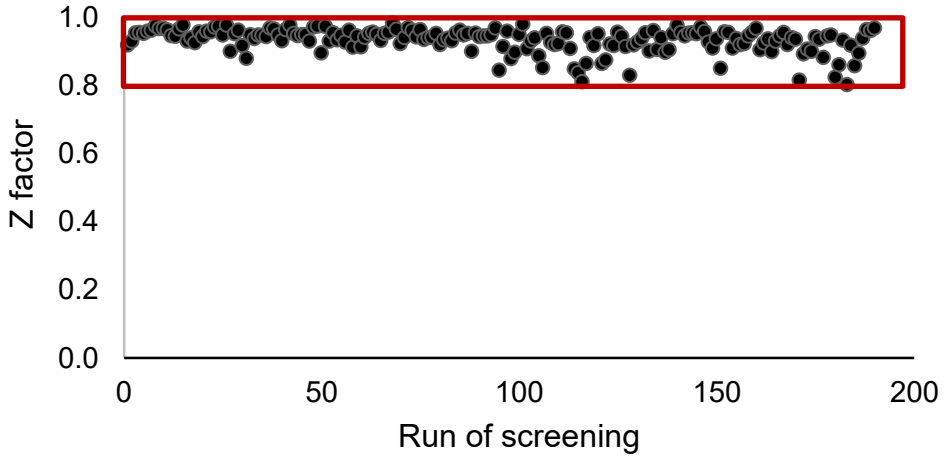
Screening assay quality evaluation

Z = 1	ideal assay
$1 > Z \geq 0.5$	excellent assay
$0.5 > Z \geq 0$	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**

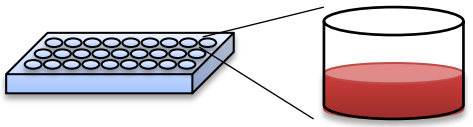
Progress 2017

Screening of Active Extract

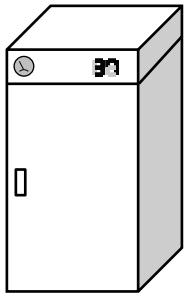
Anti-malaria

Cell-based Screening

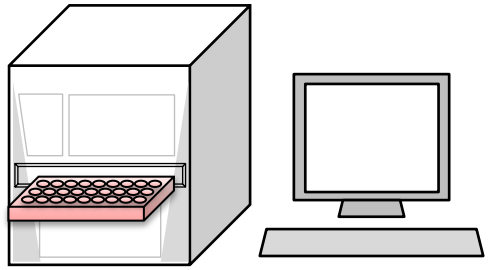
Objective: To search extract with inhibitory activity to proliferation of malaria parasite cell



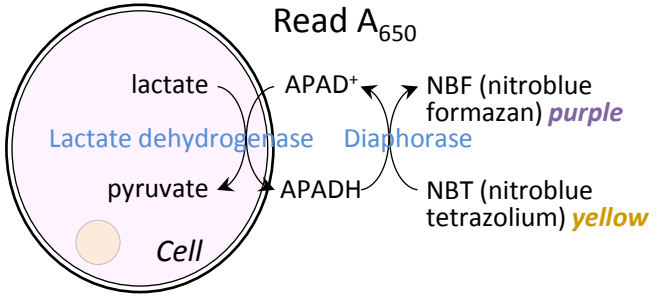
Sorbitol-treated *P.falciparum* cell culture + extract
0.3% parasitemia, 3% hematochrit (blood type=0(+)) in RPMI (+) (10% Albumax®), culture volume 100 µl



Incubation
37°C, 5% CO_2, 5% O_2, 48 h



LDH assay
Read A_{650}



Calculation of inhibition rate

$$\text{Inhibition rate } (\%) = \left(1 - \frac{A_s - A_p}{A_n - A_p}\right) \times 100\%$$

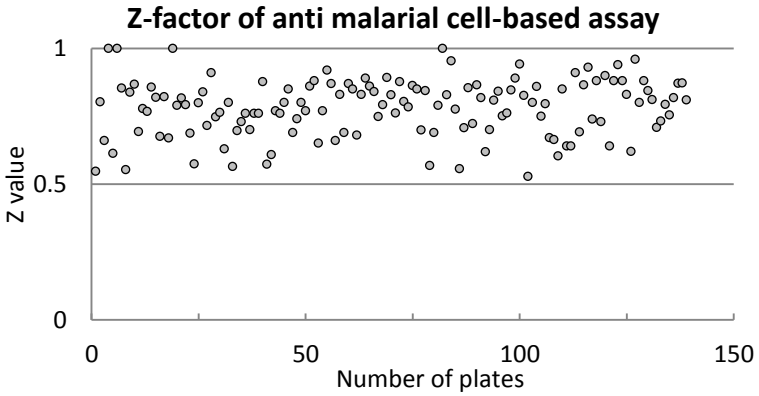
As = Sampel absorbance
Ap = Positive control absorbance (atovaquone)
An = Negative control absorbance (DMSO)

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

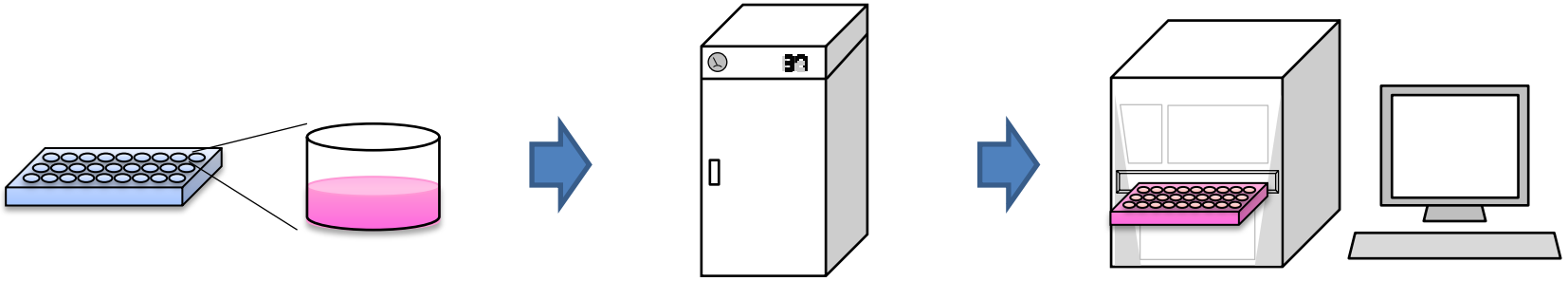
Screening performance



Z value of screening > 0.5
→Screening result is reliable

Toxicity Assay

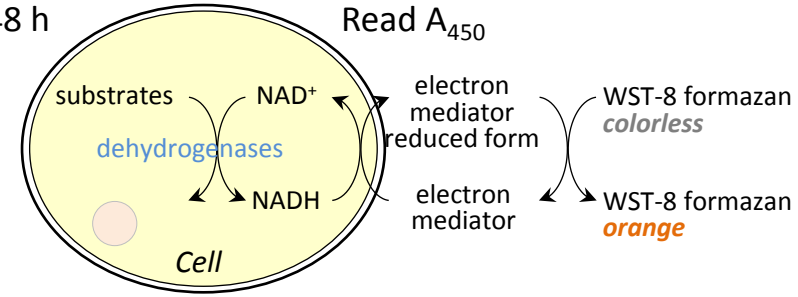
Objective: To evaluate toxicity of active extract that inhibit proliferation of malaria parasite against mammalian cell



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**

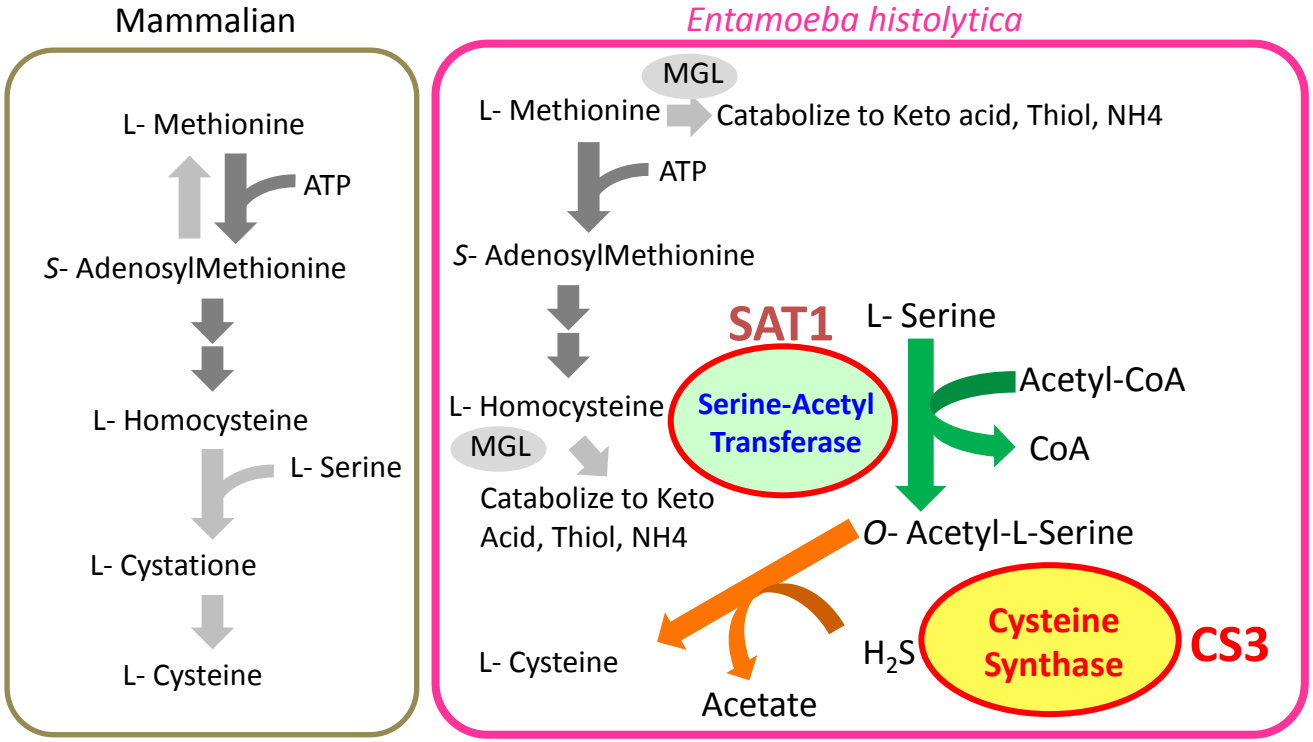
Toxicity assay reduced hit rate from 14.1% to 12.1%

Objective: To search extract with antiamebic activity

Enzyme-based screening

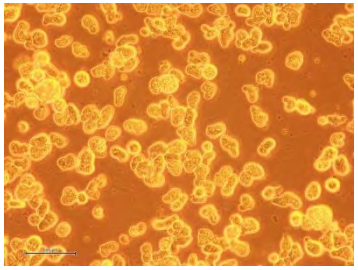
Screening target: extracts with inhibitory activity for SAT1 and CS3

Cysteine biosynthesis pathway



Cell-based screening

Screening target: extracts with inhibitory activity for proliferation of *Entamoeba histolytica* HM-1:IMSS cl6



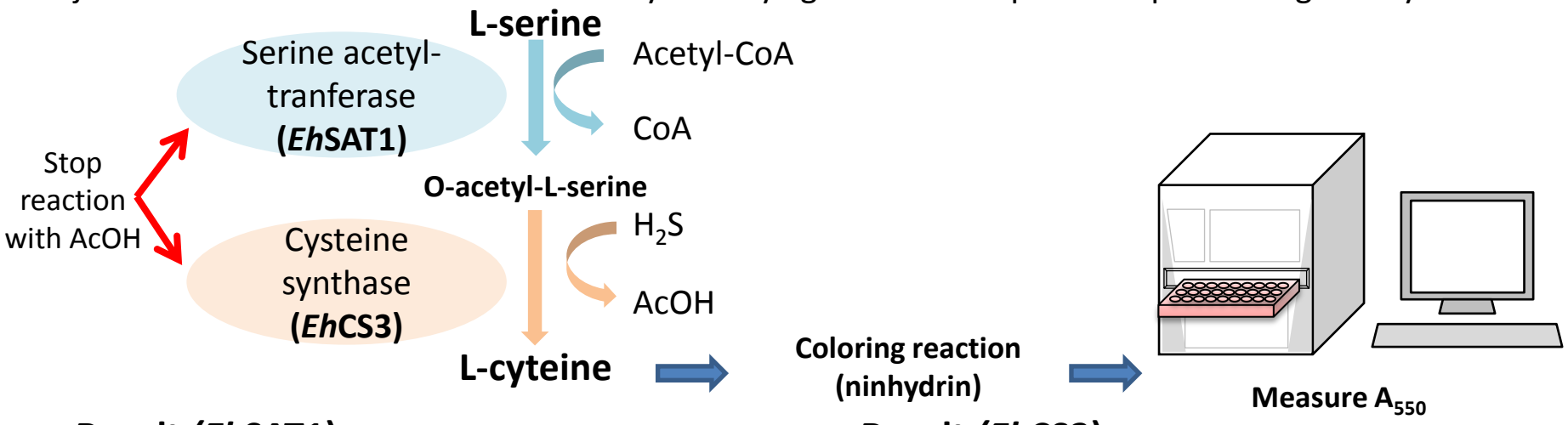
Progress 2017

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

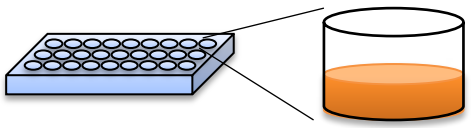
Result (*Eh*CS3)

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 (%)			

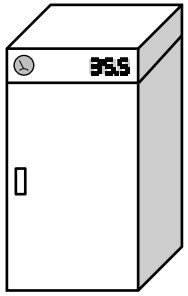
Total extract screened = 4.600

Cell-based Screening

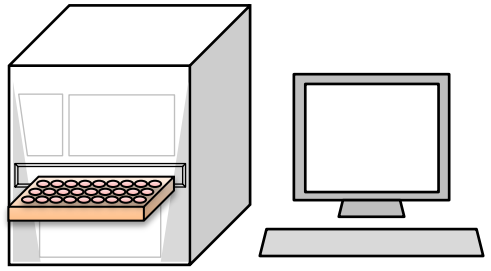
Objective: To search extract with inhibitory activity to proliferation of amebic parasite cell



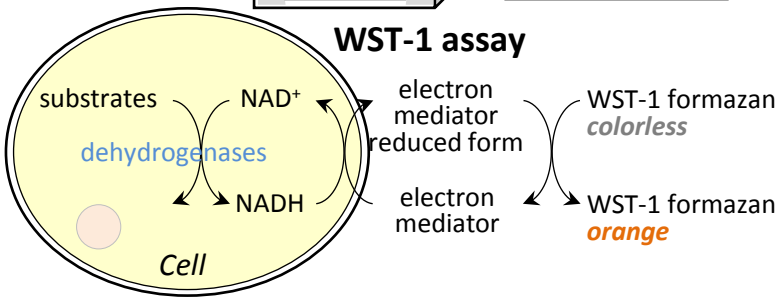
***E.histolytica* cell culture + extract**
Initial cell number = 8000 cells in BIS medium, culture volume 200 µl



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

Progress 2017

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

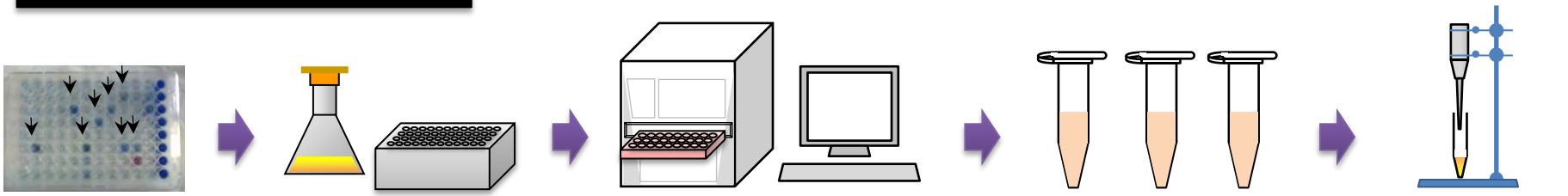
Budget Arrangement

Progress 2017

Purification

Objective: To obtain purified compound with antimalarial/antiamebic activity

General purification flow

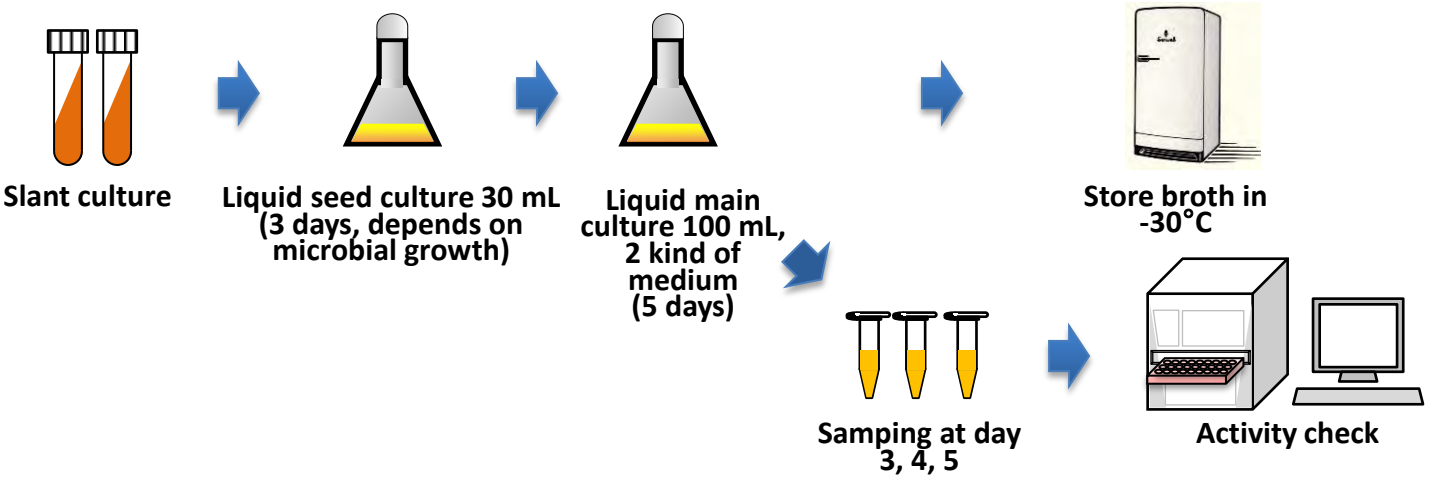


Hit → Reconfirmation extract production → Activity check → Pre-extraction Test → Purification

Pre-extraction Test: to evaluate properties (stability, polarity) of active extract

Reconfirmation extract production

Objective: To prepare *hit* extracts by reculturing the producer (microbial strain) for confirming their activities and for preliminary purification process



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**

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)

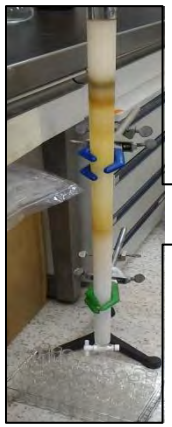
↓

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)

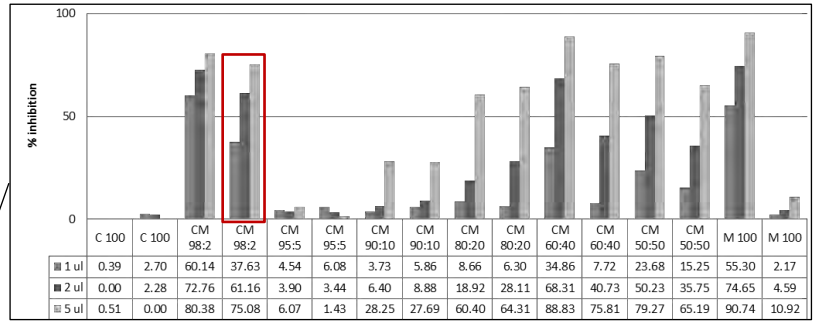
↓ **Sephadex LH-20 open column (MeOH)**
3 ml x 120 fractions

Fr. 28 + Fr. 29 (11.1 mg)

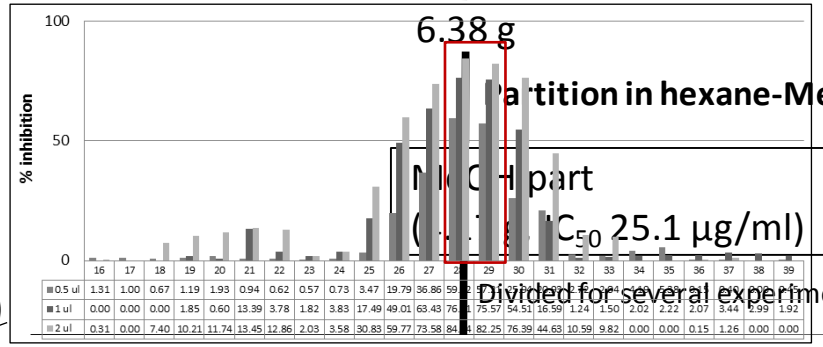
↓ **^{1}H-NMR**

Linoleic acid (IC_{50} 3.89 µM)

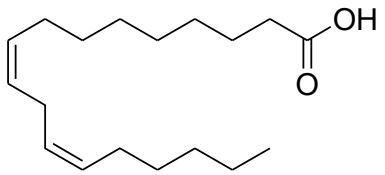
(Amila et.al., 2017)



6.38 g



Divided for several experime



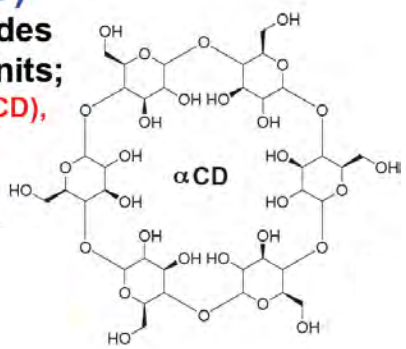
Linoleic acid
IC_{50}= 3.89 µM

PfMQO

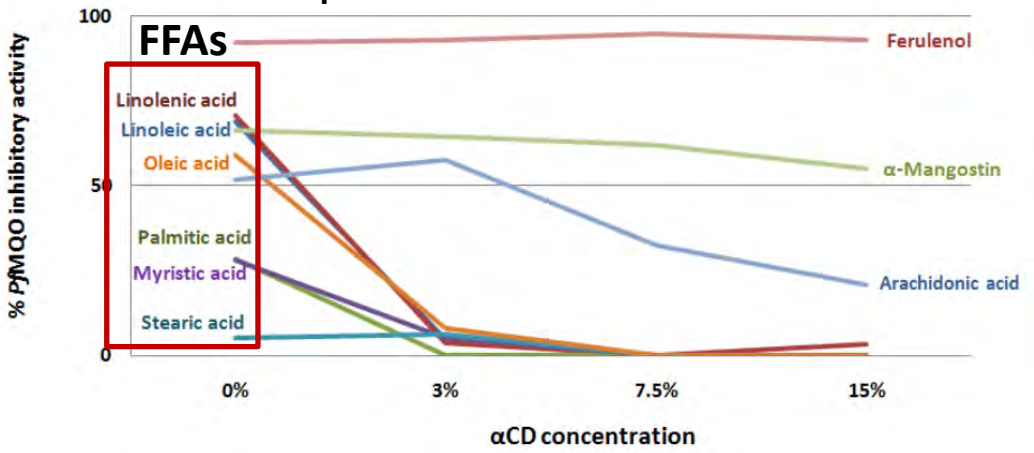
Dereplication of Free Fatty Acids (FFAs) from extracts

Cyclodextrin (CD)

Cyclic oligosaccharides containing glucose units;
6 units (αCD), 7 units (βCD), 8 units (γCD)

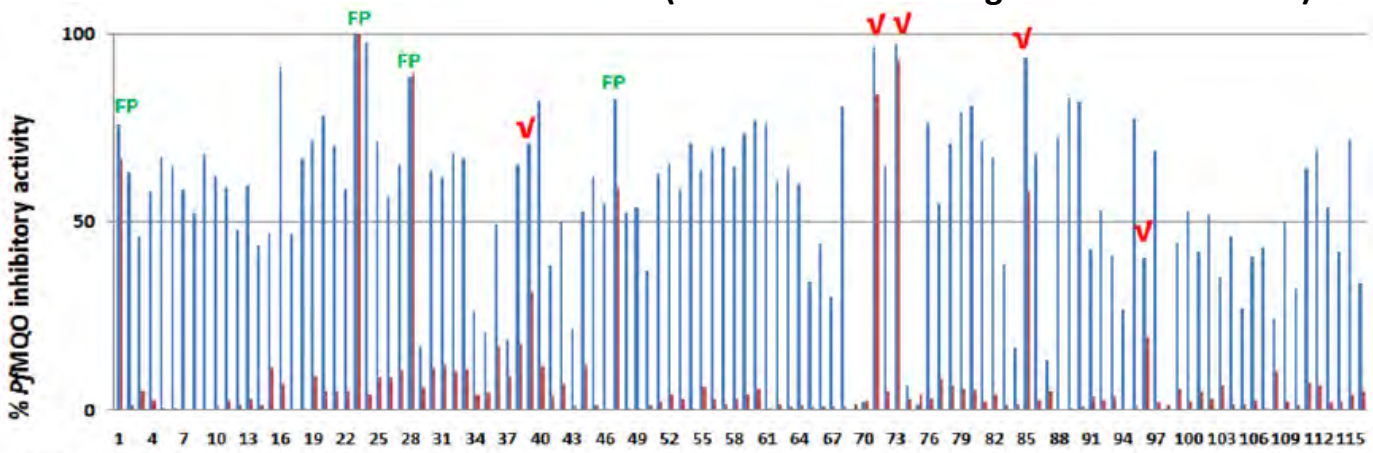


PfMQO inhibitory activity of FFAs and other inhibitors in supernatant after αCD treatment



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

(Amila et.al., 2017)

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

Progress 2017

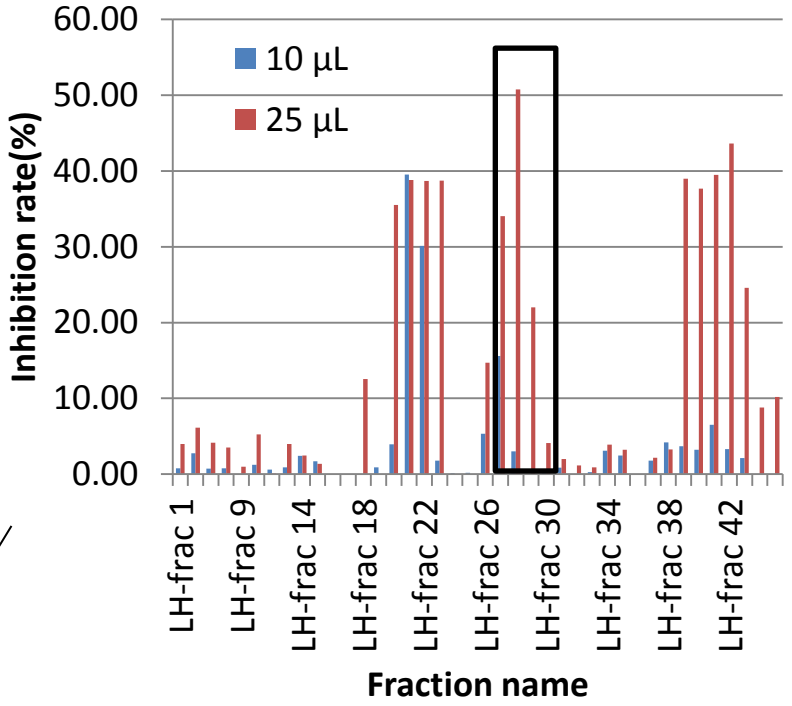
Purification

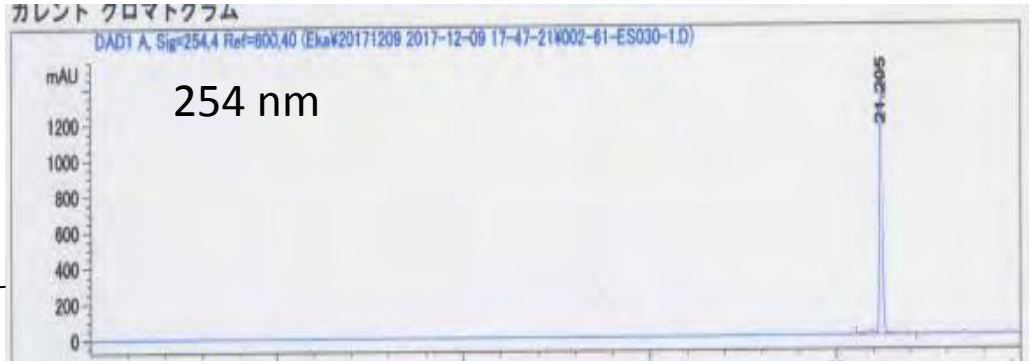
Anti-malaria

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)	√	√	√		
2	F.2182	√	√ (Silica)	√	√	√		
3	F15.2274	√	√ (Silica)	√	√	√		
4	F15.2383							No activity
5	F15.2236	√						
6	F.2046	√						
7	F15.2179	√						
8	F15.2584	√						
9	F.2182	√						
10	F15.2299	√						
10	F15.2299							
11	F15.2274	√						

Currently being purified

Will be proceeded for purification

Progress 2017

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

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

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

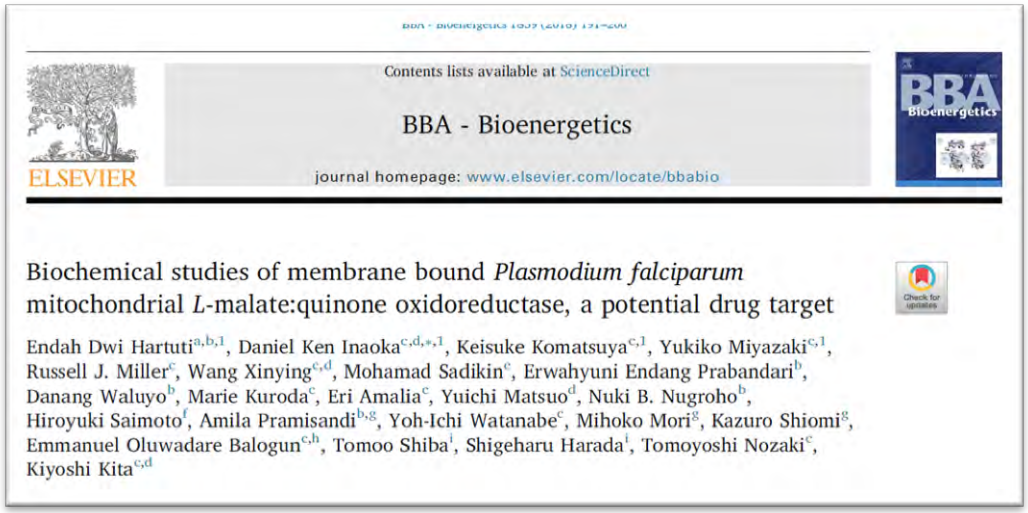
- UIN provided local plant extracts to be assayed for anti-malarial activity
- Currently, 2 active plant extracts are being purified (with inhibitory activity against PfMQO)

Progress 2017

Publication and Seminar

Publication on BBA Bioenergetics

- First publication written by Indonesian researcher as the first author



Contents lists available at ScienceDirect

BBA - Bioenergetics

journal homepage: www.elsevier.com/locate/bbabio

Biochemical studies of membrane bound *Plasmodium falciparum* mitochondrial *L*-malate:quinone oxidoreductase, a potential drug target

Endah Dwi Hartuti[a,b,1], Daniel Ken Inaoka[c,d,*,1], Keisuke Komatsuya[c,1], Yukiko Miyazaki[c,1], Russell J. Miller[c], Wang Xinying[c,d], Mohamad Sadikin[c], Erwahyuni Endang Prabandari[b], Danang Waluyo[b], Marie Kuroda[c], Eri Amalia[c], Yuichi Matsuo[d], Nuki B. Nugroho[b], Hiroyuki Saimoto[f], Amila Pramisandi[b,g], Yoh-Ichi Watanabe[c], Mihoko Mori[g], Kazuro Shiomi[g], Emmanuel Oluwadare Balogun[c,h], Tomoo Shiba[i], Shigeharu Harada[i], Tomoyoshi Nozaki[c], Kiyoshi Kita[c,d]

Presentation in The 9th International Seminar of Indonesian Society for Microbiology, Palembang (November 14-15, 2017)

- Presented 4 topics related to the project achievements

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

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**	

Planning 2018

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

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)

Thank You

JCC THIRD YEAR

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

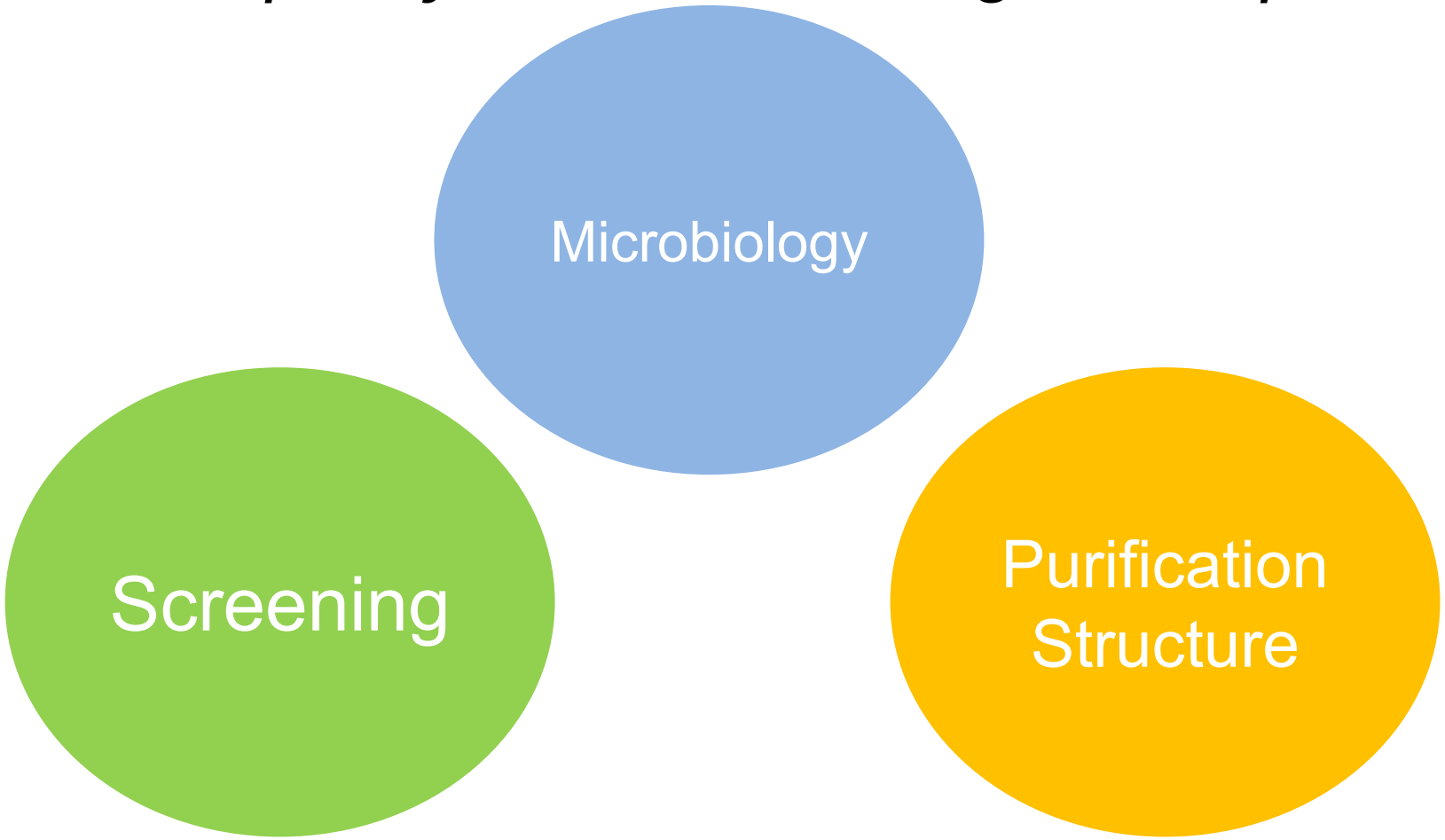
Identified problems/needs and solutions

Tomo NOZAKI
The University of Tokyo
CHIEF ADVISOR

BPPT, Jakarta, January 31th, 2018

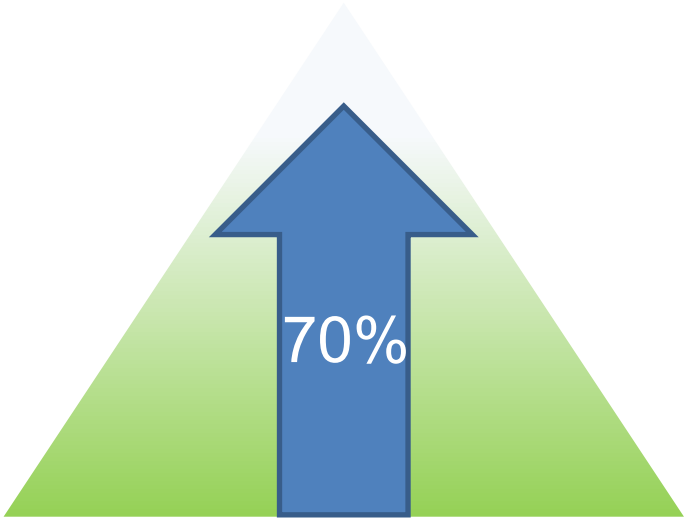
Goals of the project

1. *Identify >1 lead compounds with anti-malarial and anti-amebic activities in vivo*
2. *Build capacity needed for drug development*

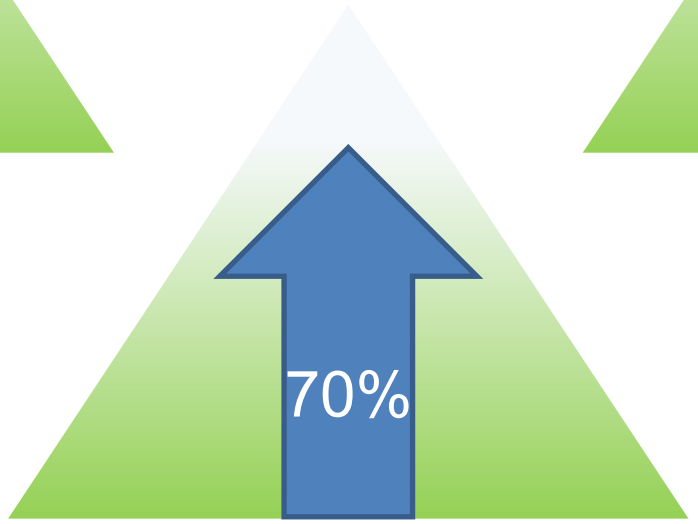


Accomplishment of goals

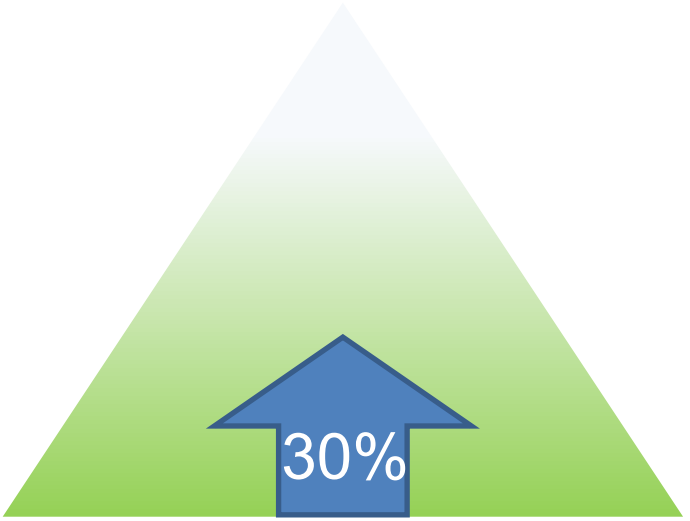
Research Capacity Building – Microbiology



Microbiology
Sampling/Isolation/Culture



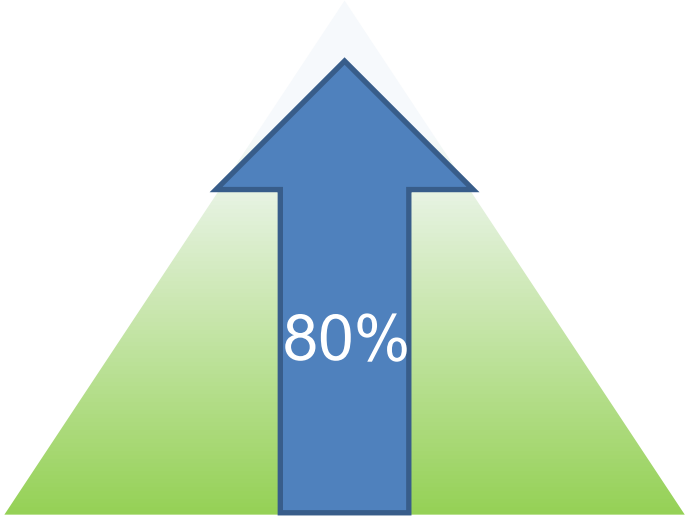
Microbiology
Extract production
Data management



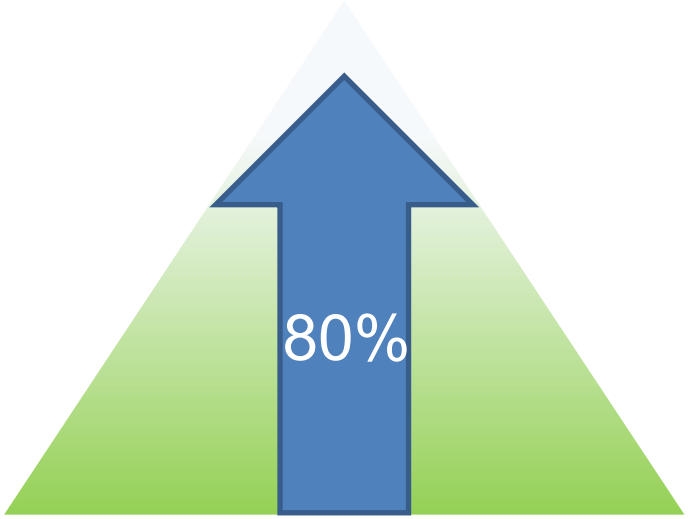
Microbiology
Classification/Taxonomy

Accomplishment of goals

Research Capacity Building – Screening



Screening
Enzyme-based assay

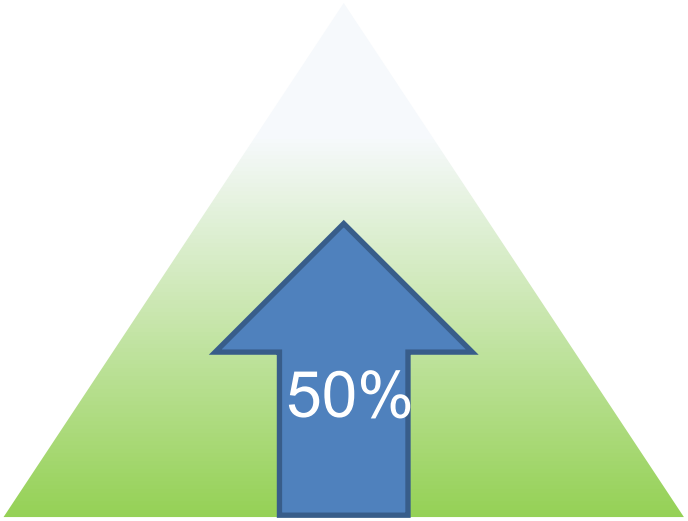


Screening
Cell-based (phenotypic) assay

Accomplishment of goals

Research Capacity Building

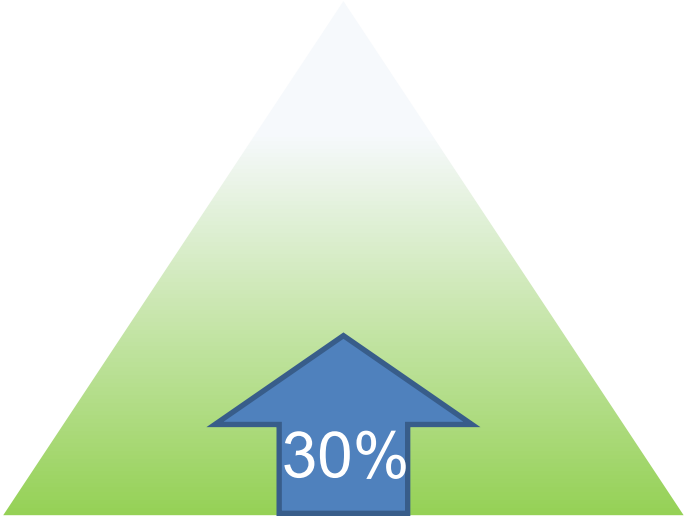
– Purification and structural elucidation



Purification

Liquid partition

Chromatography



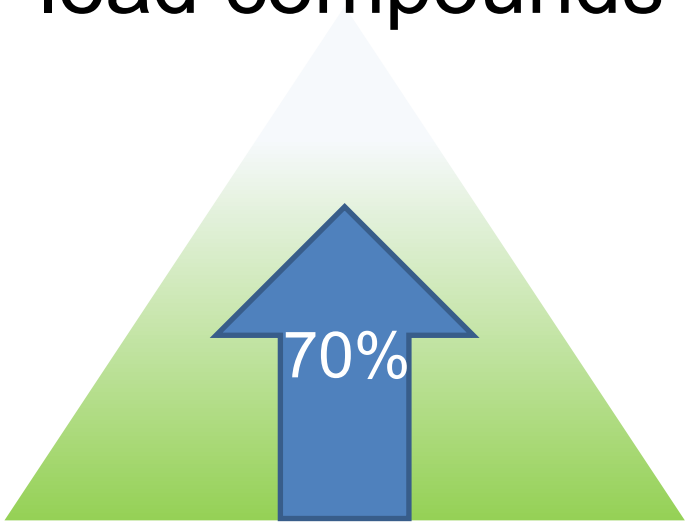
Structural elucidation

Mass spectrometry

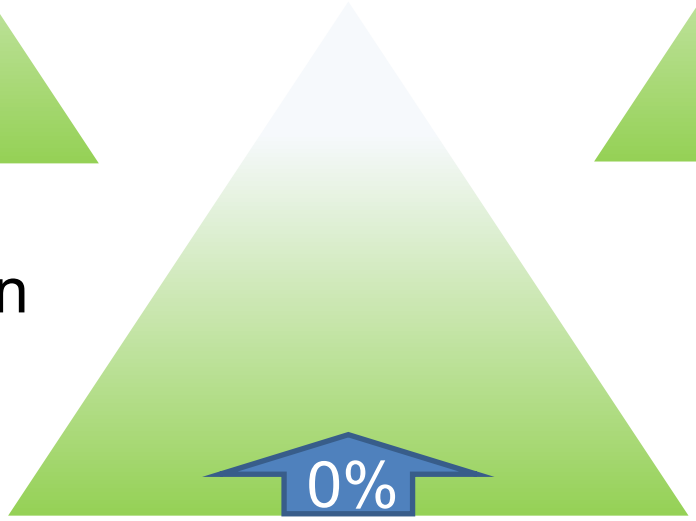
Nuclear magnetic resonance

Accomplishment of goals

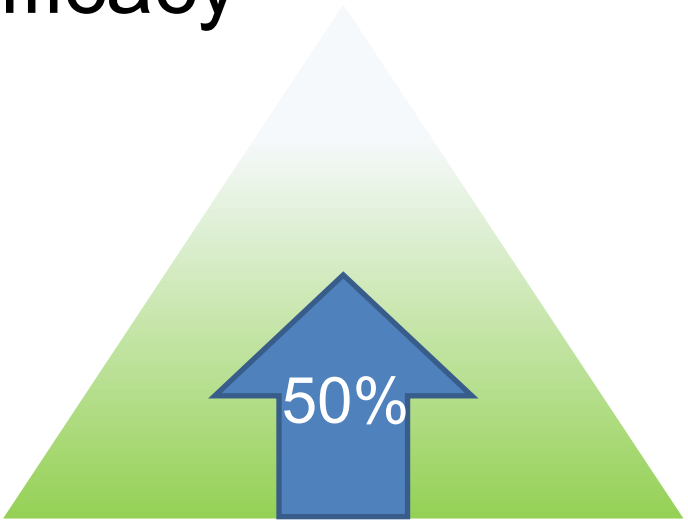
Identification of anti-malarial and anti-amebic lead compounds with in vivo efficacy



Antimalarial
Lead identification



In vivo Efficacy confirmation



Anti-amebic
Lead identification

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

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)

Report activities of ITD-UNAIR

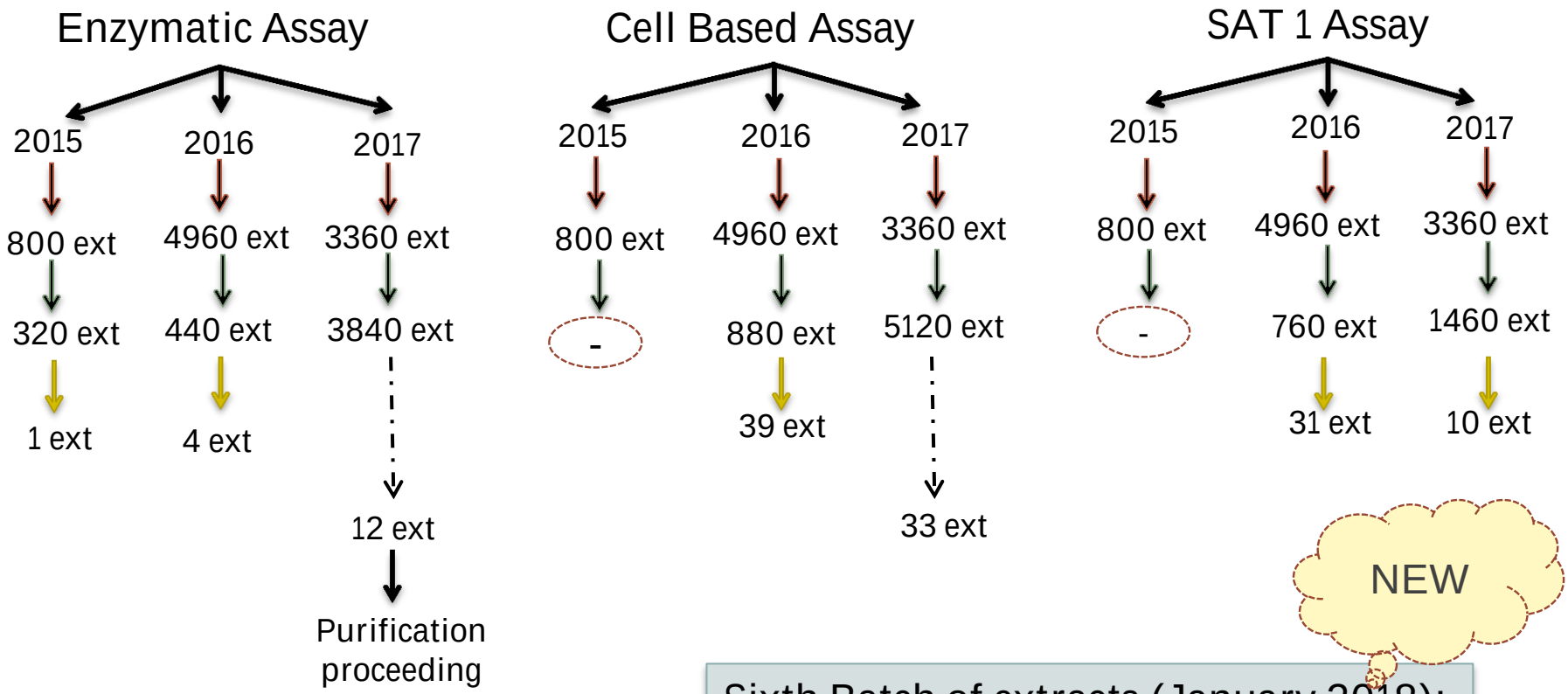
"Project for Searching Lead Compounds of anti-Malarial and Anti-Amebic Agent by Utilizing Diversity of Indonesian Bio-Resources"

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

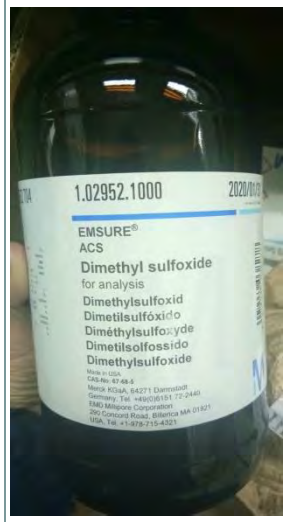
Training Anti-Malarial

Purification process

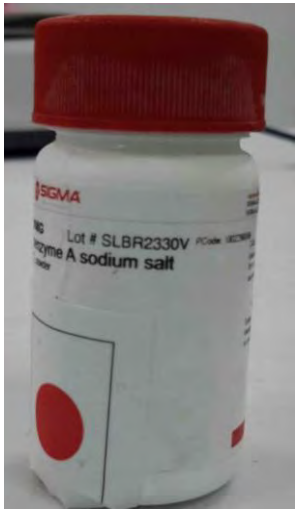
Enzymatic assay

Reconfirmation extract

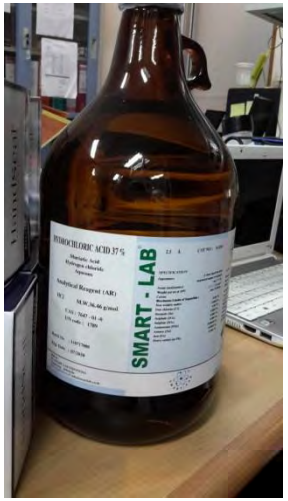
Consumables-Reagens



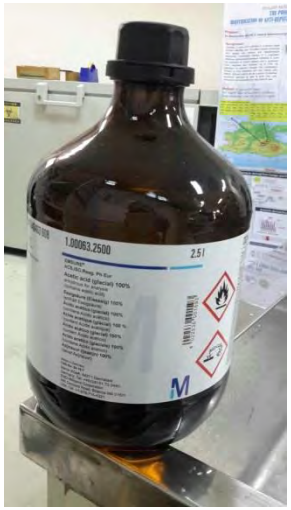
DMSO



Acetyl CoA

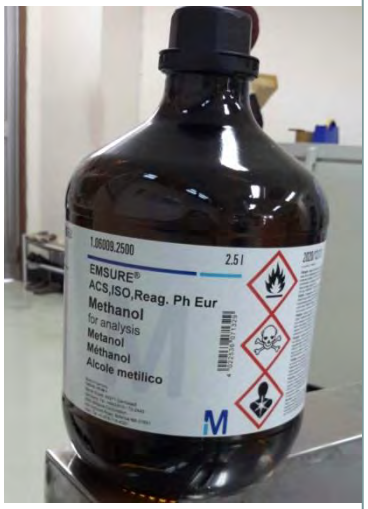


HCl 37%



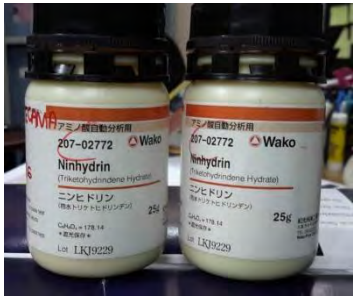
Acetic Acid

Ethanol

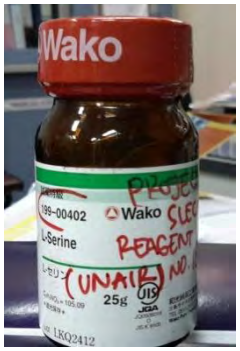


Methanol

WST-1



Ninhydrin



L-serine

Bovine Serum

OPTI-MEM

Consumables-Plasticware

Well Plate-Round Bottom

Autoclave Bag

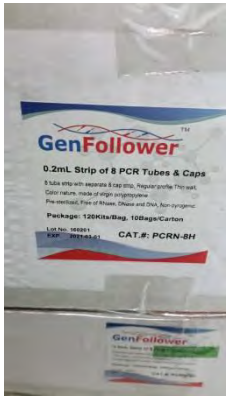
Microtube Racks

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

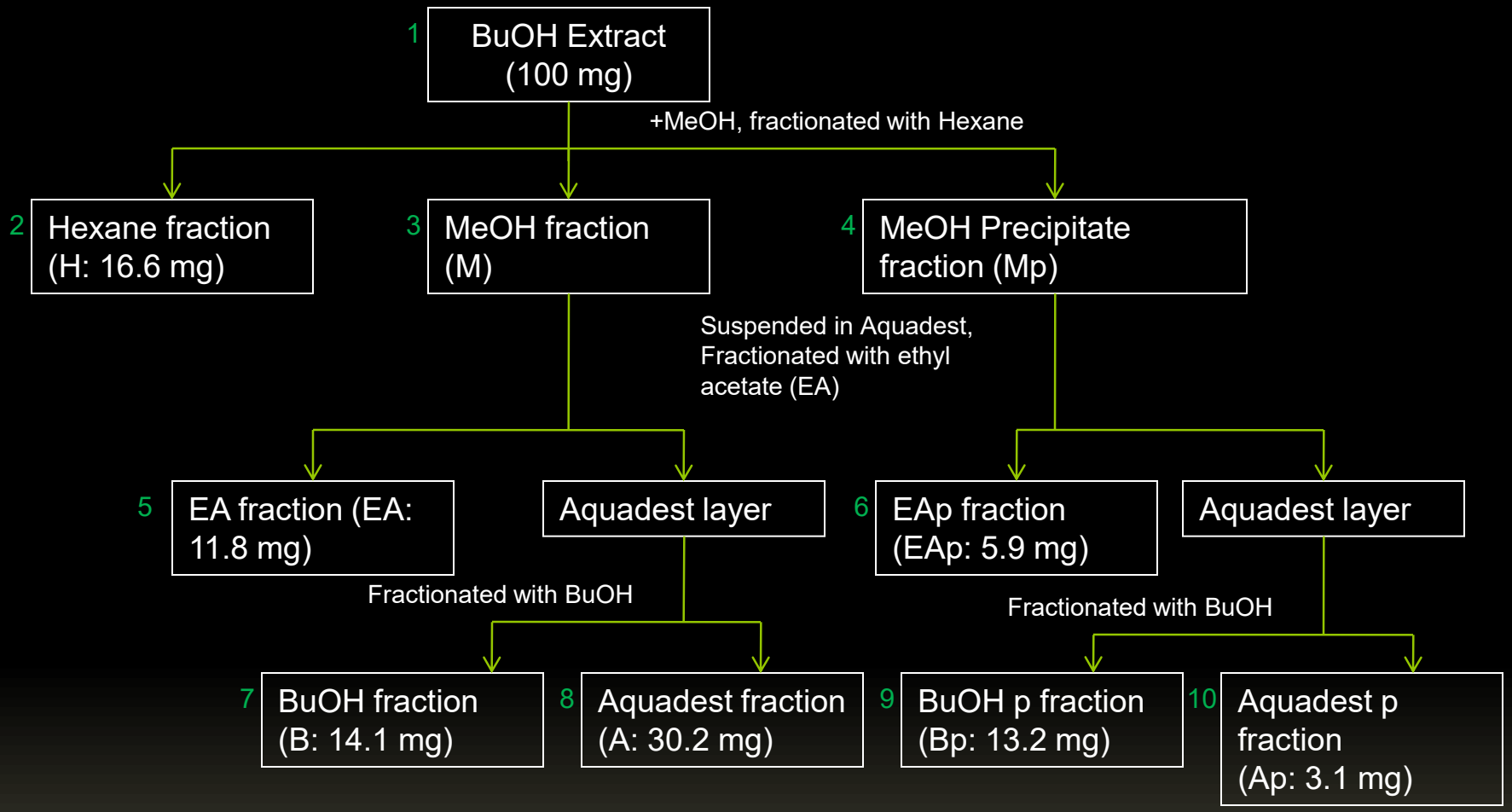
Takaya Sakura

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)



Samples 1-10 → Enzymatic assay

BPPT

JICA
Japan International Cooperation Agency

LIPI
Indonesian Institue of Science

Airlangga University

東京大学 THE UNIVERSITY OF TOKYO

北里大学 KITASATO UNIVERSITY

長崎大学 NAGASAKI UNIVERSITY

MicroBiopharm Japan

AMED
Agency for Medical Research and Development

The 4th 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 2018 and Planning 2019

Danang Waluyo
Project Co-manager

Sari Pacific Hotel, Jakarta
January 29th, 2019

Content

1. **Target Review and Research Flowchart**

2. **Progress 2018**
   a. Microbes Isolation and Extract Preparation
   b. Screening of Active Extract
   c. Purification of Active Compound
   d. Other Activities
   e. Budget Arrangement

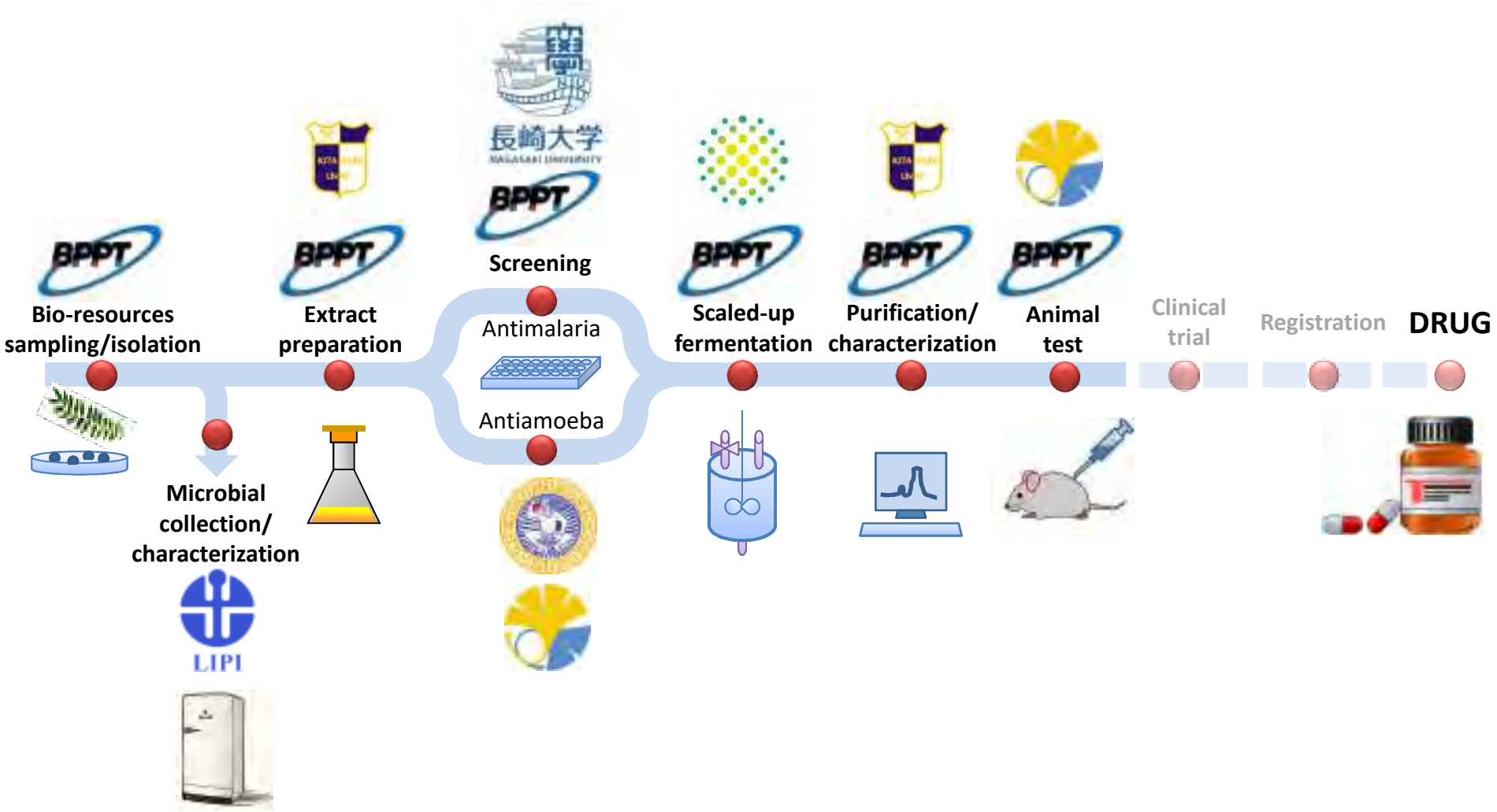
3. **Planning 2019**
   a. Research Activities
   b. Training
   c. Budget Arrangement
   d. Project Management

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 2017
Blue: partially achieved 2017

Research Flowchart



SATREPS Project 5 yrs
(FY 2015-2019)

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

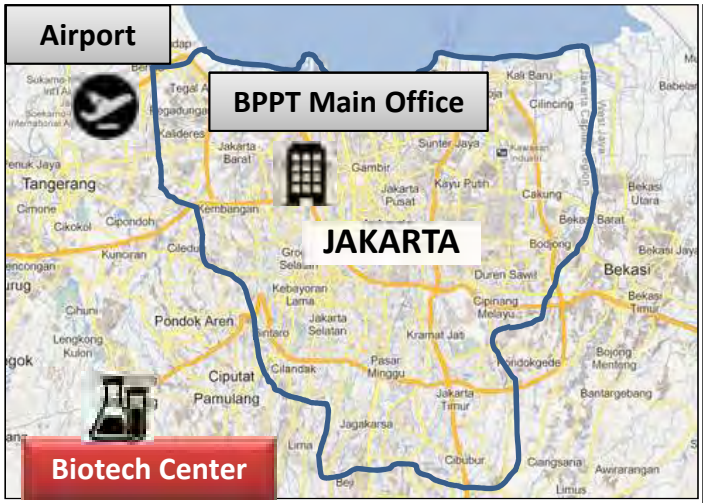
Purification of Active Compound

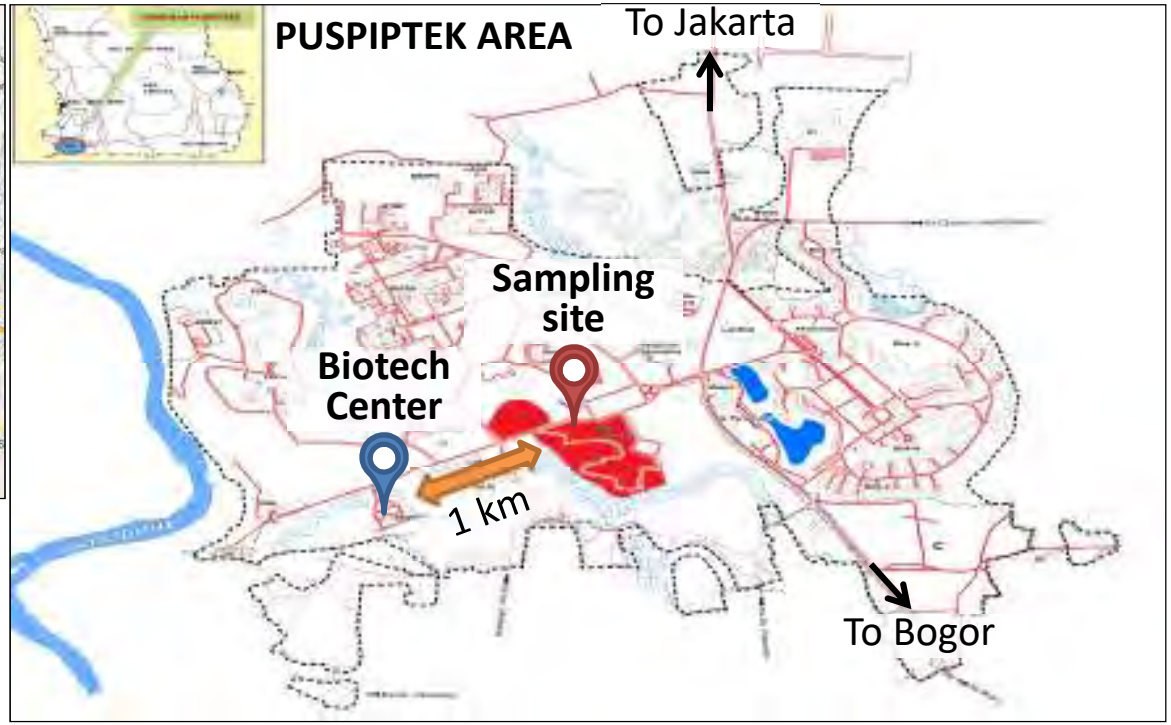
Other Activities

Budget Arrangement

Progress 2018

Field Exploration





Sampling point

Location : Puspiptek (Botanical Garden)
Coordinate : 6°20'36.7"S 106°40'39.4"E
Date : May 8-9, 2018
Temp./RH : 28-31°C, 80%

Sample obtained

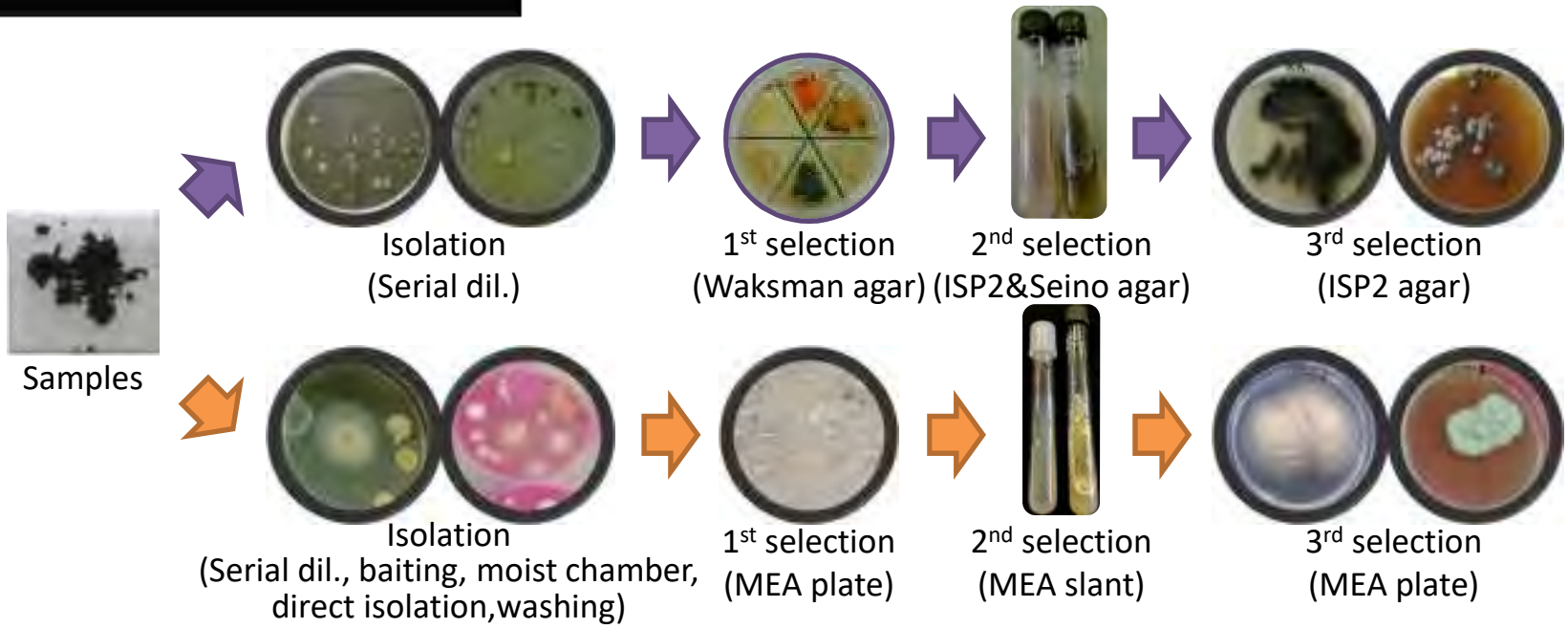
Type : Soil, litter, mushroom, insect dead body, etc.
Location : Terrestrial, wet surface, reservoir
Total number : 122 samples

Progress 2018

Microbial Isolation

Objective: To isolate microbial strain from source samples

General microbial isolation method



Result

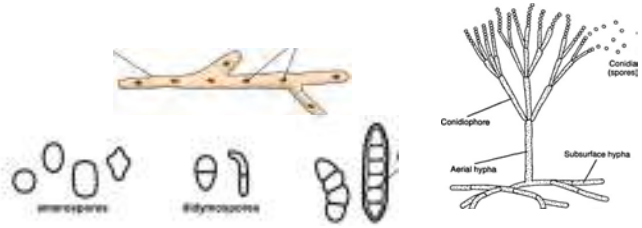
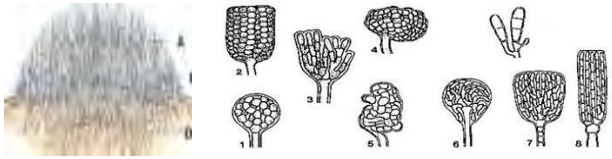
Target	Location	Number of isolated sources	Number of isolates*
Fungi	Puspiptek	83	632
	Togen (2017)	8	136
Actinomycetes	Puspiptek	37	444
	Togen (2017)	8	76
		TOTAL	**1288**

* Currently isolation is still continued

Progress 2018

Microbial Identification

Objective: To identify microbial strain (newly isolated, revived from frozen stock, interesting isolates, hit producing isolates)

	Fungi	Actinomycetes
Morphology-based	Shape of hyphae, conidial form, structure of conidiophore 	Chain of sporophore, aerial and agar hyphae, substrate mycelia, spore production within sporangia 
Molecular-based	16S rDNA	28S rDNA

Result

Target	Method	Number of Identified isolates*
Fungi	Morphology-based	1244
	Molecular-based	50
Actinomycetes	Morphology-based	793
	Molecular-based	2

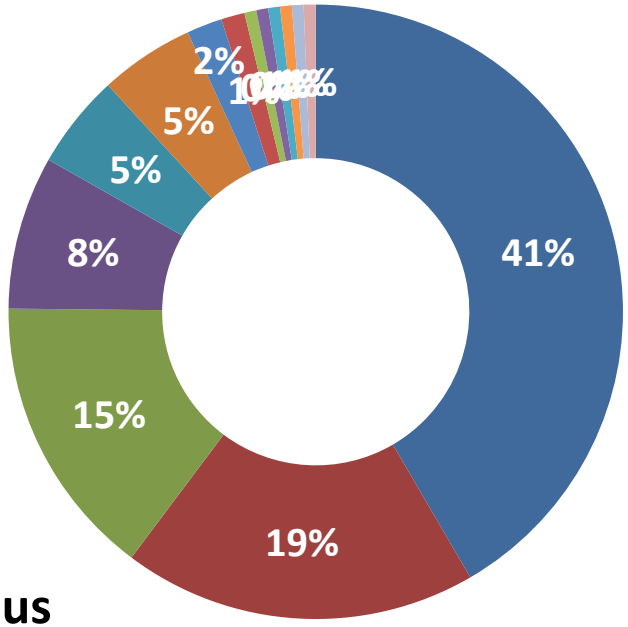
* Currently identification is still continued

Progress 2018

Microbial Identification

Result 2017

Sampling location: Togean

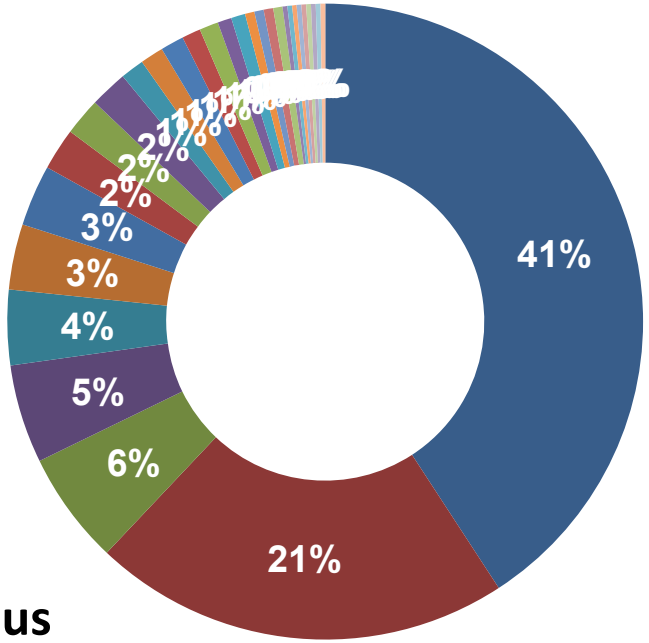


- No conidia
- *Paecilomyces*
- *Penicillium*
- *Aspergillus*
- *Fusarium*
- *Talaromyces*
- *Mortierella*
- *Verticillium*
- *Beauvaria*
- *Cephalosporium*
- *Metarhizium*

220 isolates, 13 genus

Result 2018

Sampling location: Puspiptek



- No conidia
- *Aspergillus*
- *Auerobasidium*
- *Cladosporium*
- *Paecilomyces*
- *Beauvaria*
- *Verticillium*
- *Nigrospora*
- *Dactilaria*
- *Gliocladium*
- *Pestalotiopsis*
- *Gilmaniella*
- *Idriella*
- *Penicillium*
- *Trichoderma*
- *Acremonium*
- *Fusarium*
- *Sphorotrix*
- *Rhizopus*
- *Phoma*
- *Curvularia*
- *Humicola*
- *Oidiodendron*
- *Geotrichum*
- *Gonytrichum*
- *Mammaria*

459 isolates, 29 genus

Progress 2018

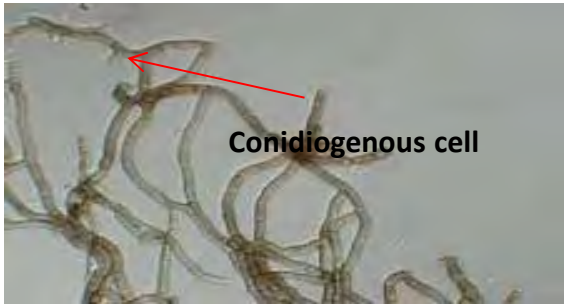
Microbial Identification

Identification of interesting microbial isolates | Fungi

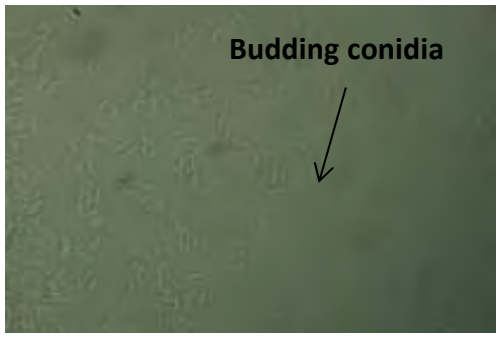
Isolate name	: BioMCC-f.PL.142	Sampling point	: Kupang
Isolation source	: Plant Litter (leaves)	Bioactivity	: MQO inhibitor
Isolation method	: Moist chamber method	Extract code	: F15.1645
Isolation time	: May 2, 2005	DNA analysis result	: 96% similarity to *Aureobasidium*

Micromorphology of BioMCC.f.PL.142 (Fungi)
MEA and PDA medium slide culture, incubate 25^0 C for 7 days

Chlamydospores Brown, 1-celled, cylindrical (young chlamydospores) and ellipsoidal and globose (old) shape,
size (3-)5-9(11)x(3-)5-7(-10) µm



- Conidiogenous cells blastic type, intercalary on hyphae.
size 1 – 2 (3)x 2-3,5 µm
- Hyphae thick, brown color, non septa , size (2,9) 3,9-5,9 µm

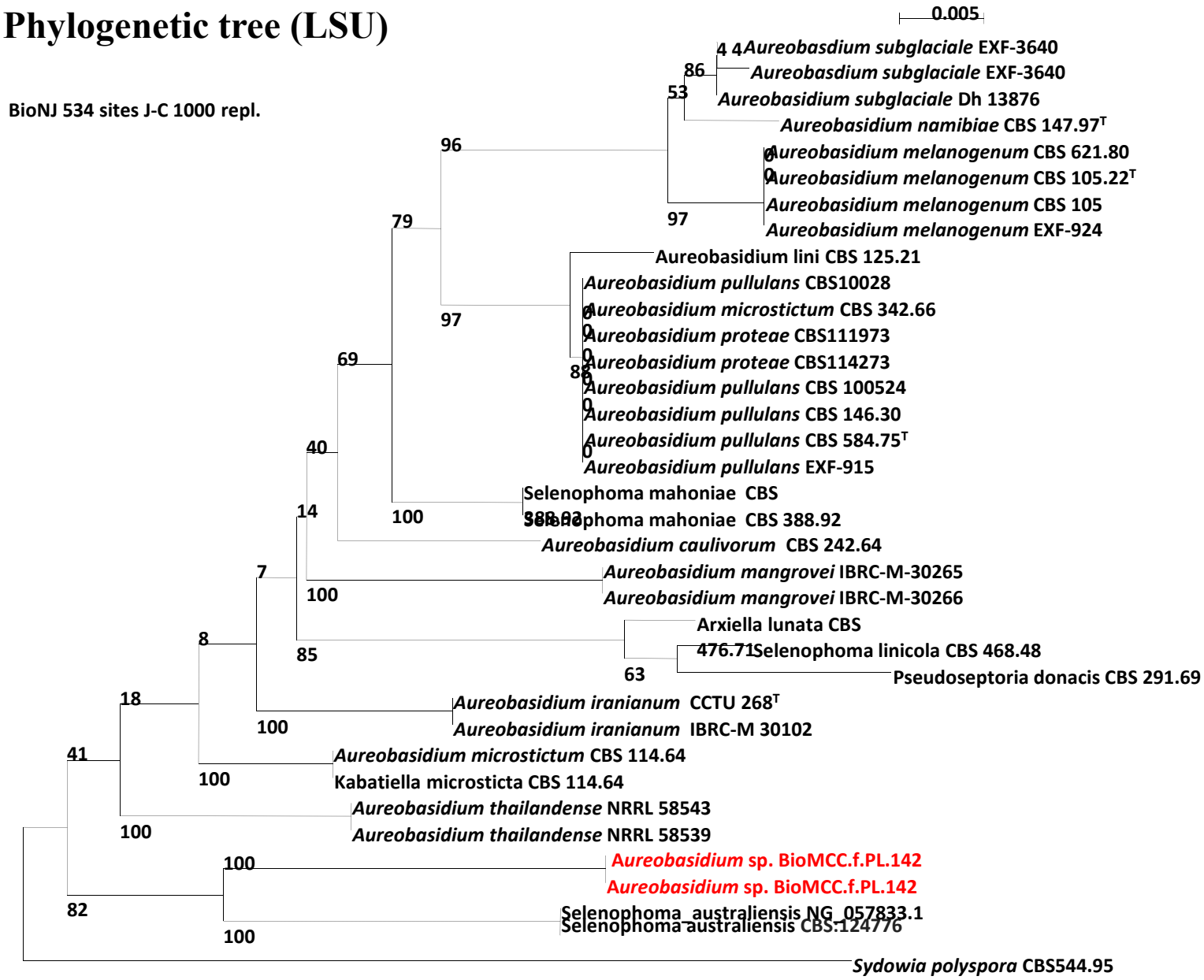


Conidia blastic, smooth, hyaline, 1-celled, ben or curved, typically lunate shaped or less often boomerang, very variable in size.
Size conidia (5-)7-9(-13)x 2-3 µm.
Budding conidia seen

Progress 2018

Microbial Identification

Phylogenetic tree (LSU)



Most probably new strain in genus *Aureobasidium*

Progress 2018

Microbial Identification

Identification of interesting microbial isolates | Actinomycetes

Isolate name	: BioMCC-a.T.2931	Sampling point	: Flores
Isolation source	: Soil	Bioactivity	: -
Isolation method	: Wet soil	Extract code	: -
Isolation date	: Sep 5, 2006	DNA analysis result	: 97% similarity to *Actinoplanes brasiliensis*

Chemotaxonomy of Strain BioMCC-a.T.2931

1. Major menaquinone is MK-9 (H_4) (79%) followed by MK-9 (H_6) (21%), analyzed by LC-MS
2. Cell wall DAP is *meso*-diamonipimelic acid (*meso*-DAP)
3. Whole cell sugars in the strain are glucose, xylose, and arabinose
4. Acyl type of the strain is glycolyl type
5. The strain contains phosphatidyl ethanolamine (PE), and phosphatidyl inositol (PI)
6. The strain doesn't contain mycolic acid

DNA-DNA HYBRIDIZATION

plate

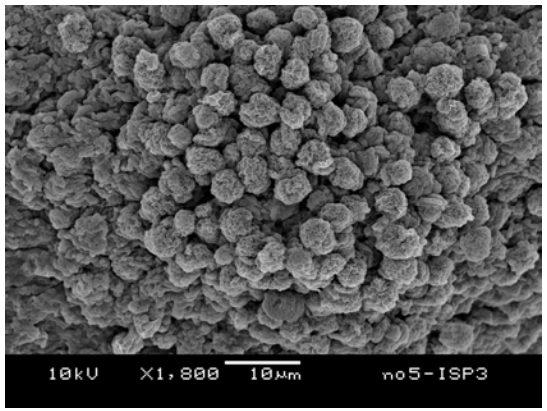
probe	3rd	5.5	nbrc 13938	nbrc 13994	nbrc 110975	nbrc 110796
	5.5	100	14.1	10.8	9.9	13.5
	nbrc 13938	216.7	100			
	nbrc 13994	22.8		100		
	nbrc 110975	10.7			100	
	nbrc 110796	7.5				100

2nd	5.5	nbrc 13938	nbrc 13994	nbrc 110975	nbrc 110796
5.5	100	40	38	67	36
nbrc 13938	30	100			
nbrc 13994	37		100		
nbrc 110975	65			100	
nbrc 110796	73				100

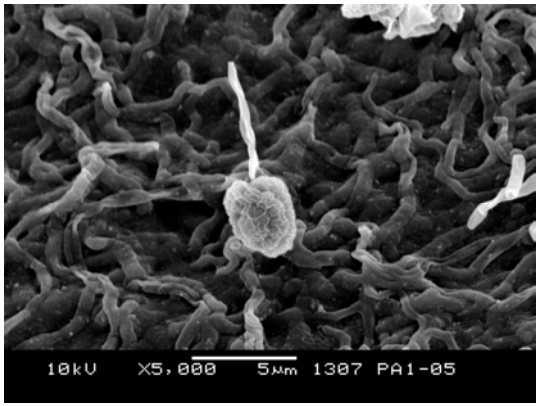
1st	5.5	nbrc 13938	nbrc 13994	nbrc 110975	nbrc 110796
5.5	100	16	27.4	14.9	15.6
nbrc 13938	6	100			
nbrc 13994	28.8		100		
nbrc 110975	8			100	
nbrc 110796	5				100

Most probably new species in genus ***Actinoplanes***

Scanning Electron Microscope



Immature sporangium (2 weeks, on ISP 3)

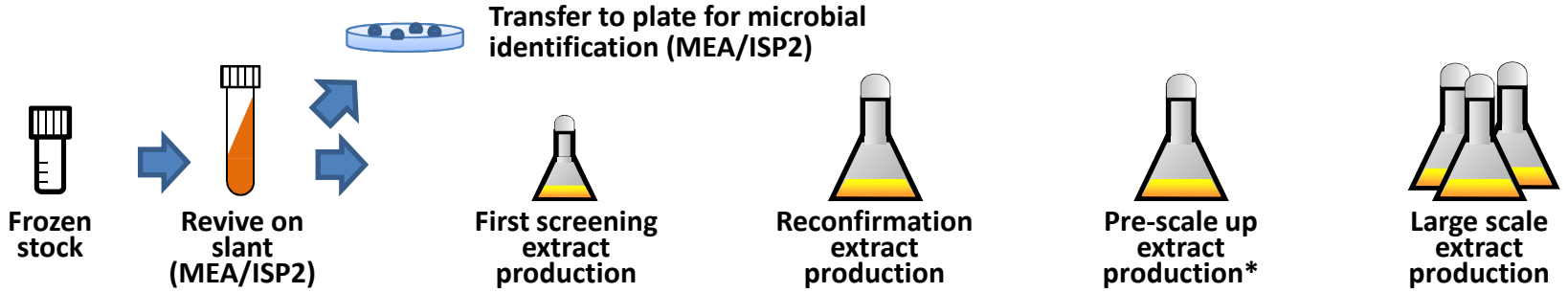


Mature sporangium (3 weeks, on ISP 7)

Progress 2018

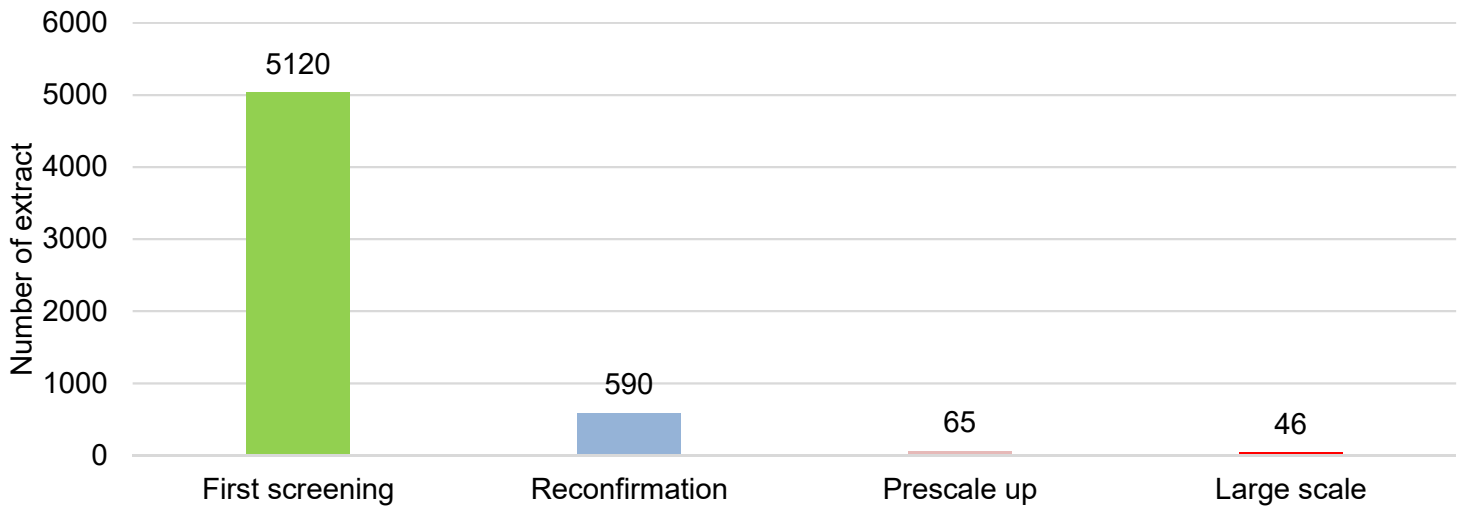
Extract Production

Objective: To produce extracts of natural resources for screening



Culture scale	30 mL	100 mL	100 mL	1000~5000 mL (100 mL x 10 / 50 flasks)
Extract volume	1 mL	600 µL	600 µL	1000~5000 mL
Number of medium	6 medium (fungi), 4 medium (actinomycetes)	2 medium	1 medium	1 medium
Temperature	28˚C			
Sampling point	-	Day 3,4,5	Day 3,4,5,6,7	Day 3 ~ harverst

Result



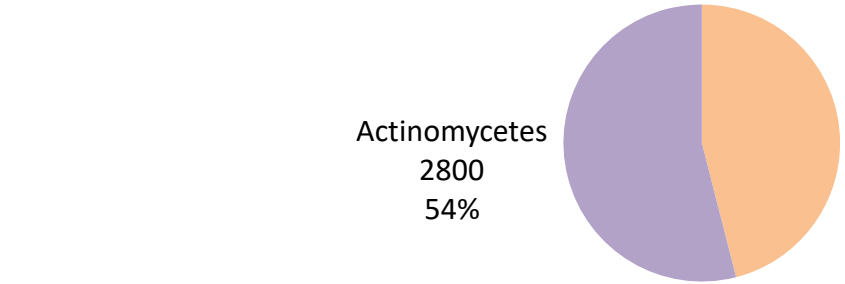
* Pre-scale up extract production was applied from July 2018

Progress 2018

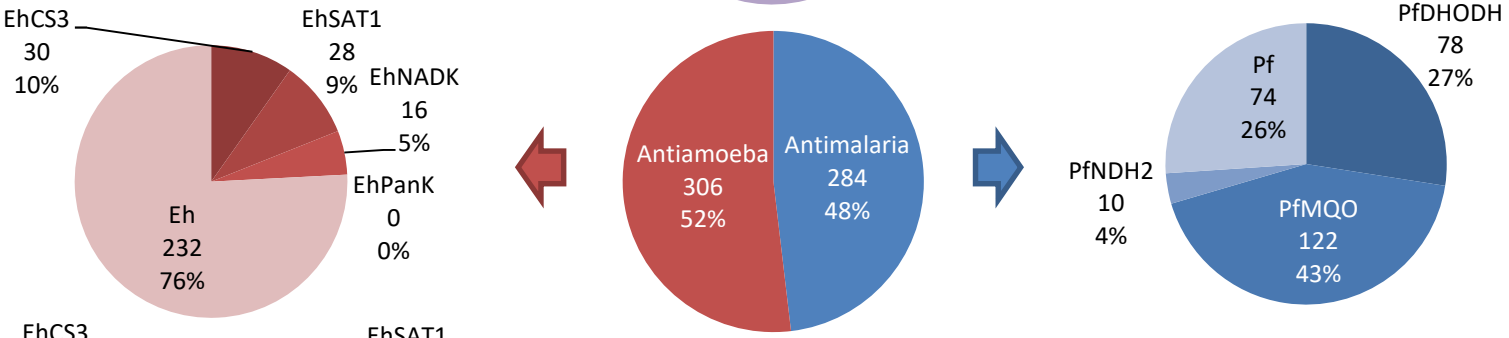
Extract Production

Result

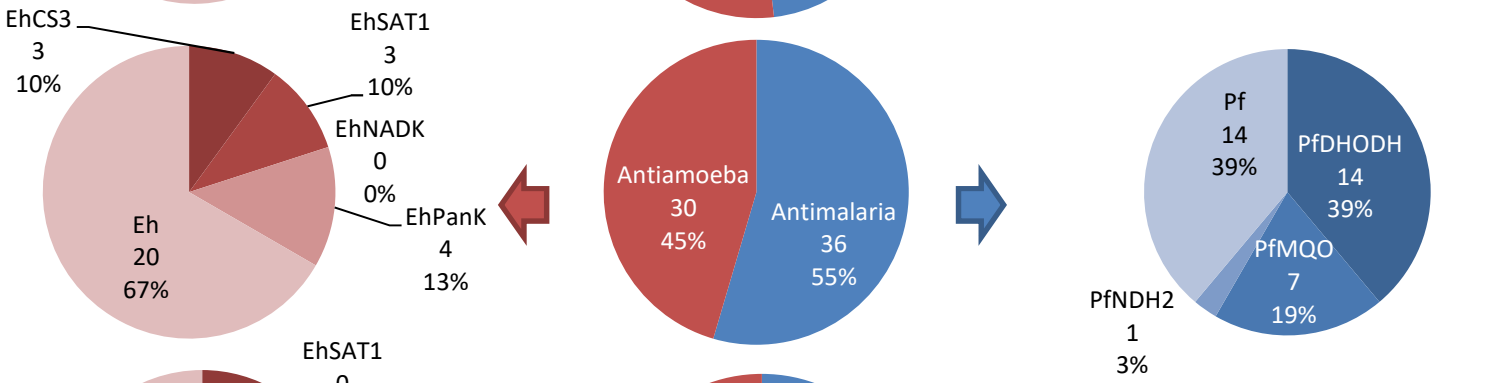
First screening extract production



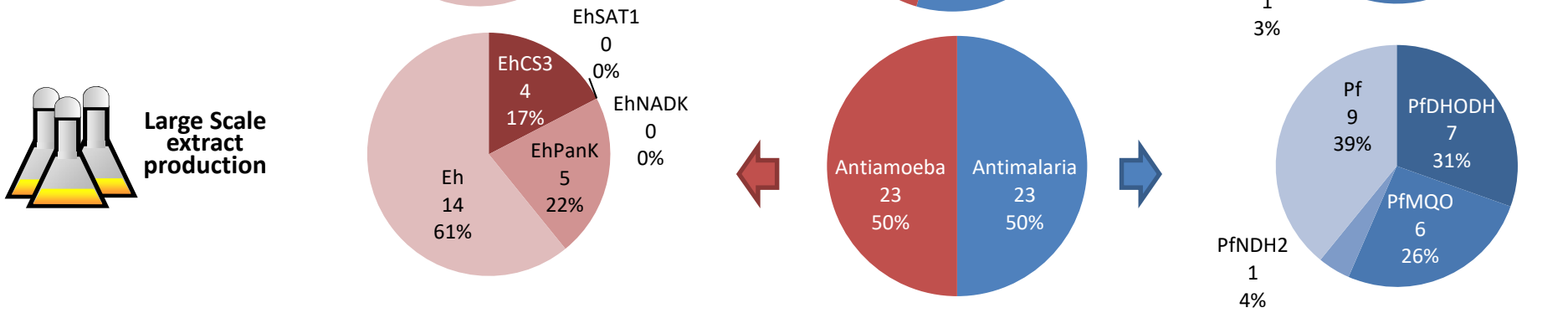
Reconfirmation extract production



Pre-scale up extract production



Large Scale extract production



Progress 2018

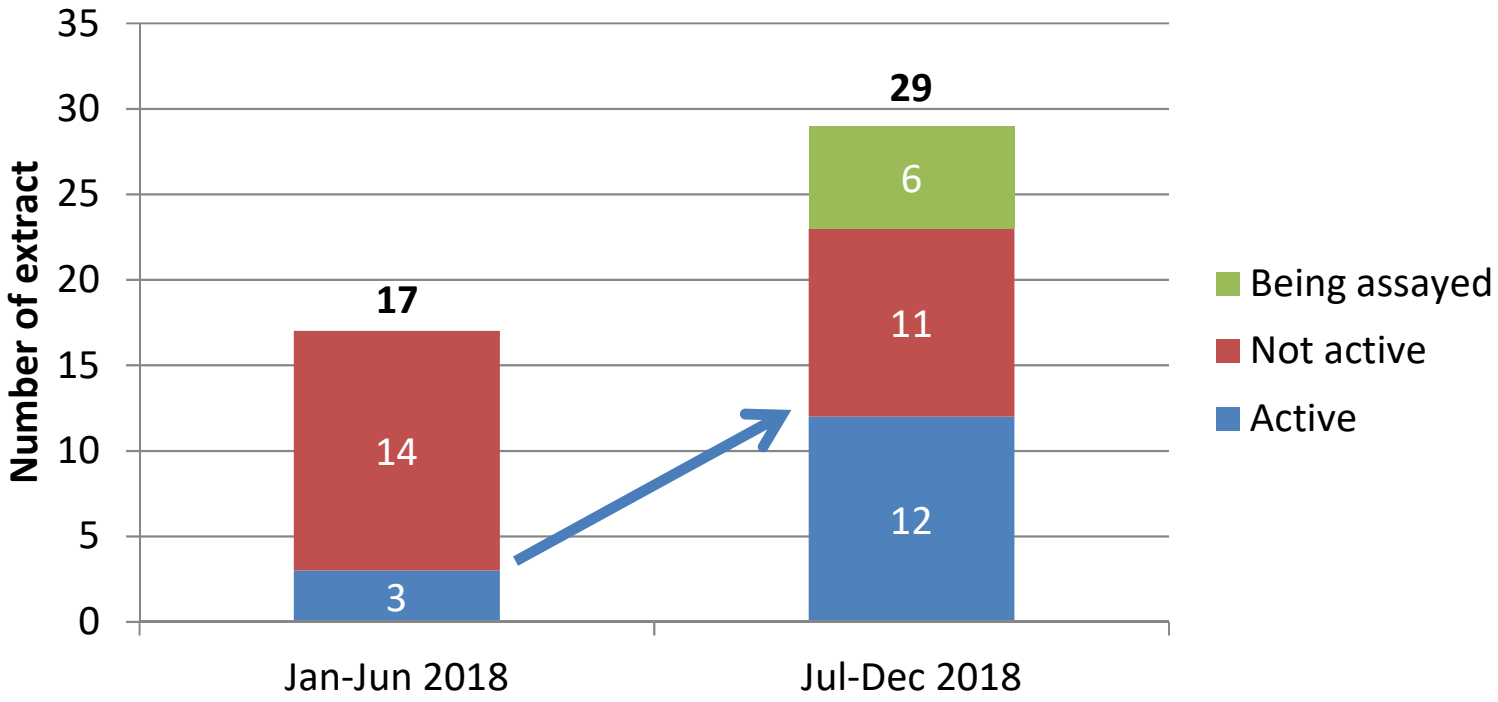
Extract Production

Pre-scale up (PSU) extract production

Objective: to improve reproducibility of active extract production

→Shorten time lag between small scale and large scale culture

→PSU extract production was introduced since July 2018



Reproducibility **17%** **41%**

Reproducibility of active extract for purification was **increased significantly** after PSU extract production was introduced

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

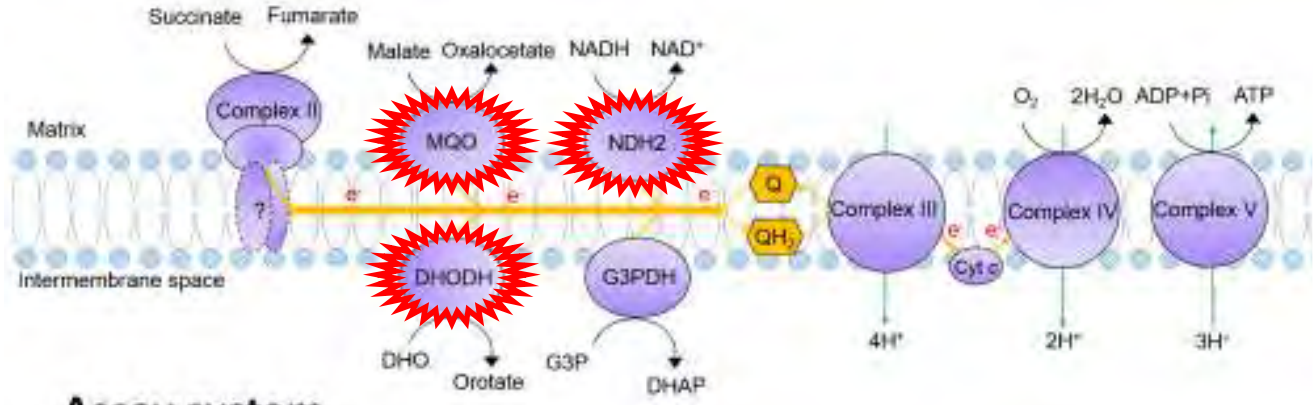
Budget Arrangement

Progress 2018

Enzyme-based screening | **Anti-malarial screening**

Objective: To obtain stable microbial extracts show selective antimalarial activity against target enzyme

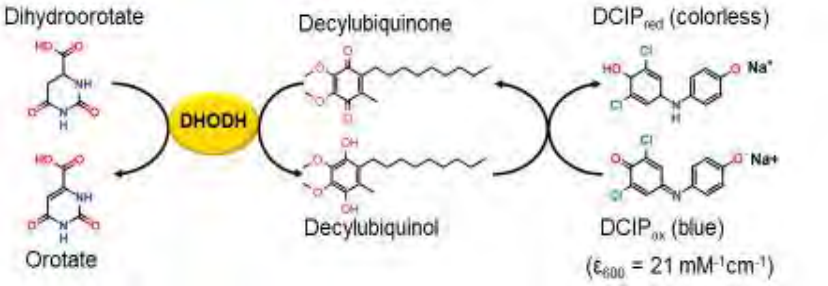
Electron transport chain in *P. falciparum*



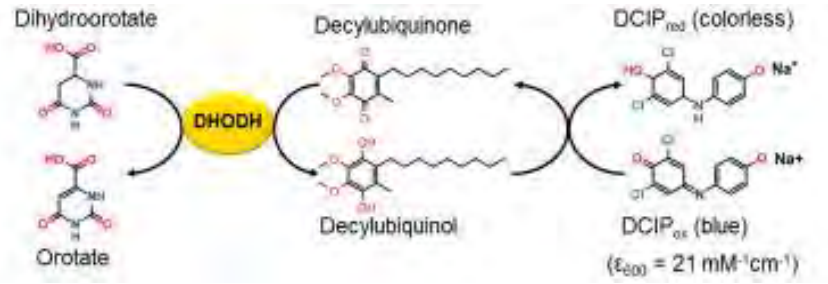
MQO: Malate:Quinone Oxidoreductase; DHODH: Dihydroorotate (DHO) dehydrogenase; G3PDH: Glycerol-3-Phosphate (G3P) Dehydrogenase; DHAP: DiHydroxyAcetone Phosphate; NDH2: Type II NADH Dehydrogenase; Q: Oxidized Quinone; QH_2: Reduced Quinone; Cyt c: Cytochrome c; SQOR: Sulfide:quinone oxidoreductase; EFTDH: Electron-transfer Flavoprotein Dehydrogenase; MDH: Malate dehydrogenase (NAD^+).

Assay system

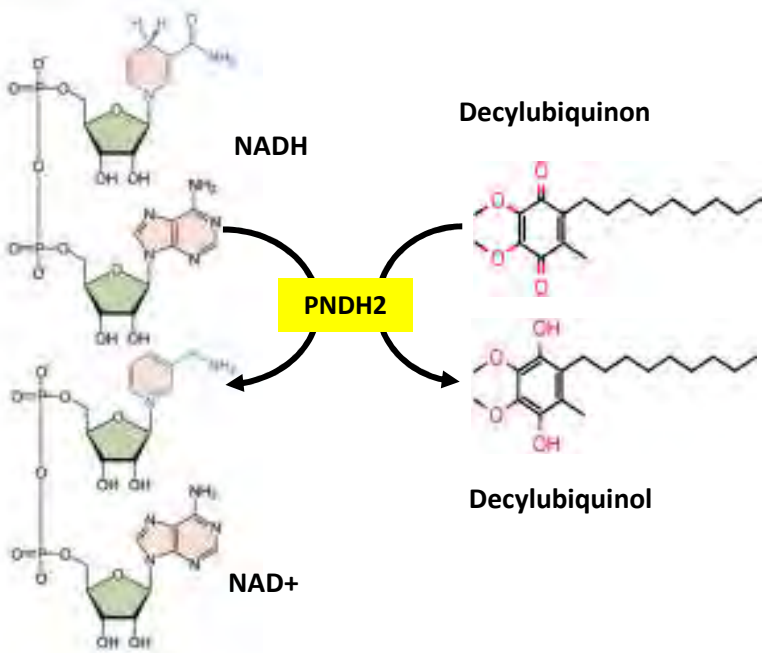
Target enzyme: *Pf*DHODH



Target enzyme: *Pf*MQO



Target enzyme: *Pf*NDH2

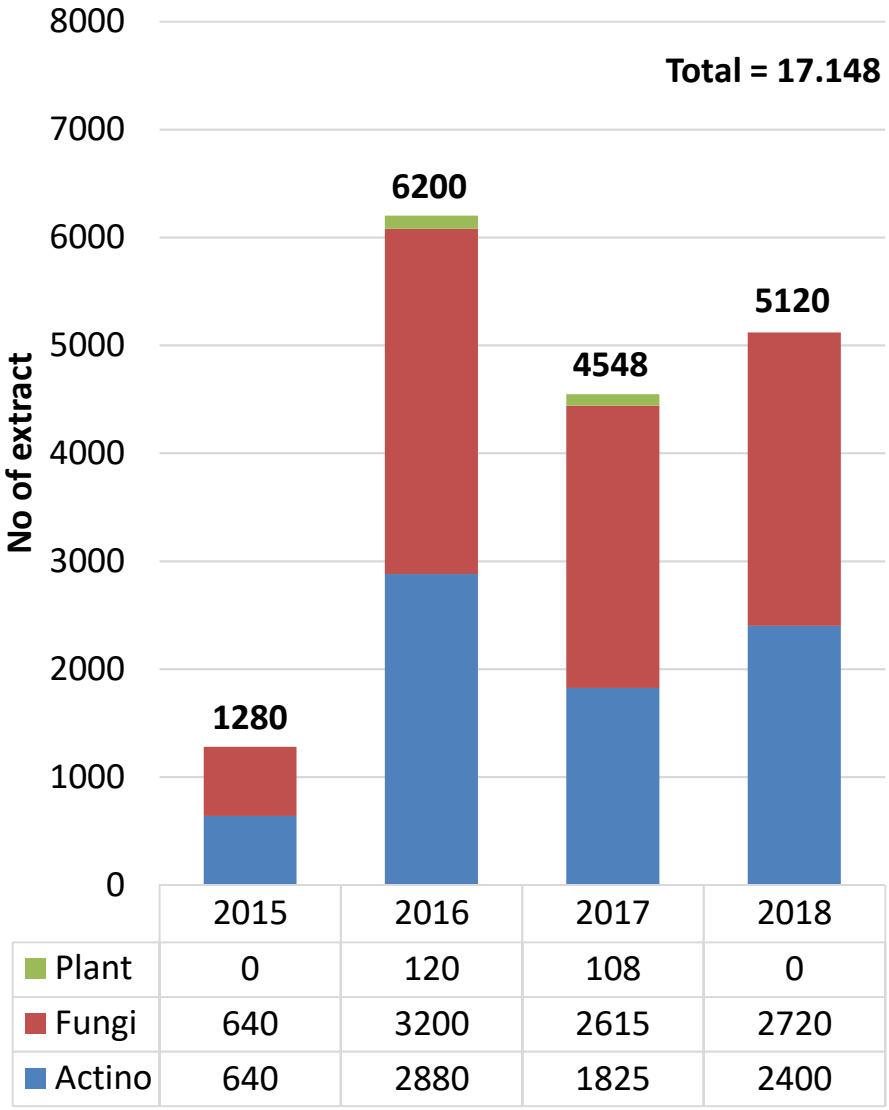


Progress 2018

Enzyme-based screening **Anti-malarial screening**

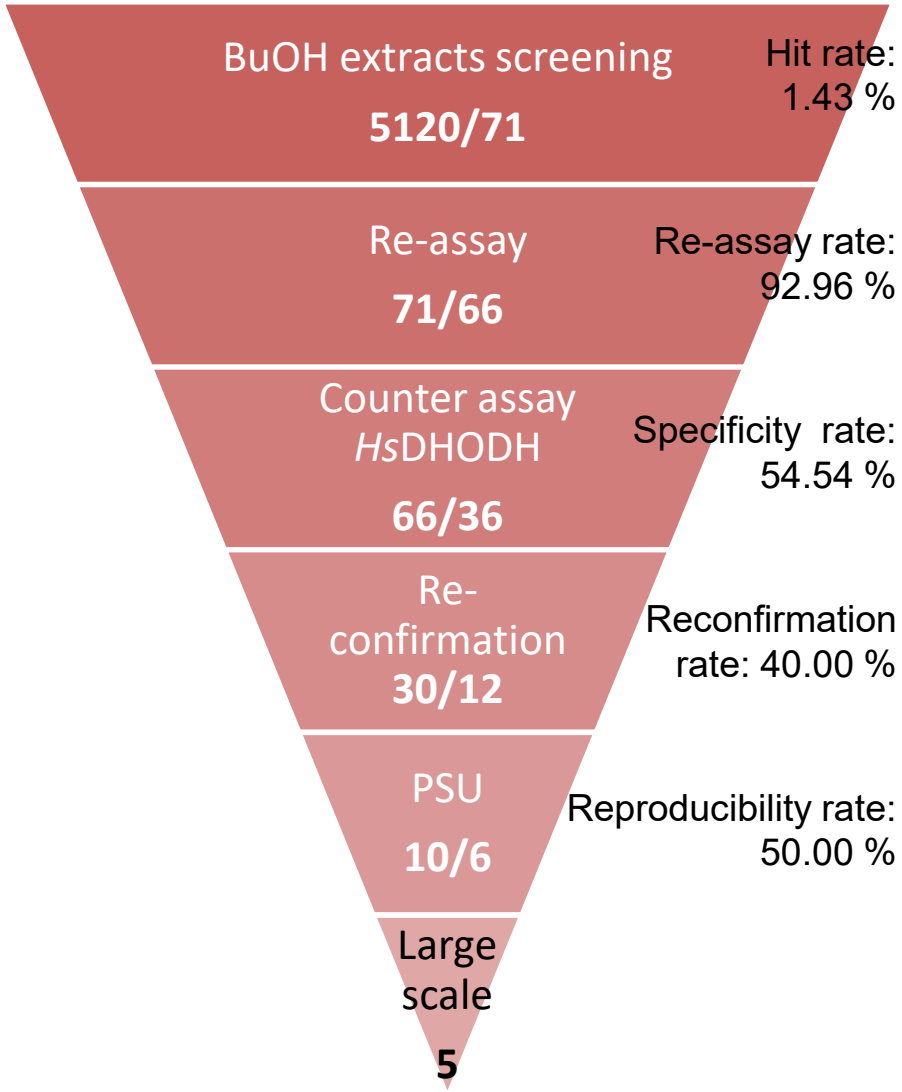
Result

*Pf*DHODH screening



	2015	2016	2017	2018
Plant	0	120	108	0
Fungi	640	3200	2615	2720
Actino	640	2880	1825	2400

Achievement in 2018

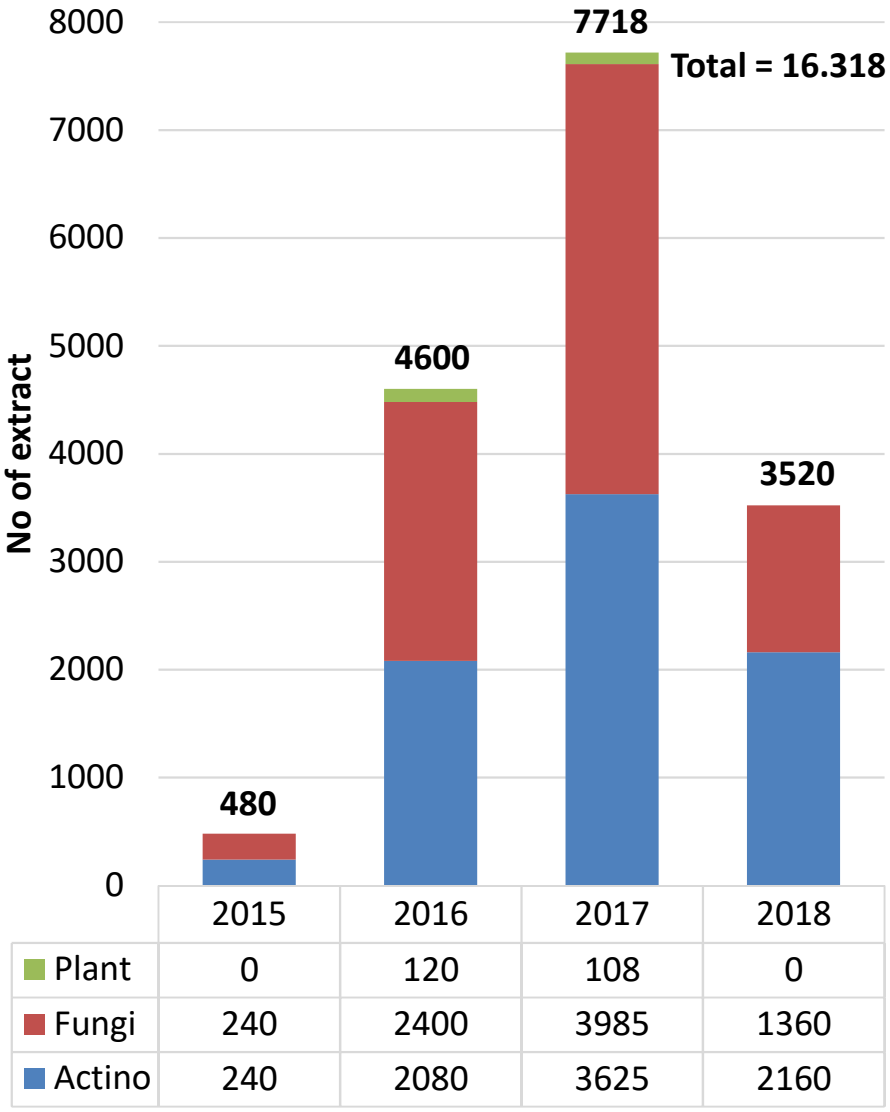


Progress 2018

Enzyme-based screening **Anti-malarial screening**

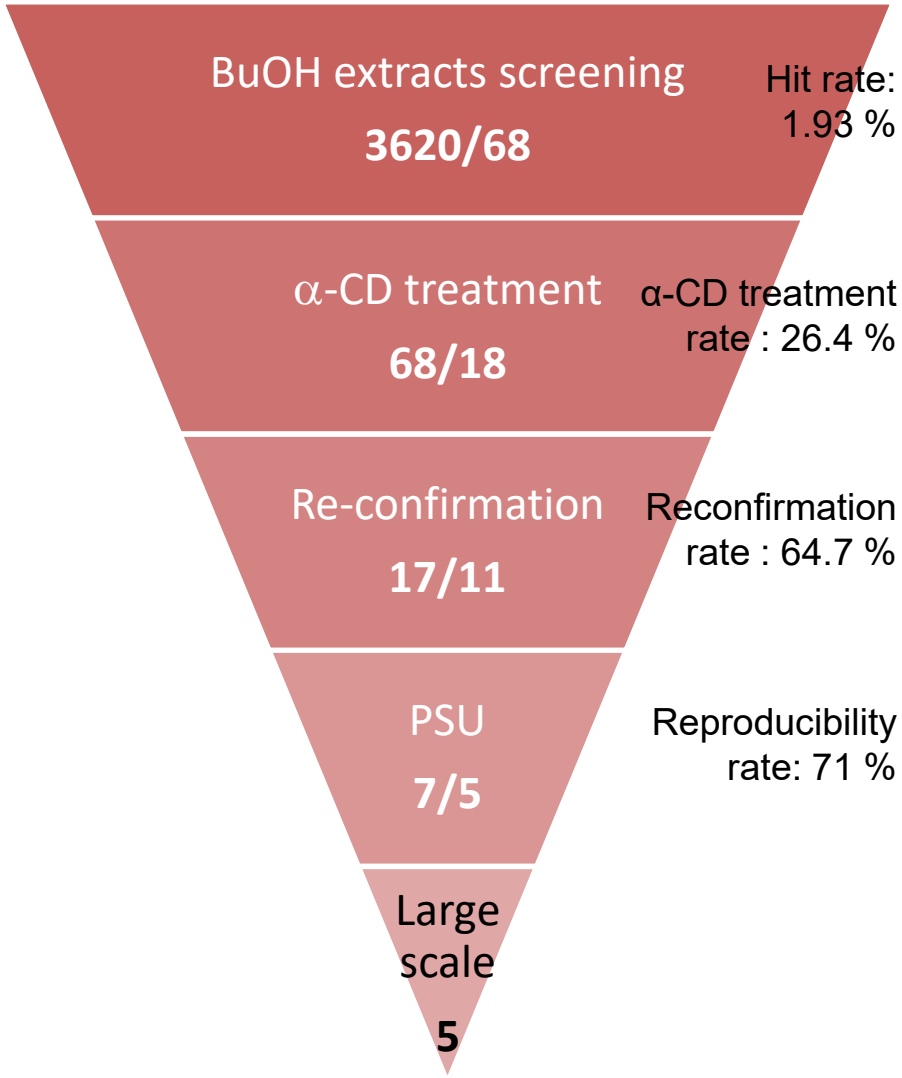
Result

*Pf*MQO screening



	2015	2016	2017	2018
Plant	0	120	108	0
Fungi	240	2400	3985	1360
Actino	240	2080	3625	2160

Achievement in 2018

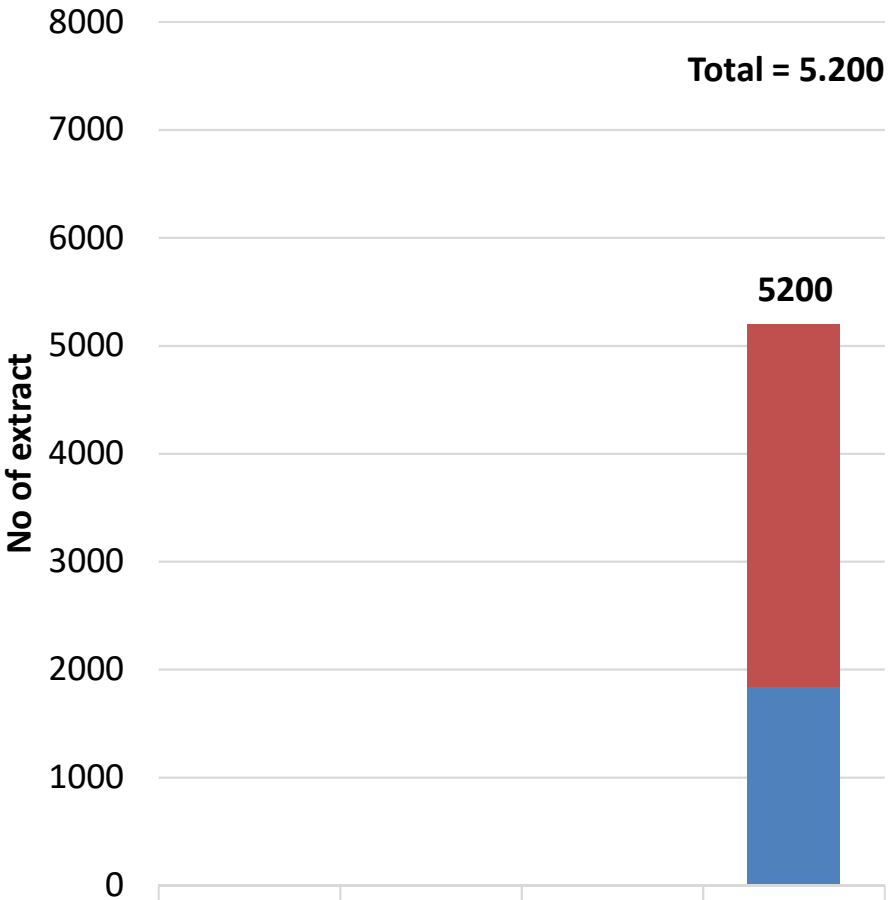


Progress 2018

Enzyme-based screening | **Anti-malarial screening**

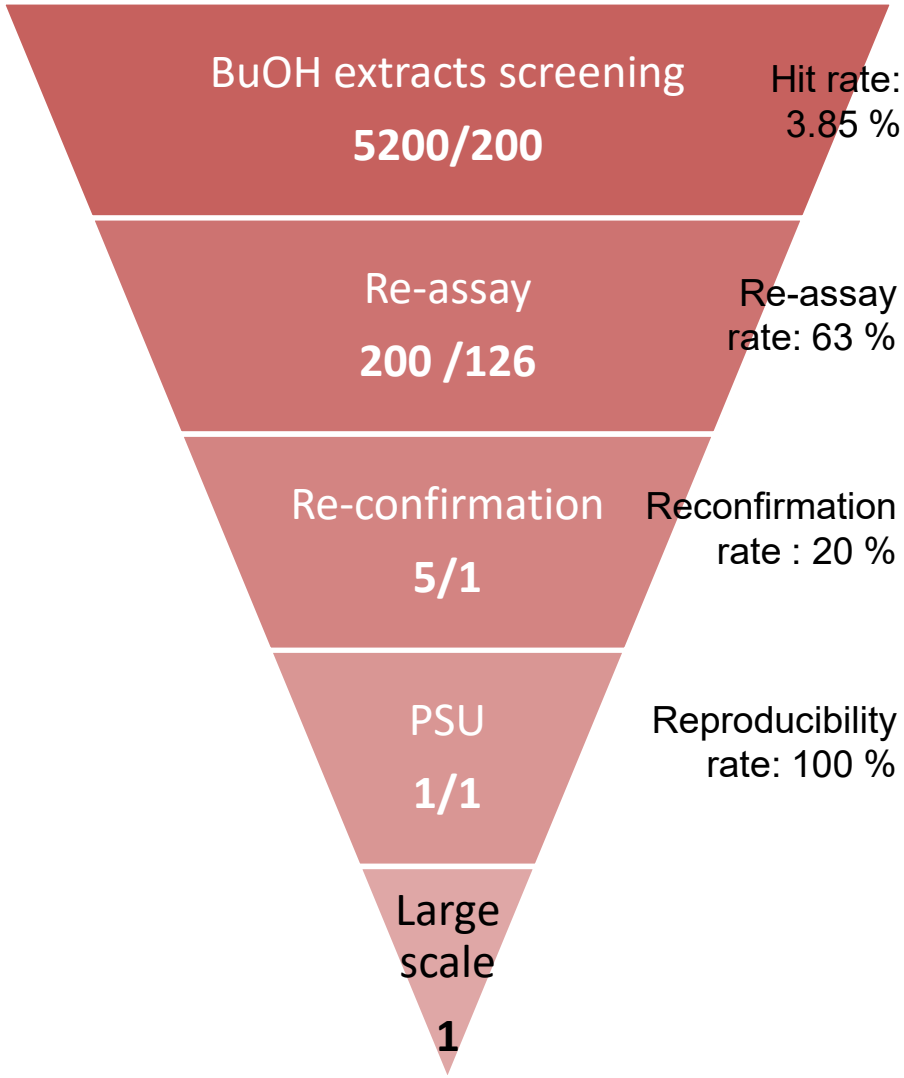
Result

*Pf*NDH2 screening



	2015	2016	2017	2018
Plant	0	0	0	0
Fungi	0	0	0	3360
Actino	0	0	0	1840

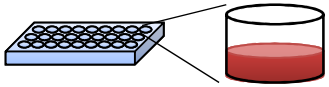
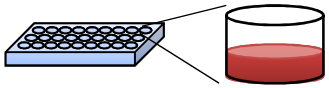
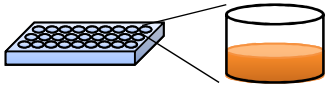
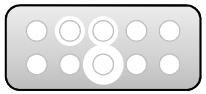
Achievement in 2018



Progress 2018

Cell-based screening **Anti-malarial screening**

Objective: To search extract with inhibitory activity to proliferation of malaria parasite cell

	Objective	Method	Assay Method
First screening	Searching for active microbial extracts	*Pf* 3D7 cell + Microbial extract 	LDH assay
Re-assay	Confirming activity of active extracts	*Pf* 3D7 cell + Active microbial extract 	LDH assay
Toxicity assay	Searching non-toxic active extracts	*Hs* DLD1 cell + Active microbial extract 	WST-8 assay
Dereplication	Searching active extract with non-frequent hit	Gram (+) bacteria + Active non-toxic microbial extract 	Halo-forming assay

Hit

Progress 2018

Cell-based screening **Anti-malarial screening**

Result

	Condition	Number of extract	Number of active extract	Screening rate
First screening	Initial parasitemia=0.3% (ring-form tropozoit) Hematochrit=3% Media=RBC (O+)+Albumax+RPMI Control=Atovaquone, DMSO (max 1%) Extract amount=2.500x dil. (final) Threshold=100%	10.160	713	7%
Re-assay	(Same as first screening)	713	463	65%
Toxicity assay	Initial cell number=$2.5x10^4$ (exponential phase) Media=DMEM+FBS Control=No cell, DMSO (max 1%) Extract amount=25x dil. (final) Selectivity=100x Threshold=50%	463	188	25%
Dereplication	Target=*Bacillus subtilis* ATCC 6633 Media=Nutrient agar Control=Chloramphenicol, DMSO Thershold=no halo (visual observation)	188	83	44%
Hit				
Reconfirmation		35	28	80%
Pre-scale up		10	9	90%
Scale up		8	6	75%

Hit rate = 0.82%

Progress 2018

Cell-based screening

Anti-amebic screening

Objective: To search extract with inhibitory activity to proliferation of amebic parasite cell

Anti-amebic screening result will be reported by AU

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

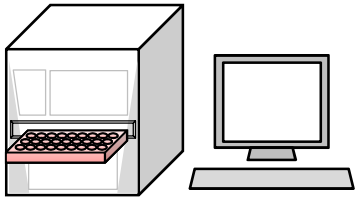
Progress 2018

Purification of active compound

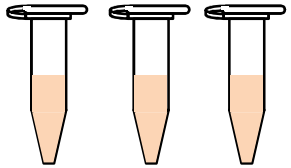
Objective: To obtain purified compound with antimalarial/antiamebic activity

General purification workflow

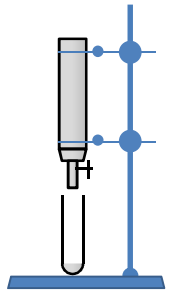
Large scale extract



Activity check
(butanol extract)



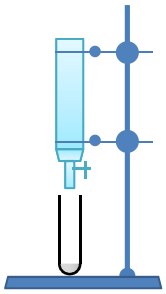
Pre-extraction Test
(PET)
to evaluate properties (stability, polarity) of active extract



Main purification
(activity guided)

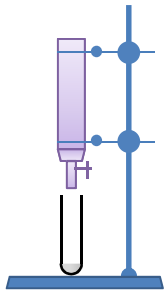
Typical main purification workflow

Extraction with
suitable solvent
(based on PET result)



Partition with
silica column

Partition with
preparative TLC
(silica) or
preparative
HPLC (ODS)



Partition with
size exclusion
(LH-20) column

Purified
compound

Progress 2018

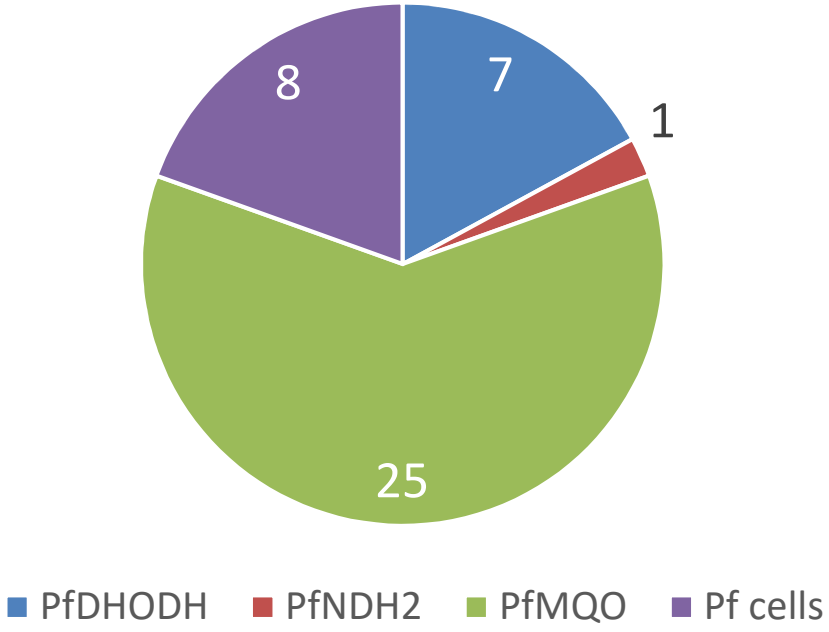
Purification of active compound

Anti-malarial active compound

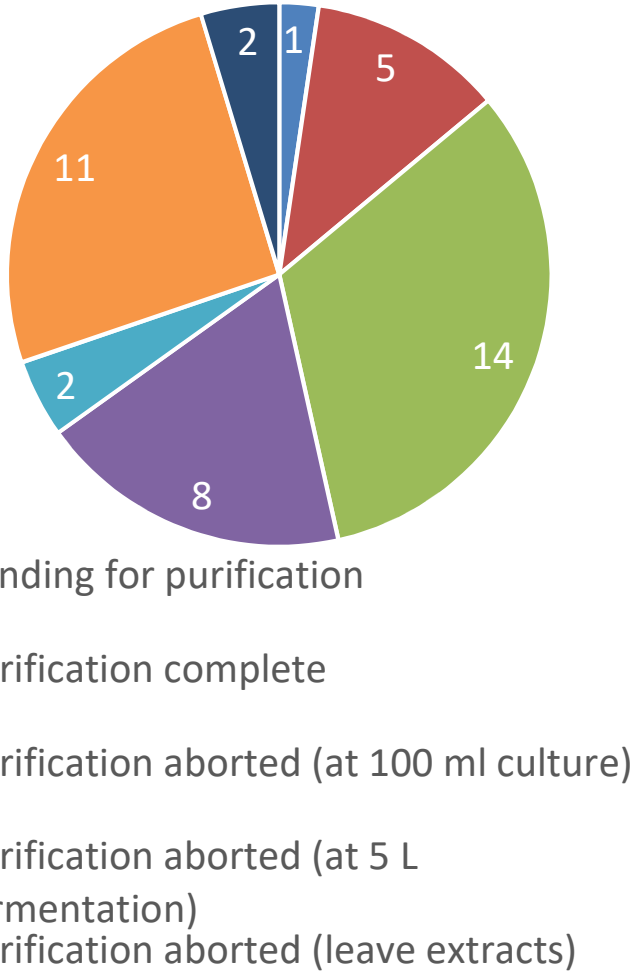
Purified Active Compunds

Total purified extracts = 41

Number of extract for purification in 2018 based on their activity



Resume of extract status for purification in 2018

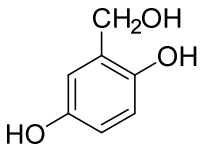
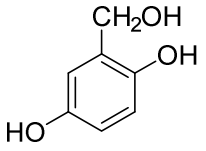
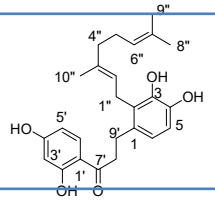
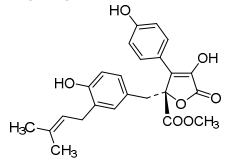
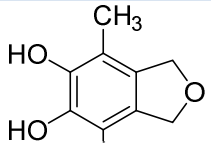
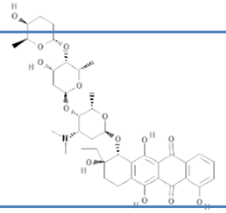


Progress 2018

Purification of active compound

Anti-malarial active compound

Structure Elucidated Active Compounds

Extract Code	Isolate Code	Source	Sampling Point	Isolation Method	Isolate Name	Compound Name	Structure	Activity
F15.1158	BioMCC-f.T.7495	Soil	Ambon	Wet method	*Aspergillus assiutensis* (99% similarity)	2,5 dihydroxy benzoil alcohol		*Pf*DHODH
F15.3082	BioMCC-f.T.5350	Soil	Pangan-daran	Wet method	*Aspergillus sp.* (morphology)	2,5 dihydroxy benzoil alcohol		*Pf*DHODH
Bread fruit (leave)	-	Plant	Tangsel	-	*Artocarpus altilis*	3,4 2',4'-tetrahydroxy-2-geranylchalcone		*Pf*MQO
F15.2274	BioMCC-f.T.1757	Soil	Flores	Lithium chloride method	*Aspergillus sp.* (morphology)	Butyrolactone-I		*Pf*DHODH
F15.2438	BioMCC-f.T.4328	Soil	Jepara	Wet method	*Aspergillus neoflavipes* (99% similarity)	1,3 dihydro- 7 ethyl-4,5,6-isobenzophurantriol		*Pf*MQO (false positive compound)
A21.1497	BioMCC-a.T.3335	Soil	Madura	Acid treatment method	*Streptomyces sp.* (morphology)	Cosmomycin		*P.falciparum*

Progress 2018

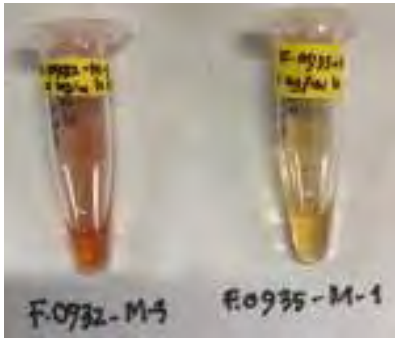
Purification of active compound | **Anti-amebic active compound**

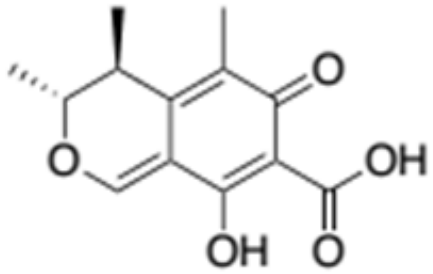
Extract code: F.0935

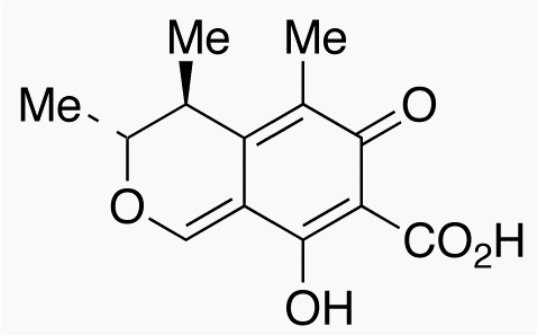
Isolate name	: *Penicillium citrinum*	Isolation time	: May 2, 2005
Isolate code	: BioMCC-f.mo.043	Sampling point	: Banjarmasin
Isolation source	: Marine organism	Bioactivity	: *E.histolytica* cell growth
Isolation method	: …	DNA analysis result	: 100% similarity *P.citrinum*

IC_{50} determination of identified compounds and its standard against *E. histolytica* cell

compounds	µg/ml	µM
F.0932-M-3	3.9 ± 0.2	15.6 ± 0.8
F.0935-M-1	8.1 ± 0.4	32.3 ± 1.6
Citrinin standard (Toronto, C523500)	40.8 ± 2.1	163.1 ± 8.4







F.0932-M-3; F.0935-M-1 *Citrinin standard (Toronto, C523500)*

* Purification was conducted at The University of Tokyo

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

Progress 2018

Training

Training in Japan

BPPT

No	Nama	Title	Period	Venue
1	Danang Waluyo	Determination of target for drug discovery	Mar 1-23, 2018	The University of Tokyo
2	Eka Siska	Purification of active compound	Sep 3-29, 2018	Kitasato University
3	Evita Chrisnayanti	Purification of active compound	Sep 24 – Oct 20, 2018	Kitasato University
4	Avi Nurul Oktaviani	Identification and characterization of Actinomycetes	Sep 3 – Dec 22, 2018	Kitasato University
5	Kristiningrum	Identification and characterization of Fungi	Oct 31 – Nov 29 , 2018	Kitasato University
6	Danang Waluyo	Determination of target for drug discovery	Nov 12 – Dec 7, 2018	The University of Tokyo

AU

No	Nama	Title	Period	Venue
1	Dr. Myrna Adianti	Cell toxicity assay and new enzyme assays for antiamebic compound discovery	Jan 8-29, 2018	The University of Tokyo
2	Ms. Hilkatul Ilmi	Cell toxicity assay and new enzyme assays for anti-Malaria discovery	Nov 4 – Dec 1, 2018	Nagasaki University
3	Ms. Lidya Tumewu	Structure elucidation of active compound	Sep 2-30, 2018	The University of Tokyo

Progress 2018

Training

On-site Training

No	Name	Institution	Topic	Period
1	Prof. Tomoyoshi NOZAKI	Univeristy of Tokyo	Progress Monitoring	25 Januari - 6 Feb 2018 6 - 15 Maret 2018 8 - 16 Mei 2018 27 Juni – 4 Juli 2018 9 – 13 September 2018 27 Nov – 7 Des 2018
2	Dr. Azuma WATANABE	MicroBioFarm Japan	Isolation, Purification and Structure Analysis of Chemical Compounds	30 Jan – 3 Feb 2018
3	Prof. Kazuro SHIOMI	Kitasato University	Isolation, Purification, and Structure Analysis of Chemical Compounds	28 Jan – 3 Feb 2018
4	Dr. Kazuyuki DOBASHI	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	21 Jan – 2 Feb 2018 19 April – 16 Mei 2018 25 Juli – 17 Agustus 2018 21 Nov - 13 Desember 2018
5	Dr. Mihoko MORI	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	24 Jan – 10 Februari 2018 7 – 18 Mei 2018 22 Agustus – 6 Sept 2018
6	Dr. Ken Daniel INAOKA	Nagasaki University	Malaria (Investigation and Analysis)	28 Jan - 3 Februari 2018 2 Juli – 13 Juli 2018
7	Dr. Takaya SAKURA	Nagasaki University	Malaria (Investigation and Analysis)	28 Jan – 3 Feb 2018 7 – 18 Mei 2018 2 – 13 Juli 2018
8	Dr.Michio YAMASHITA	University of Tokyo	Isolation, Purification, and Structure Analysis of Medical Compounds	28 Jan - 24 Feb 2018 24 Juni – 21 Juli 2018
9	Dr. Katsuhiko ANDO	Kitasato University	Collection and Isolation of Microbial Resources	7 - 18 Mei 2018 26 Agust – 7 Sept 2018
10	Dr. Toru OKUDA	Kitasato University	Isolation, Purification and Structure Analysis of Chemical Compounds	14 – 18 Mei 2018 27 – 31 Agustus 2018
11	Dr. Toshiyuki TOKIWA	Kitasato University	Isolation, Purification, and Structure Analysis of Medical Compounds	28 – 31 Agustus 2018

Progress 2018

Training

Impact

Microbial isolation and identification

- Increased capability of morphology-based identification
  →Diversity of newly isolated microbes were increased
- Increased capability of identification of interesting microbe
  →Identification of new microbial species were performed

Extract production

- Increased reproducibility of active extract production
  →Number of extracts those lost their activities were reduced
- Increased capability on managing microbial extract
  →Request-based extract production management system was established

Screening system

- Increased capability on development of target for drug screening
  →A new screening system was proposed and developed (anti TB)

Purification and elucidation of active compound

- Increased capability on active compound purification
  →Number of purified and structure-elucidated active compounds were increased

Progress 2018

Technical meeting

Objective: To evaluate and monitor progress of the project

Scientific meeting

- 5 times (Feb 1, Mar 12, Jul 2, Oct 3, Nov 28)
- Agenda: Progress report and problem solving
- Supervised by Project Advisor

Weekly meeting

- Once a week (every Thursday)
- Agenda: Progress report of each team
- Supervised by Project Co-manager

Annual meeting

- Twice (Feb 14, Dec 20)
- Agenda: Evaluation and planning the project
- Supervised by Project manager and co-manager

Progress 2018

Networking

Airlangga University

- July 5th, 2018
- Technical discussion on progress of anti-amebic screening

LIPI

- Oct 31th, 2018
- Technical discussion on microbial preservation and sharing of microbial isolates for screening

Gadjah Mada University

- Nov 2nd, 2018
- Initiation of collaboration on development of anti-cancer agents

Progress 2018

Networking

Obihiro University of Agriculture and Veterinary Medicine

- Collaboration on development of anti-toxoplasmolysis agents by utilizing Indonesian bio-resources
- MTA was signed on Aug 25th, 2017
- More than 3800 microbial extracts were screened by end of 2018
- Currently, reconfirmation extracts are being produced

The University of Tokyo

- Collaboration on development of anti-tuberculosis agents by utilizing Indonesian bio-resources (together with Airlangga University, funded by TB Alliance USA)
- MTA was signed on July, 2018
- More than 3500 microbial extracts were shared for first screening
- Currently, screening are being performed in AU

Brawijaya University

- Providing training on *in vitro* anti-malarial assay (LDH assay)
- Training was conducted on Sep 4-7, 2018 at BTC-BPPT
- Attended by 2 trainees

Progress 2018

Microbial Isolation, Identification, and Extract Production

Screening of Active Extract

Purification of Active Compound

Other Activities

Budget Arrangement

Progress 2018

Budget and Expenses

BC for SLeCAMA project 2018

- Budget = Rp. 418.444.000

Insinas MoRTHE 2018

- Budget = Rp. 175.000.000

→ **Total = 593.444.000**

Description	Expenses (Rp.)	Note
Chemical & laboratory supplies	197.962.400	Incl. gases and liquid gases
Salary	204.174.080	Salary for not permanent BC member
Travel	43.675.100	Field trip, visit AU&LIPI
Equipment	137.162.000	AC, Printer
TOTAL	**582.973.580**	

Planning 2019

Planning 2019

Project Planning

1. Microbial isolation and identification
   →Isolation of microbial strain (from Bawean Island)
   →identification/taxonomy studies of isolated microbes

2. Extract production
   →Improving extract production management system
   →Improving reproducibility of microbial active extract

3. Screening
   →Continuing screening of extracts (plants, microbes

4. Purification
   →Establishment of new dereplication method

5. Efficacy test
   →Testing active compound in animal model (to be done under collaborative research with Brawijaya University)

Planning 2019

Activity Planning

1. Field trip for microbial sampling
   →Time: April 23-26, 2019 (tentative)
   →Venue: Bawean Island

2. International symposium
   →Time: Mid October, 2019
   →Venue: Jakarta (tentative)

3. Publication
   - Scientific journal: submission of at least 2 papers into scientific journal
   - Conference: participating in Asian Mycological Congress (Oct 1-4, 2019, Mie, Japan)

Networking Planning

Brawijaya University: Efficacy test of anti-malarial active compound
Gadjah Mada University: Screening of microbial extracts with specific anti-cancer activity
Obihiro Univ. of Agric.Vet.Med: Purification of anti-toxoplasmolysis agents

Planning 2019

Budget Arrangement

- BPPT allocated budget for FY 2019 as much as **Rp. 699.998.000**

- 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. 317.000.000**

Description	BPPT Budget (Rp.)	Note
Salaries	184.320.000	Salary for not permanent BC member
Reagents and consumables	218.800.000	Incl. gases and liquid gases
Travel	135.417.000	Transportation (airfare, sea, ground), accomodation, daily allowance
Equipment	75.000.000	Laboratory bench, etc.
Meeting	86.461.000	JCC Meeting, International symposium
TOTAL	**699.998.000**	

Target Review (2018)

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 2018
Blue: partially achieved 2018

Thank You

SATREPS **SLeCAMA Project**

JCC fourth year

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

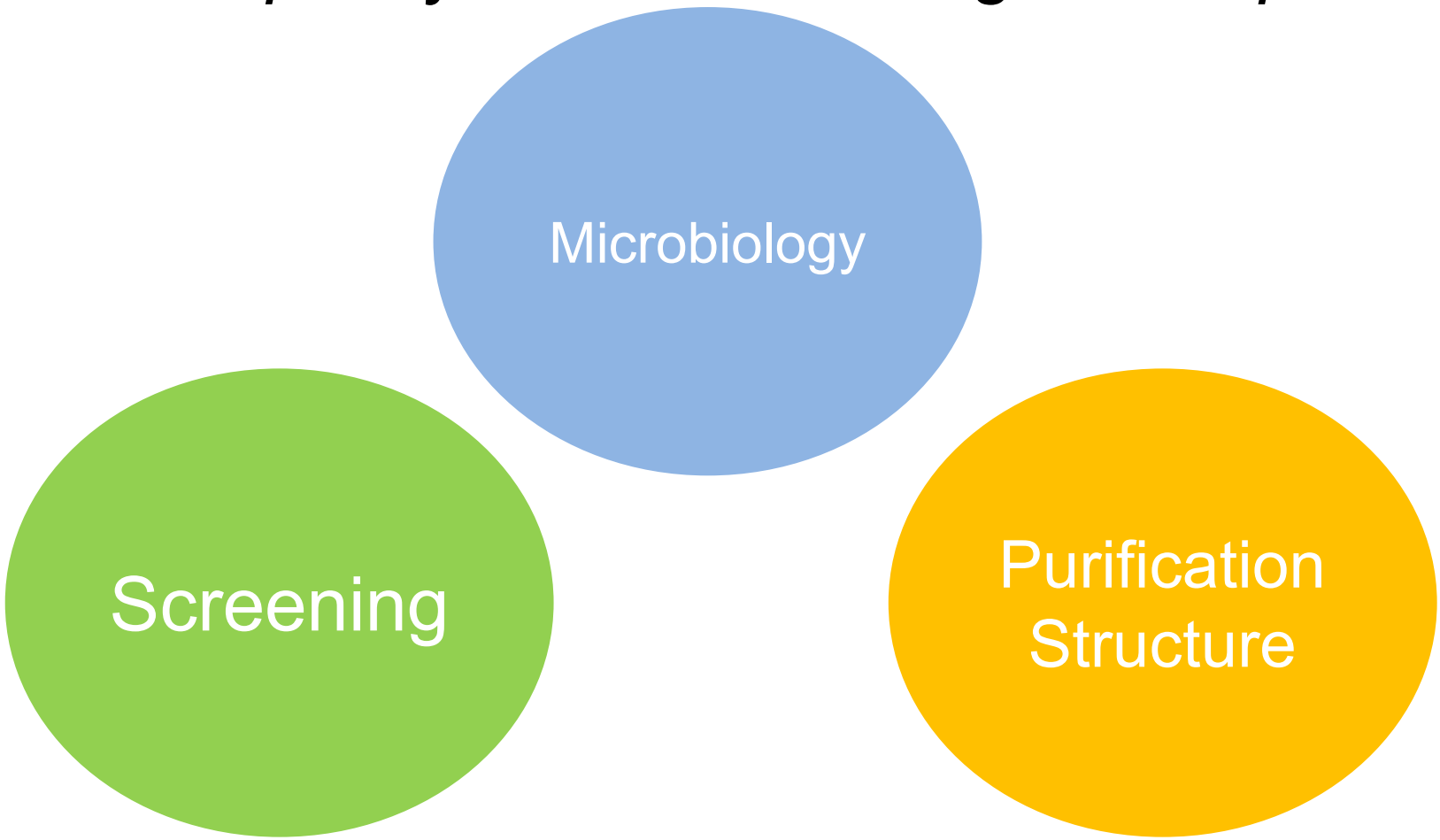
Achievements, needs, and solutions

Tomo NOZAKI
The University of Tokyo
Chief Advisor

Jakarta, January 29th, 2019

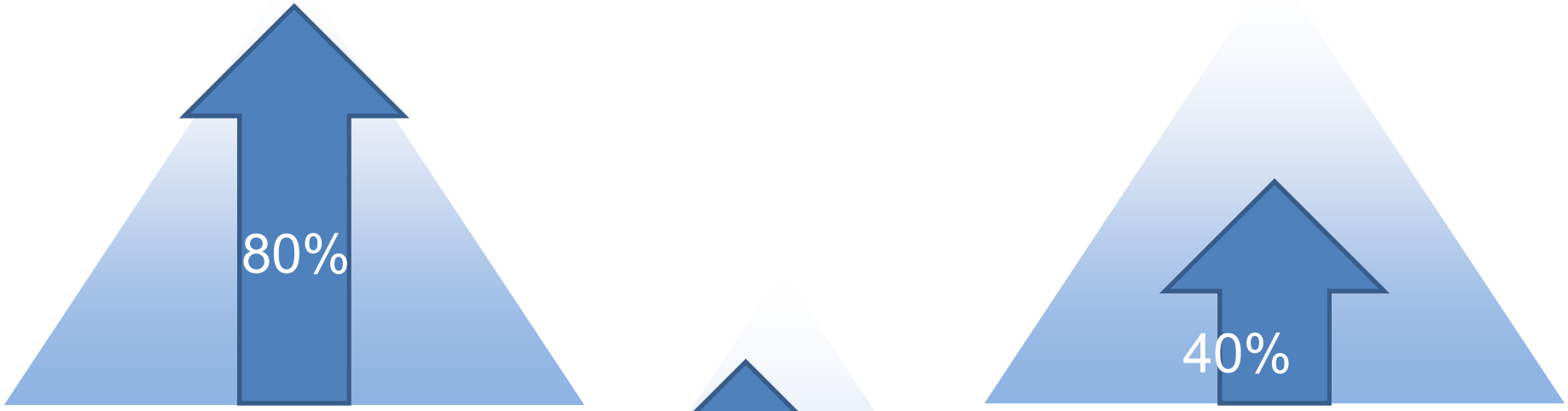
Goals of the project

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



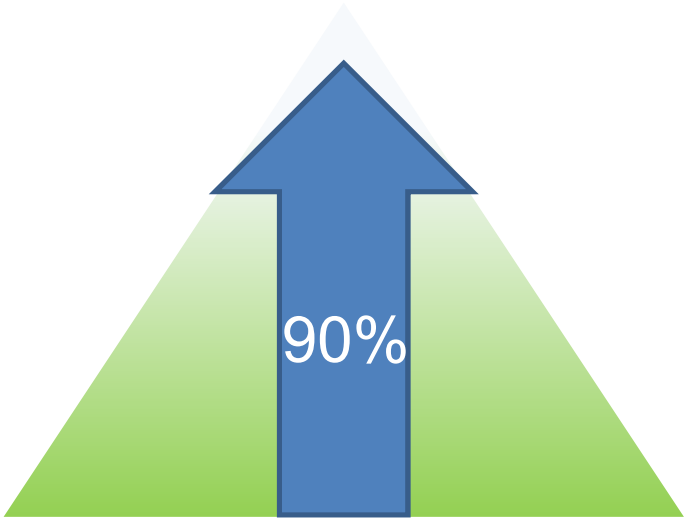
Microbiology
Sampling/Isol
ation/Culture

Microbiology
Classification
/Taxonomy

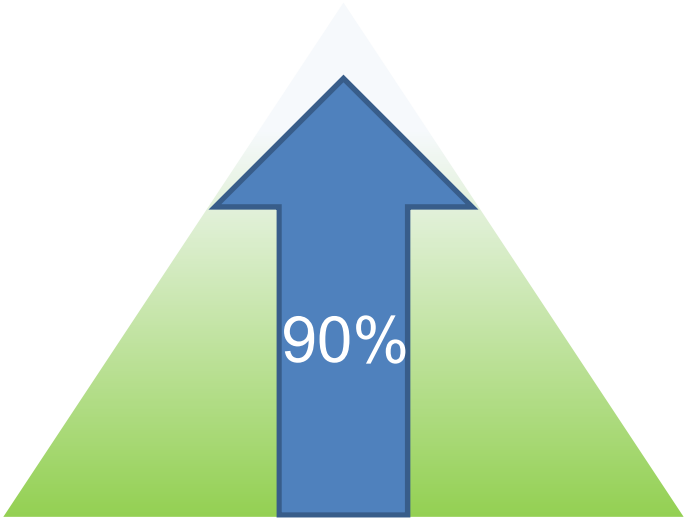
Microbiology
Extract production
Data management

Accomplishment of goals

Research Capacity Building – Screening



Screening
Enzyme-based assay

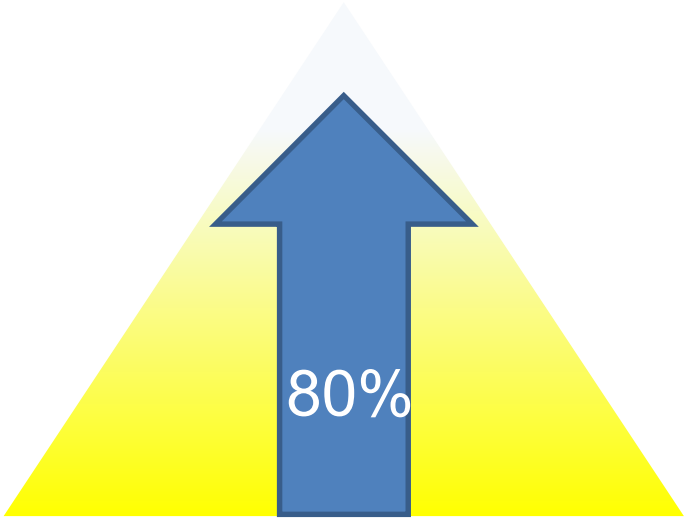


Screening
Cell-based (phenotypic) assay

Accomplishment of goals

Research Capacity Building

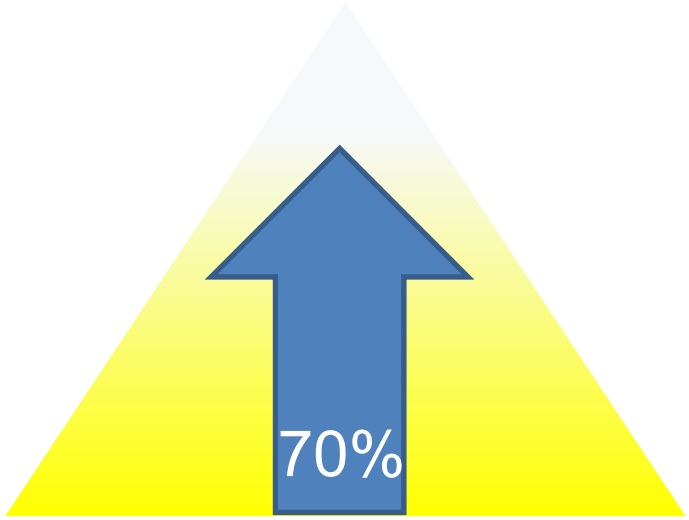
– Purification and structural elucidation



Purification

Liquid partition

Chromatography



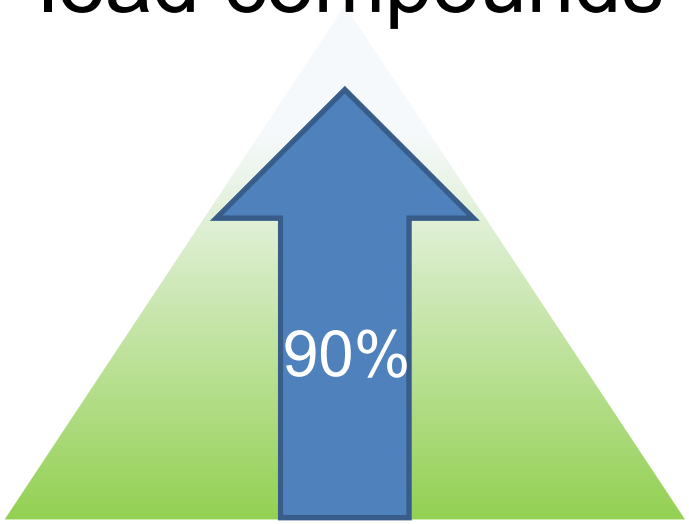
Structural elucidation

Mass spectrometry

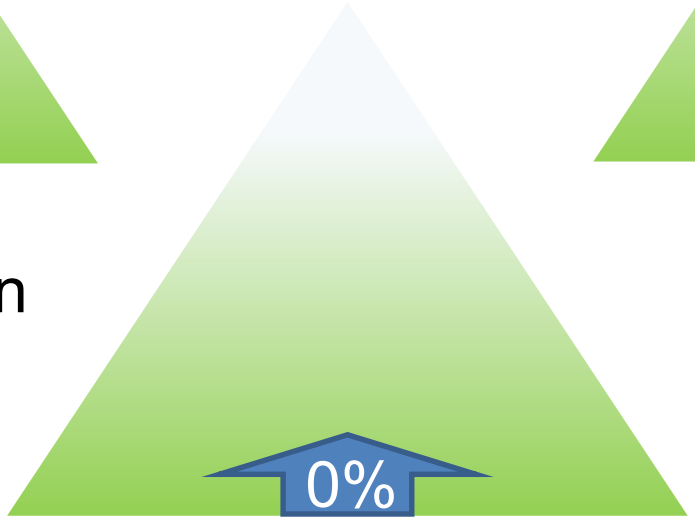
Nuclear magnetic resonance

Accomplishment of goals

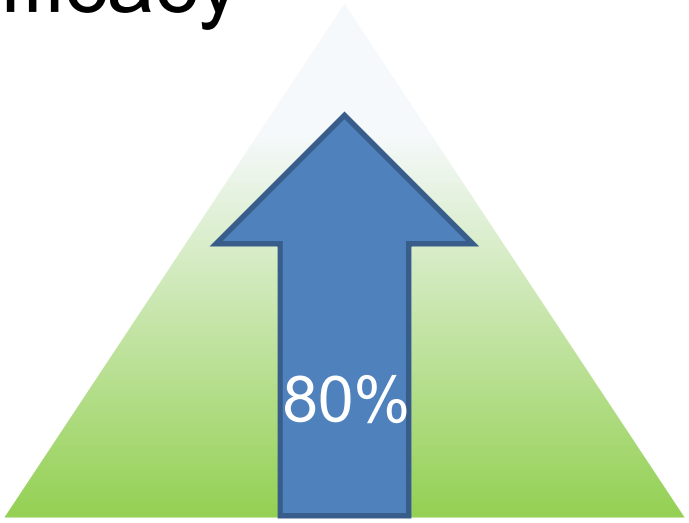
Identification of anti-malarial and anti-amebic lead compounds with in vivo efficacy



Antimalarial
Lead identification

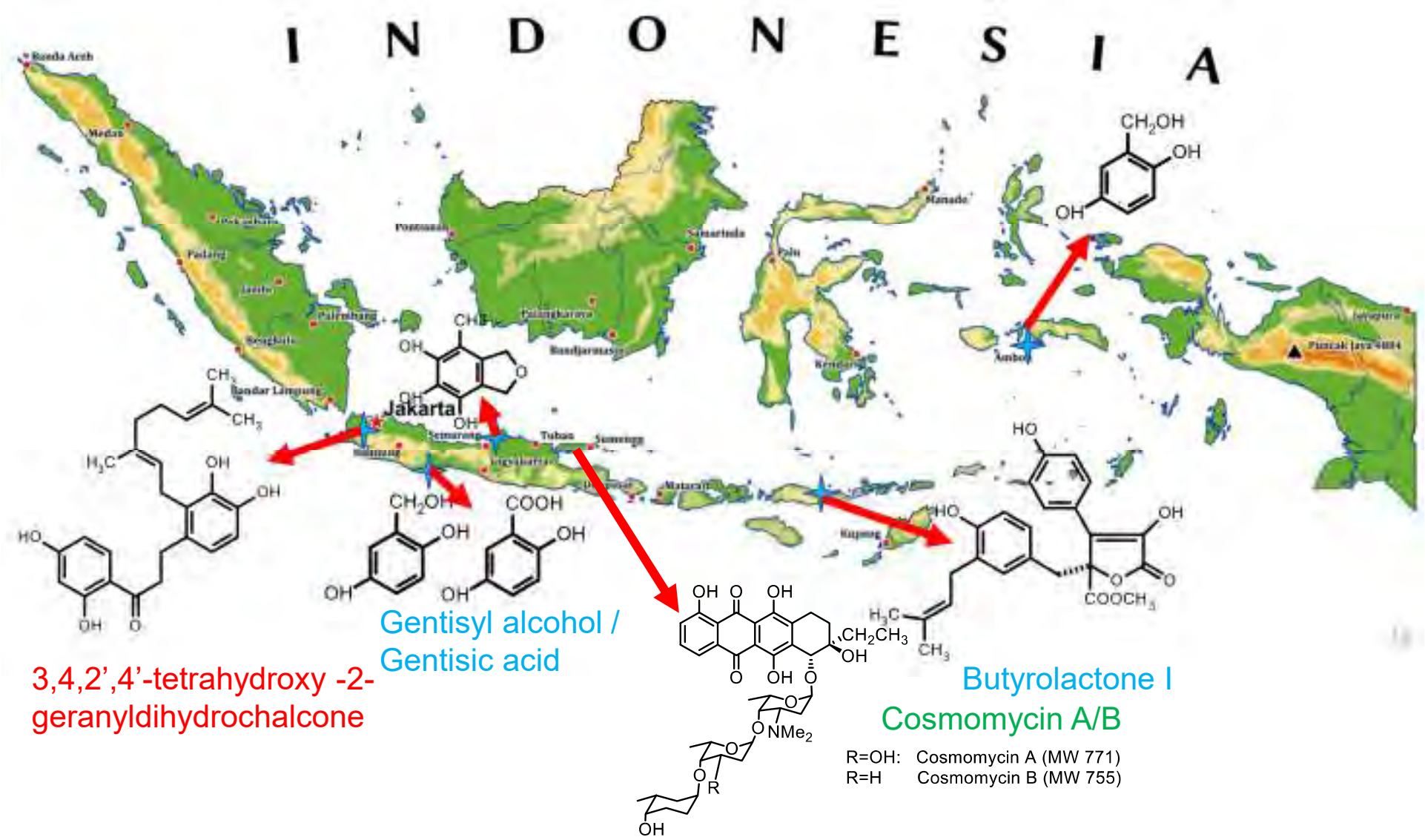


In vivo Efficacy confirmation



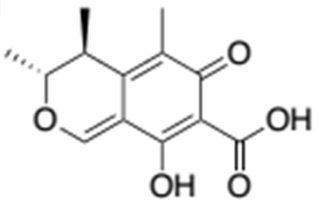
Anti-amebic
Lead identification

Highlights (2018) of Antimalarial discoveries: DHODH and MQO inhibitors and anti-proliferative compounds



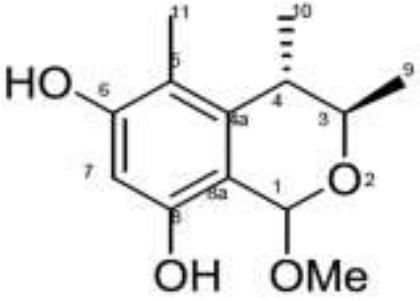
Highlights (2018) of Antiamebic discoveries: anti-proliferative compounds

Citrinin



Chemical Formula: $C_{13}H_{14}O$
Exact Mass: 250.08
Molecular Weight: 250.25

Decarboxylated citrinin



Problems / needs (Jan, 2018)

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

Suggested solutions to the needs (Jan 2018) and the current status (Jan 2019)

1. Characterization/archiving of microbial strains…..Enhance training for taxonomy…..Not satisfactory (particularly at molecular levels); Further improvement needed.
2. Exploitation of new targets and introduction of new screening platforms…New enzyme targets need to be selected and explored ….. Satisfactory; several target enzymes added.
3. Prioritization of identified hits for purification…Ranking of hits by selectivity index, counter-screening, taxonomy of isolates, preliminary extraction test….Partially satisfactory; Dereplication methods need to be developed.
4. Broadening of the bottleneck process(es) (purification/structure elucidation)…Inclusion of additional purification stations needed (Unair and UTokyo)….Satisfactory; New problems identified = A bottleneck was not restricted to large scale culture production; Schedule sharing started.

Inclusion of dereplication step during screening

De`rep`li`ca´tion

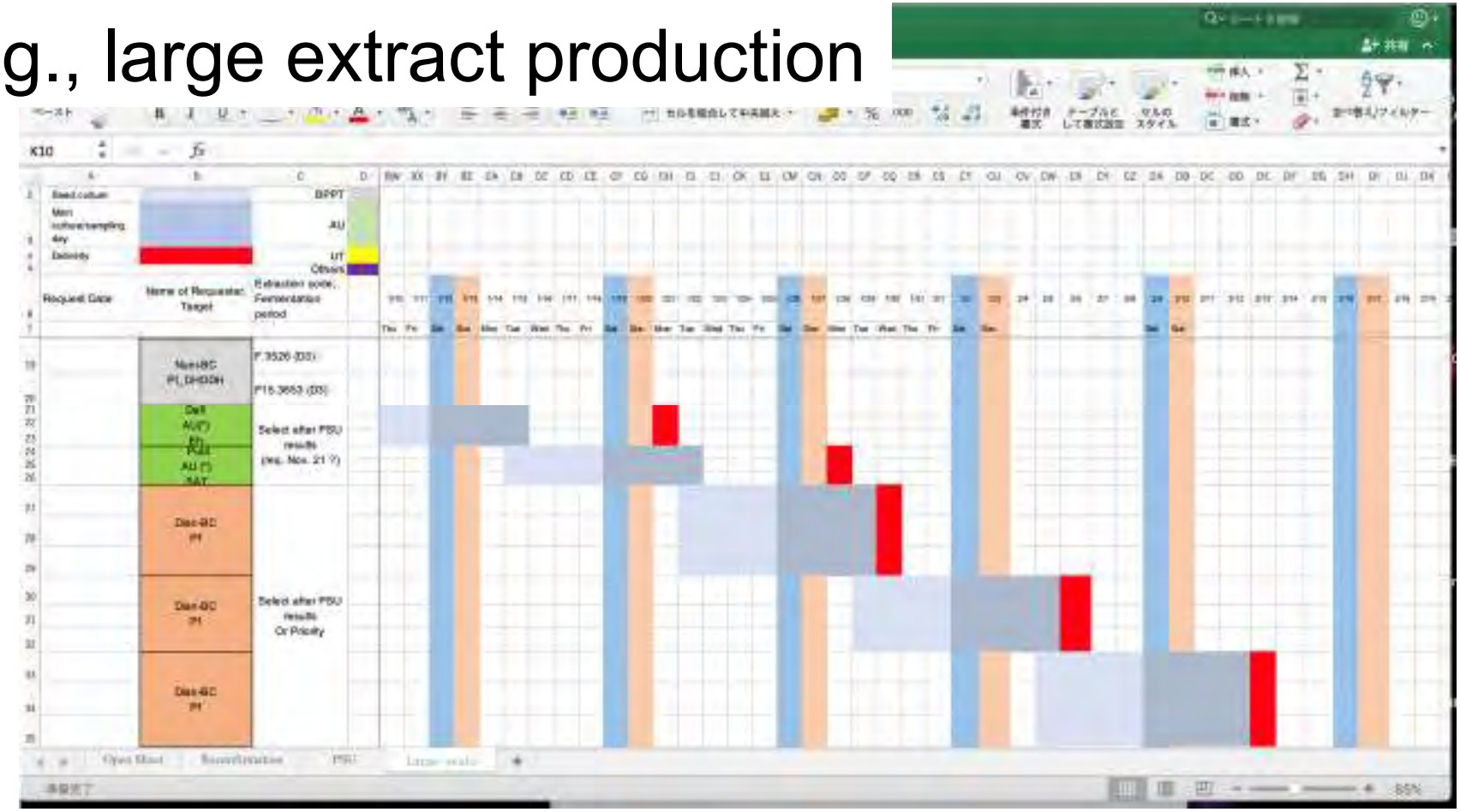
n. 1. *(Biochem.)* the process of testing samples of mixtures which are **active** in a screening process, so as to recognize and eliminate from consideration those active substances already studied; - a stage subsequent to the preliminary screening in the process of discovery of new pharmacologically active substances in mixtures of natural products; - also called counterscreening. See screening.

"Not to repeat discoveries of previously known compounds (including frequent hits)"

1. Use of other references (negative control organisms) (counter-screening)
2. TLC and PDA/HPLC profile-based identification (database?)
3. Preference to uncommon microbes

Schedule management and sharing

e.g., large extract production



1. The schedule is updated every Friday and shared on the last Friday of the month among all team members
2. Helps other teams plan ahead
3. Helps visualization of bottleneck processes

Suggested solutions to the needs (Jan 2018) and the current status (Jan 2019)

5. Cordination between BC/Airlangga/InaCC……Periodical mutual visits / joint meetings for data and method sharing; cross depositing of microbes…..Partially conducted
6. Establishment and development of a drug develop consortium (networking)……Utilization of next JCC meeting or International Symposium …..2nd International Symposium held in October
7. Broadening of disease areas….toward other infectious diseases (e.g., TB/HIV/Helicobacter/Hepatitis/Dengue) and non-communicable diseases (e.g., cancers/obesity/hypertension….) …..Patially conducted and further planned
8. Sustainable development of the capacity…..Continuous funding >5 years, continuous oversea collaboration…..New application to sustain the activity will be filed this year.

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

- 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

Achievements in capacity building in 2018

Training in Japan

- 4 Long-term (3-5 years) trainees (incl. other funding sources)
- 9 short-term (1-3 months) trainees
  - Microbe characterization
  - Purification

Training in Indonesia

- 29 dispatches of 11 Japanese experts (1-8 weeks)

Plan for capacity building in 2019

Training in Japan

- 7 Long-term (3-5 years) trainees (incl. other funding sources) (two more after 2020)
- 5-6 short-term (1-2 months) trainees
  - Microbe characterization
  - Purification

Training in Indonesia

- 20 dispatch of Japanese experts (1-8 weeks)
- International symposium

In summary…..

We had so many difficulties,….but

We have been doing great!

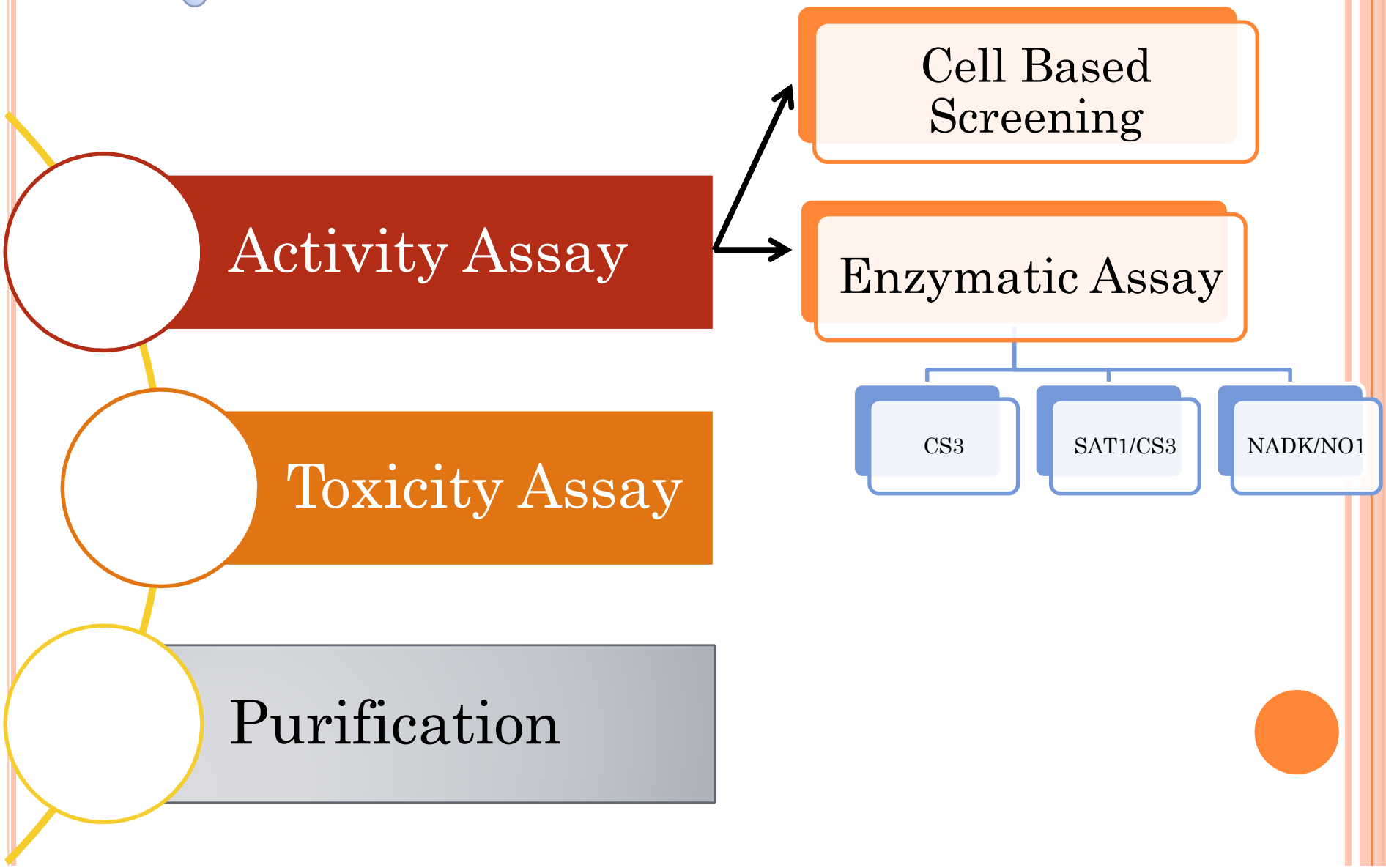
Let us achieve what we aimed at!

Activities Report of ITD-UNAIR

"Project for Searching Lead Compounds of anti-Malarial and Anti-Amebic Agent by Utilizing Diversity of Indonesian Bio-Resources"

January 29, 2019

MAIN WORK at ITD-UNIVERSITAS AIRLANGGA



Extract from BPPT

Cell Based Screening

2016-2018 → 12380 → 1 active non toxic → On progress purification

Enzymatic Assay (CS3)

2015-2017 → 5400 → 1 active → On progress purification

Extract from BPPT

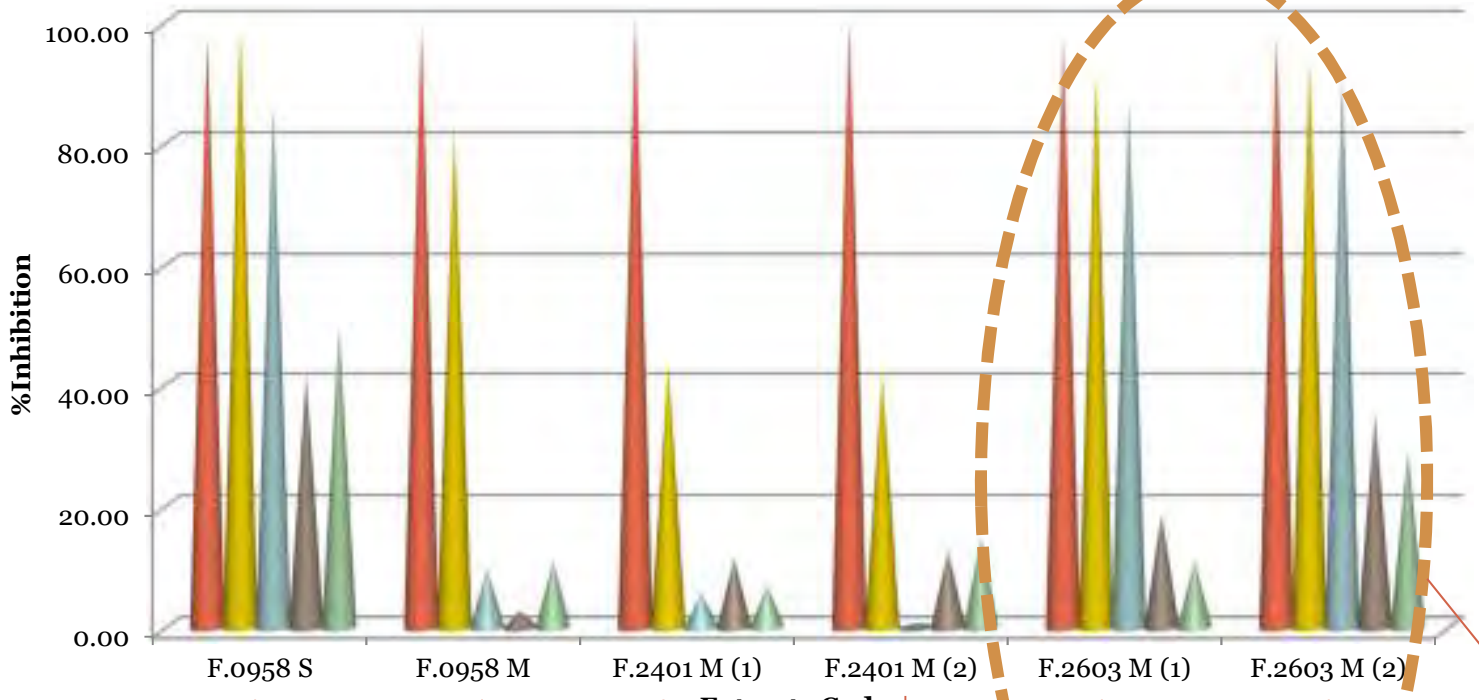
Enzymatic Assay
(CS3/SAT1 Coupled Assay)

2016-2018 → 6600 → 2 active → Large Scale Requested

Enzymatic Assay
(NADK/NO1 Coupled Assay)

2018 → 7260 → 50 hits

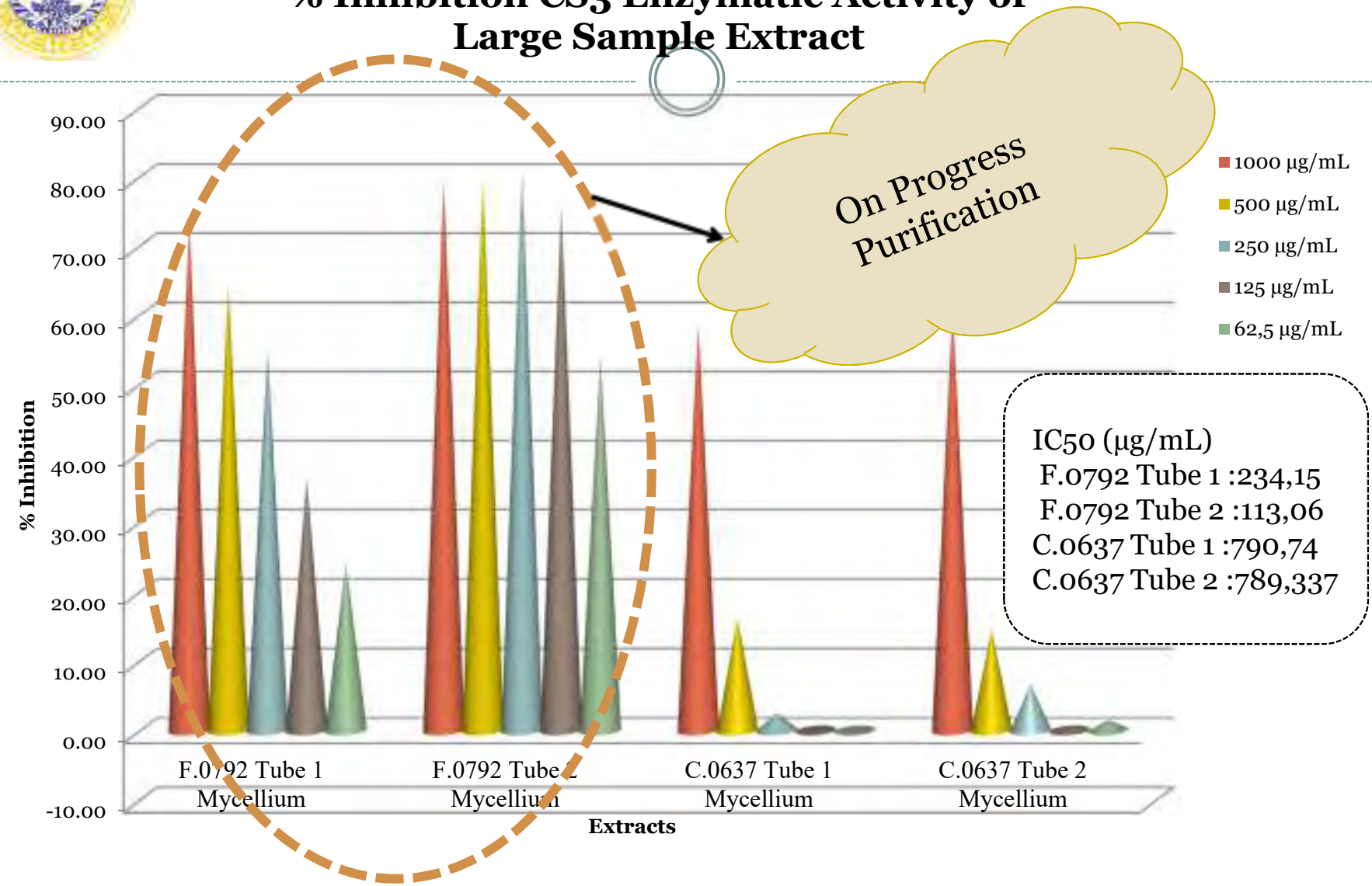
Activity and Toxicity Assay (Huh7it) Large Scale Extracts for Cell Based



	F.0958 S	F.0958 M	F.2401 M (1)	F.2401 M (2)	F.2603 M (1)	F.2603 M (2)
CC_{50} µg/mL	46.75	>1000	>1000	>1000	>1000	>1000
IC_{50} µg/mL	1.2	34	252	662	2.77	0.88

On Progress Purification

% Inhibition CS3 Enzymatic Activity of Large Sample Extract



IC50 (µg/mL)
F.0792 Tube 1 :234,15
F.0792 Tube 2 :113,06
C.0637 Tube 1 :790,74
C.0637 Tube 2 :789,337

TRAINING/TECHNOLOGY TRANSFER in 2018

Myrna Adianti, Ph.D

January 2-29, 2018 at University of Tokyo

Cell Toxicity Assay and New Enzyme Assay for Antiamebic Compound Discovery

Lidya Tumewu, M.Farm, Apt

September 2-29, 2018 at The University of Tokyo

Structure Elucidation of Compounds (Purification of F.0935.S as Antiamoeba)

Hilkatul Ilmi, M.Si

Nov 4 –Dec 2 2018 at Nagasaki University

Cell Toxicity Assay and New Enzyme Assay for Antimalarial Discovery

Instrument 2018

Victor Nivo Plate Reader

BOD Monitor

Ultrasonic Cleaner

THANK YOU

Cell Based Assay Report

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2016-207	5120	182	122	39	7	4	-	Requested LS
2018	7260	137	13	5	4	4	2	1 active non toxic

Enzymatic Assay (CS3) Report

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2016-207	5120	60	22	10	4	1	1	On progress purification

Enzymatic Assay (CS3/SAT1 Coupled Assay)

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2016-2017	2220	41	10	1	-	-	-	Not Growth
2018	4380	26	5	3	2	2	-	Request LS

Enzymatic Assay (NADK/NO1 Coupled Assay)

Year	Primary Screening	Hit extracts	Received Reconfirm	Hit Extracts	Received PSU	Hit Extracts	Received Large Scale	Status
2018	7260	50	-					Request Reconfirm

Toxicity MTT Assay (Huh7it)

Year	Hit Primary Screening	Hit Reconfirm	Hit PSU	Hit Large
2018	356	3	4	2

Extract Primary from BPPT

- Extracts (2016-2017):
  - 64 deep well-plate (5120 dry extract) ⟶ 182 hits cell based and 60 hits CS3 enzymatic assay (Total 242 hits)
- Extracts (January 2018)
  - 56 deep well-plate (4380 dry extract) ⟶ 112 hits cell based, 26 hits CS3/SAT1 Coupled Assay and 41 hits NADK/NO1 Coupled Assay (Total 179 hits)
- Extracts (May 2018)
  - 36 deep well-plate (2880 dry extract) ⟶ 25 hits non toxic cell based and 9 hits NADK/NO1 Coupled Assay (Total 34 hits)

Extract Reconfirmation from BPPT

- Extracts (2017):
  - 122 extracts for cell based and 27 extracts for enzymatic assay (CS3) (Total 149 extracts) ⟶ 25 hits active non toxic for cell based and 17 hits CS3 (Total 42 hits)
- Extracts (2018)
  - 13 extracts for Cell based assay ⟶ 5 active non toxic
  - 5 extracts for Enzymatic assay (SAT1/CS3 Coupled assay) ⟶ 3 hits active
  - 5 extracts for Enzymatic assay (CS3) ⟶ 1 hits active

  (Total received 23 extracts and get total 13 hits active)

Extract PSU from BPPT

- Extracts (September 2018):
  - 13 extracts for Cell based assay → 4 active non toxic
  - 4 extracts for Enzymatic assay (CS3) → 1 active
- Extracts (November 2018)
  - 12 extracts for Cell based assay → 8 active
  - 11 extracts for Enzymatic assay (CS3) → 2 active
  - 2 extracts for Enzymatic assay (SAT1/CS3 Coupled Assay) → 2 active
- Extract (December 2018)
  - 4 extracts for Cell based assay → 3 active non toxic

(Total 46 received and get 20 hits)

Extract Large Scale from BPPT

- Extracts (October 23, 2017):
  - 12 dry extracts for Enzymatic assay (CS3) ⟶ 1 active (on progress purification)
- Extracts (October 29, 2018):
  - 1 extracts (F.0958) for Cell based assay ⟶ Supernatant (IC50 1,2 µg/mL and CC50 46,75 µg/mL or toxic) and Mycellium (IC50 34 µg/mL and CC50 >1000 µg/mL or non toxic)
- Extracts (December 22, 2018):
  - 1 extracts (C.0637) for Enzymatic assay (CS3)
  - 2 extracts (F.2603 & F.2401) for Cell based assay ⟶ F.2603 active non toxic and F.2401 no activity non toxic

BPPT
BADAN PENGKAJIAN DAN PENERAPAN TEKNOLOGI

JICA
Japan International Cooperation Agency

LIPI
Indonesian Institue of Science

Airlangga University

東京大学
THE UNIVERSITY OF TOKYO

北里大学
KITASATO UNIVERSITY

長崎大学
NAGASAKI UNIVERSITY

MicroBiopharm Japan

AMED
Agency for Medical Research and Development

The 5th 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

Project Completion Report

(Draft)

Danang Waluyo

Project Co-manager

Swiss-Belhotel BSD, Tangerang Selatan

January 9th, 2020

Content of Project Completion Report

I. Basic Information of the Project

1. Country
2. Title of the Project
3. Duration of the Project
4. Background
5. Overall Goal and Project Purpose
6. Implementing Agency

II. Results of the Project

1. Results of the Project
2. Achievements of the Project
3. History of Project Design Matrix (PDM) Modification

III. Results of Joint Review

1. Results of Review based on Development Assistance Committee (DAC) Evaluation Criteria
    a. Relevance
    b. Effectiveness
    c. Efficiency
    d. Impact
    e. Sustainability
2. Key Factors Affecting Implementation and Outcomes
3. Evaluation on the Results of the Project Risk Management
4. Lessons Learnt

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

1. Prospects to Achieve Overall Goal
2. Plan of Operation and Implementation Structure of the Indonesian Side to Achieve Overall Goal
3. Recommendation for the Indonesian Side
4. Monitoring Plan from the End of the Project to Ex-post Evaluation

Annex

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**

   Utilization of Indonesian biological resources for drug development are urgently needed to overcome health problem in Indonesia (especially infectious diseases, particularly malaria and amebiasis) and to increase its economic competitiveness by transforming from bioresources-based comparative economic activities to innovation-based competitive economic activities
5. **Overall Goal and Project Purposes**

Overall Goal:

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

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:** **BPPT, Airlangga University, LIPI**

II.1. Result of the Project

Input by the Japanese Side

(1) Amount of input: **JPY 300,000,000**

(2) Expert dispatched
Number of persons: **114 persons** (all were short-term dispatch, until end of Dec 2019)
Major activities:
- Microbial isolation and identification
- Establishing screening system
- Purification and structure identification

(3) Receipt of training participants:
Number of persons: **3 persons for PhD course, 52 persons for short course** (all were from Indonesia and had training in Japan)
Major training items:
- Microbial isolation and identification
- Establishing screening system
- Purification and structure identification

(4) Equipment provision: **JPY 103,000,000**
Major supplies: BSCs, trays for shaker incubator, microplate reader, spectrophotometer, centrifuge, ultracentrifuge, incubators, , trays for shaker incubator, HPLCs, rotary evaporators, microscopes, sonicator

(5) Overseas activities cost: **JPY 52,000,000**
Major contents: Short term training ,business trip, long term training (PhD course)

II.1. Result of the Project

Input by the Indonesia Side

(1) Counterpart assignment: **Total 42 persons**

BPPT : 31 persons
AU : 6 persons
LIPI : 5 persons

(2) Provision of offices and other in-kinds

BPPT : Office and lab space, BSL-2 facilities, equipments (fermentation, extract production, purification and structure analysis), microbial collection
AU : Lab space, equipments
LIPI : Microbial collection

(3) Other items borne by the Government of Indonesia (FY 2015-2019):

Budget: **Rp. 2,861,916,940**

- BPPT = Rp. 2,640,107,000
- AU = Rp. 221,809,940

Major expenses: Consumables and lab supplies, lab furniture, equipment, travel expenses, salaries

Notice: There was no major modification compared to initial plan.

II.1. Result of the Project

Activities

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

- **>18000 extracts were screened for inhibitor of PfMQO, PfDHODH, PfNDH2, PfDPCK**
- **>100 active extracts were obtained**

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

- **Inhibitory activity of all extracts to the proliferation of *P.falciparum* were measured**
  → Secondary screening were not continued according to change of screening strategy as suggested by the experts
- **>20 active extracts showed inhibitory activity to the proliferation of *P.falciparum* (double hit)**

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

- **>12000 extracts were screened for inhibitory activity of proliferation of *P.falciparum***
- **>100 active extracts were obtained**

II.1. Result of the Project

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

- **>80 extracts with anti-malarial activity were objected for active compound isolation and purification**
- **10 anti-malarial active compounds were obtained**

1.5. Establishment of mass production system of the lead compound candidates

- **Large-scale extract production system was established using shaking-flask method**
- **Large-scale extract production based on jar fermenter and flash preparative chromatography was examined**

1.6. Determination of chemical structures of the lead compound candidates

- **Chemical structure of 9 compounds with anti-malarial activity were elucidated**
- **1 compound is being structure elucidated (probably novel compound)**

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

- **Efficacy of one anti-malarial active compound (gentisyl alcohol) was tested using 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 an antimalarial active compound (borrelidin) was discussed**

II.1. Result of the Project

Activities

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 extract to the *Entamoeba histolytica*-derived site-specific recombinant enzyme

- **>9700 extracts were screened for inhibitor of EhCS3, EhSAT1/CS3, EhNADK/NO1**
- **47 active extracts were obtained**

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

- **Inhibitory activity against proliferation of *E.histolityca* of all extracts used for enzyme-based screening was examined**
- **7 active 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

- **>16000 extracts were screened for inhibitory activity of proliferation of *P.falciparum***
- **44 active extracts were obtained**

II.1. Result of the Project

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

- **>20 extracts with anti-amebic activity were objected for active compound isolation and purification**
- **2 anti-amebic active compounds were obtained**
- **1 anti-amebic active compounds is being purified**

2.5. Establishment of mass production system of the lead compound candidates

- **Large-scale extract production system was established using shaking-flask method**
- **Large-scale extract production based on jar fermenter and flash preparative chromatography was examined**

2.6. Determination of chemical structures of the lead compound candidates

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

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

- **Efficacy of one anti-amebic active compound (fumagilin) 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 an antiamebic active compound (fumagilin) was discussed**

II.1. Result of the Project

Activities

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

- **>3600 newly isolated microbes were added and registered into microbial collection**
- **>20000 microbial extracts and >360 plant extracts were produced and registered in extracts library**

3.2. Establishment of screening systems

- **3 enzyme-based (PfDHODH, PfMQO, PfNDH2) and 1 cell-based anti-malarial screening systems were established**
- **3 enzyme-based (EhCS3, EhSAT1/CS3, EhNADK/NO1) and 1 cell-based anti-amebic screening system were established**
- **Establishment of dereplication method**

3.3. Establishment of culture and evaluation systems

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

3.4. Introduction of technologies of isolation and purification

- **Introduction and implementation of pre-extraction test (PET) for determining purification startegies**
- **Isolation and purification method to obtain active compound were introduced and implemented**
  →liquid-liquid extraction method, column chromatography, HPLC (analytical, semi-preparative, recycle), TLC (analytical, semi-preparative)

II.1. Result of the Project

3.5. Introduction of technologies of chemical structure elucidation

- **Implementation of HPLC spectrum and LC-MS analysis method for estimating molecular weight of the compound**
- **Introduction of Natural Product Dictionary for estimating chemical structure of the compound based on its UV profile**
- **Introduction of NMR analysis for structure elucidation of the compound**

II.1. Result of the Project

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**
- **Research collaboration on development of anti-toxoplasmolysis drug from Indonesian microbial resources was established between BPPT and Obihiro University of Agriculture and Veterinary Medicine**
  →MTA for transferring microbial extracts for anti-toxoplasmolysis screening was signed
- **Research collaboration on efficacy test of anti-malarial active compound in animal model was established between BPPT and Brawijaya University**
- **Research collaboration on development of anti-tuberculosis agents was established between BPPT, Airlangga University and The University of Tokyo**
  →MoU and MTA for transferring microbial extracts for anti-tuberculosis screening was signed
- **Research collaboration on development of anti-cancer agents was initialized between BPPT and Gadjah Mada University**
- **Research collaboration on development of anti-malarial agents from local plants was conducted between BPPT and Islamic State University Syarif Hidayatullah**
- **Research collaboration between BPPT and Bandung Institute of Technology 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.**
- **Research collaboration between BPPT and Eijkman Institute was conducted with topic of anti-malarial activity assay of active compound from plant**
- **Research collaboration between BPPT and Gadjah Mada University was conducted with topic of anti-malarial activity assay of active compound from algae**
- **Research collaboration between BPPT, The University of Tokyo, Kitasato University, Bozo Research Institute, IPB University, and LIPI concerning on development of structure modification and pre-clinical assessment system for development of anti-infection agents was initiated**
  →LoI for this collaboration was signed

II.2. Achievement of the Project

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

Achieved in 1st year: Total 10 compounds

- **9 compounds with anti-malarial activity were obtained**
- **1 compound is being structure elucidated (probably novel compound)**

1.2. Chemical structure elucidation is completed for at least one (1) compound with anti-malarial activity by the time of the Terminal Evaluation

Achieved in 1st year: Total 10 compounds

- **Chemical structure of 9 compounds with anti-malarial activity were elucidated**
- **1 compound is being structure elucidated (probably novel compound)**

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.

Achieved in 5st year: Total 1 compound

- **Efficacy of an anti-malarial active compound (gentisyl alcohol) was tested using animal model**

II.2. Achievement of the Project

Outputs and Indicators

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

Achieved in 3rd year

- **>20 extracts with anti-amebic activity were objected for active compound isolation and purification**
- **2 anti-amebic active compounds were obtained**
- **1 anti-amebic active compounds 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

Achieved in 3rd year

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

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

Achieved in 5th year

- **Efficacy of an anti-amebic active compound (fumagilin) was tested in animal model**

II.2. Achievement of the Project

Outputs and Indicators

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

Achieved in 3rd year

- **>3600 newly isolated microbes were added and registered into microbial collection**
- **>20000 microbial extracts and >360 plant extracts were produced and registered in extracts library**

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

Achieved in 2nd year

- **3 enzyme-based (PfDHODH, PfMQO, PfNDH2) and 1 cell-based anti-malarial screening systems were established**
- **3 enzyme-based (EhCS3, EhSAT1/CS3, EhNADK/NO1) and 1 cell-based anti-amebic screening system were established**
- **Establishment of dereplication method**

II.2. Achievement 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

Achieved in 3rd year

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

Achieved in 4rd year

- **Introduction and implementation of pre-extraction test (PET) for determining purification strategies**
- **Isolation and purification method to obtain active compound were introduced and implemented**
  →liquid-liquid extraction method, column chromatography, HPLC (analytical, semi-preparative, recycle), TLC (analytical, semi-preparative)

3.5. Technologies of chemical structure analysis of compounds are introduced at the Indonesian research institute(s) by the time of the Terminal Evaluation

Achieved in 5th year

- **Implementation of HPLC spectrum and LC-MS analysis method for estimating molecular weight of the compound**
- **Introduction of Natural Product Dictionary for estimating chemical structure of the compound based on its UV profile**
- **Introduction of NMR analysis for structure elucidation of the compound**

3.6. International symposiums are held for drug discovery for two (2) times at least

Achieved in 5th year

- **International symposiums were held twice in 2017 and 2019**

II.2. Achievement of the Project

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

Achieved in 5th year

- **9 compounds with anti-malarial activity were obtained**
- **1 compound is being structure elucidated (probably novel compound)**
- **Efficacy of 1 anti-malarial active compound was tested in animal model**

2. At least one (1) lead compound with anti-amebic activity are determined on the basis of animal experiments for efficacy

Achieved in 5th year

- **>20 extracts with anti-amebic activity were objected for active compound isolation and purification**
- **2 anti-amebic active compounds were obtained, 1 anti-amebic active compounds is being purified**
- **Efficacy of 1 anti-amebic active compound was tested in animal model**

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

Will be achieved by the end of the project period

- **1 research paper was published**
- **Another 1 research paper is being submitted (already accepted)**
- **Another related publication:**
  - **2 research papers, 4 presentations in scientific conferences**

II.3. History of Project Design Matrix (PDM) Modification

1. **Change of counterpart**
   - Main counterpart institute from Indonesia was changed from Biotech Center of BPPT to Center for Pharmaceutical and Medical Technology of BPPT due to re-organization in BPPT (2016)
   - Change of main unit of LIPI from RC for Biotechnology to InaCC due to appropriateness for the project (2016)
   - Main counterpart institute from Japan was changed from Tsukuba University to The University of Tokyo due to position movement of Chief Advisor (2017)
   - Main counterpart institute from Indonesia was changed from Center for Pharmaceutical and Medical Technology to Laboratory for Biotechnology due to re-organization in BPPT (2017)

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

III. Result of Joint Review

Result of Review based on DAC (Development Assistance Committee) Evaluation Criteria

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

- **Long-term National Development Plan 2005-2025: health and drug became one field in National Prime Research Program**
  →directed to develop and implement technology for drug raw material production for import product substitution
- **Mid-term National Development Plan 2015-2019: controlling malaria is one of the government priority in field of health and infectious diseases**
- **National system of science and technology (Constitution no.11, 2019): utilization of local bioresources for advancement of science and technology**

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

- **All indicators of project purpose achievement have been achieved**
- **The capacity of Indonesian researchers and institutes on drug development is improved**
- **The active compound producers will be deposited in InaCC (previous SATREPS project outcome)**
- **Joint symposium with other SATREPS, e-ASIA, and J-GRID projects conducted in Asian countries was organized**

III. Result of Joint Review

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

- **All planned inputs have been realized**
- **All indicators have been achieved**

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

- **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 product**
- **BPPT received numbers of request to support anti-malarial assay by other research institutes in frame of research collaboration, as well as services**
- **BPPT received joint research offer from other research institutes (domestic and international).**

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

- **Technology development for reducing dependency of imported raw drug material is stated as one of research priority by the government**
- **BPPT committed to allocate budget to continue the research (Rp.400 million, FY2020)**
- **A proposal for obtaining research grant from Ministry of Research and Technology was submitted**
- **BPPT signed a LoI with LIPI, IPB, UTo, and Malaya University to conduct joint research**
- **Anti-malarial assay procedure will be proposed to become a service provided by BPPT**

III. Result of Joint Review

Key Factors Affecting Implementation of Outcomes

- **Biosafety and biosecurity system**
  → Development of drug needs a proper system to ensure the safety of researcher involved and the materials being used (pathogens, biohazards, etc.)

- **Regulations related to importation**
  → Lack of coordination between ministries results in difficulties on importation of equipment/reagents/supplies for research to Indonesia for running the project (since most of them are not produced in Indonesia)

- **Material transfer**
  → Some of technologies developed in Japan need to be verified using real sample from Indonesia before the capacity is built in Indonesia

- **Tasks 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

III. Result of Joint Review

Evaluation on the Results of the Project Risk Management

1. Risk Management Results

- **Biosafety and biosecurity**
  →Establishment of BSL-2 laboratory
  →Establishment of SOP

- **Regulations related to importation**
  →Understanding current regulations
  →Selecting local prominent vendor

- **Material transfer**
  →Exchanging MTA between involved counterparts
  →Monitoring the implementation of material transfer

- **Tasks distribution**
  →Determination of bottle neck of the process
  →Re-distribution of tasks to potential and capable counterparts

III. Result of Joint Review

2. Results of the Use of Lessons Learnt

- **Biosafety and biosecurity**
  - →Establishment of BSL-2 laboratory
    - ✓ Assay was done safely
  - →Establishment of SOP
    - ✓ Reliable and traceable data

- **Regulations related to importation**
  - →Understanding current regulations
    - ✓ Shorten importation time
  - →Selecting local prominent vendor
    - ✓ Spec-matched items

- **Material transfer**
  - →Exchanging MTA between involved counterparts
    - ✓ Technology development/transfer was done smoothly
  - → Monitoring the implementation of material transfer
    - ✓ Ensuring the impact of material transfer

- **Tasks distribution**
  - →Determination of bottle neck of the process
    - ✓ Increased in efficiency of the process
  - →Re-distribution of tasks to potential and capable counterparts
    - ✓ Speed up the process and objective achievements

III. Result of Joint Review

Lessons Learnt

- **Biosafety and biosecurity**
  - →A system for ensuring biosafety and biosecurity is indispensable
  - →Solid and obeyable SOP is the key for obtaining trustworthy data

- **Regulations related to importation**
  - →Understanding related regulations may accelerate the achievement of target
  - →Selecting local prominent vendor
    - ✓ Spec-matched items

- **Material transfer**
  - →Advancement of technology is part of successful drug development
  - →Biological resources will be protected and fully utilized through MTA

- **Tasks distribution**
  - →More efforts to improve efficiency are needed for limited resources
  - →Good collaboration will shorten the long process of drug development

IV. For the Achievement of Overall Goals after Project Completion

Prospects to Achieve Overall Goal

Overall Goal:

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

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.

Prospects

- Capacity on isolation and identification of microbial isolates was improved
  →**Core microbial library construction composed from highly diverse microbial isolates**

- Screening and assay system was implemented
  →**Development and implementation of new screening and assay system**

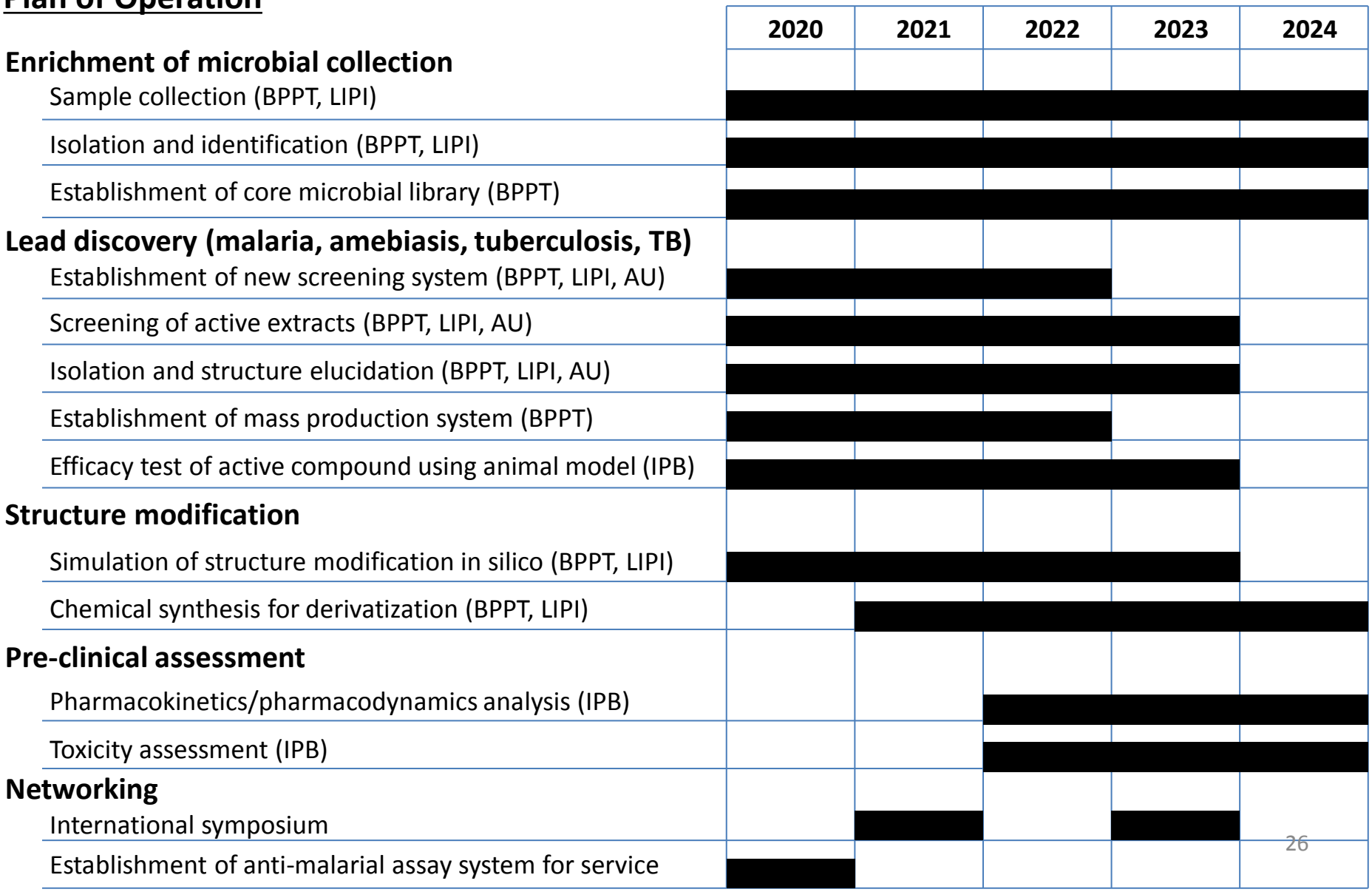
- Some of potential active compounds were isolated and identified
  →**Structure modification for lowering the toxicity level of the compounds**
  →**Pre-clinical assessment of promising lead compounds**

- Research network between institutes in Indonesia and Japan was built
  →**Research collaboration to maintain the network**

IV. For the Achievement of Overall Goals after Project Completion

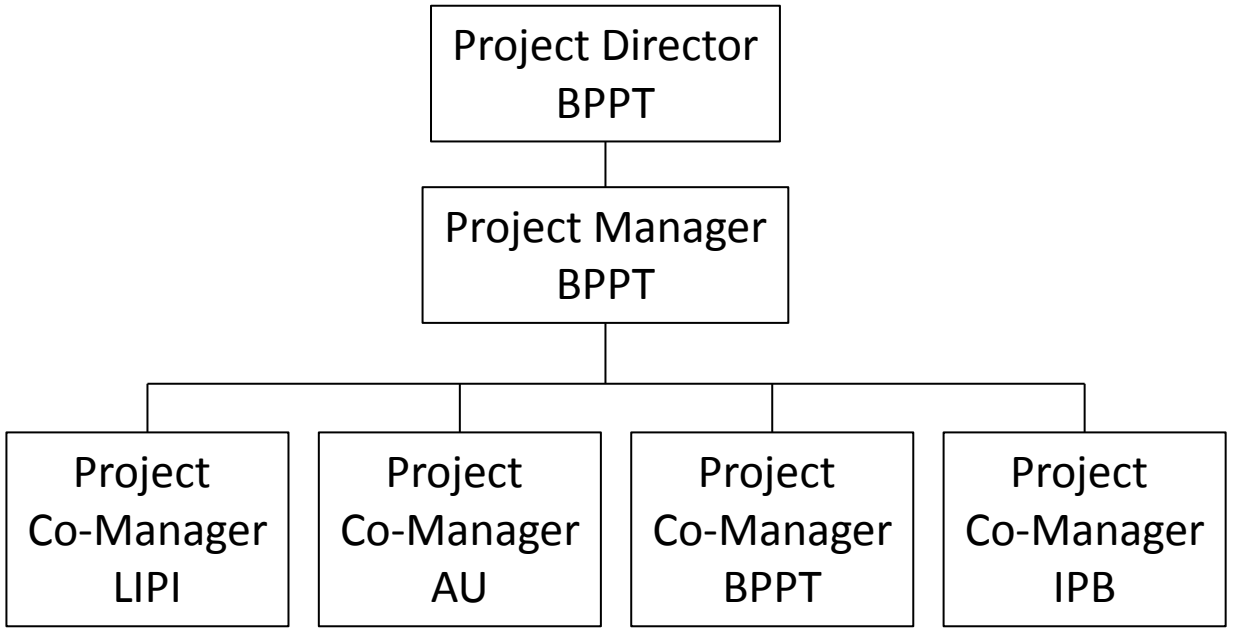
Plan of Operation and Implementation Structure of the Indonesian Side to Achieve the Overall Goal

Plan of Operation



IV. For the Achievement of Overall Goals after Project Completion

Implementation Structure



IV. For the Achievement of Overall Goals after Project Completion

Recommendations for the Indonesian Side

- Microbial collection is a precious capital for Indonesia
  → Should be well managed and fully utilized

- Sustainability is a key factor for successful drug development
  → Continuous support from top management is required
  → Promoting drug development research activities in Indonesia

- Research networks in drug development field
  → Promoting natural resources based drug discovery research activities
  → Promoting competency-based research network in Indonesia
  → Promoting A-B-G networks for social implementation of research outputs

- Research environment in Indonesia
  → Maintain and improve the quality scientific discussion among researchers/institutes

IV. For the Achievement of Overall Goals after Project Completion

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

Core microbial library construction

→ A core microbial library composed from at least 1000 microbial isolates with high diversity is established

Establishment of new screening system

→At least 1 screening system for obtaining anti-infectious diseases (malaria/amebiasis/dengue/tuberculosis) is developed and implemented

Obtaning active compound with antimalarial/antiamebiasis/dengue/tuberculosis

→At least 1 active compound with antimalarial/antiamebiasis/dengue/tuberculosis is obtained and the chemical structure is elucidated

International symposium on drug development

→An international symposium is held

Annex

ANNEX 1: Result of the Project (list of dispatched experts, list of counterparts, list of trainings, etc.

ANNEX 2: List of Products Produced by the Project (reports, manuals, handbooks, etc.)

ANNEX 3: Project Design Matrix (PDM, all versions)

ANNEX 4: Record of Discussion, Minutes of Meeting, Minutes of JCC Meeting (copy)

ANNEX 5: Project Monitoring Sheet (copy)

Thank You

SATREPS **SLeCAMA Project**

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.01（Apr. 2015 – March 2016）

Name: Prof. Tomoyoshi NOZAKI
Title: Chief Advisor

Submission Date: April 2016

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese : 12 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in the 1st year which included Chief Advisor. A coordinator was dispatched and will stay continuously in the project office

1-1-1-2. Indonesian: the Project Director, Project Manager and Project Co-managers were appointed in accordance with the R/D. Researchers were listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: 11 Indonesian researchers participated in trainings in Japanese institutes in the 1st year

1-1-2-2. In-country Training : There were following 3 trainings organized in the project with Japanese instructors.

a. Training on Isolation and Characterization of microbes
b. Training on Purification of Enzymes for Screenings
c. Training on Identification of Actinomycetes from Soil Samples

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side : BPPT prepared BSL-2 level's laboratory spaces to install new equipment in BTC-BPPT and AU prepared the laboratory space as well. BPPT provided a room for Japanese experts. Bio-resources possessed in BPPT were provided to the project.

1-1-3-2. Provision by Japanese side: Required laboratory equipment for 1st years' activities was procured in Japan and Indonesia in 2015. The disbursement for the procurement was around 63 million Japanese yen. After struggling for long time to get the import permission from Indonesian authorities, however the permission was not given finally due to a new regulation related to some equipment (freezers) issued by Min. of Trade. Therefore UT is now rearranging

the importation procedure again to be able to import the equipment.
In addition to equipment procured in Japan, some equipment were procured locally by UT with the amount of 405 million rupiah.

1-1-4. Local costs

1-1-4-1.Indonesian Side: BPPT and AU prepared annual budget for running cost in each institute with its amount around 450 million rupiah each for 2016. It could be used for employing personnel, travelling and consumables, etc.

1-1-4-2.Japanese Side: JICA Indonesia Office provided running expenses for such as employing secretary, car rental and consumables with its amount around 210 million rupiah for the 1st year.
UT locally procured and provided laboratory supplies (reagents and plastic wears) with the amount of 156 million rupiah in the 1st year.

1-2 Progress of Activities

As described in PDM, there are 3 main activities to be conducted in this Project: 1) Identification of compounds with anti-malarial activity from the extracts of Indonesian biological resources, 2) Identification of compounds with anti-amebic activity from the extracts of Indonesian biological resources, 3) Establishment of technologies and research system for drug discovery using biological resources at the Indonesian research institutes. In 2015, most of activities were carried out in Japan, since the equipment had not been installed in Indonesian institutes, yet. In general, the preliminary research was conducted by the Indonesian researchers, and further studies have been carried out by the Japanese experts for enhancement and validation. By the close technical guidance and instruction, necessary skill and knowledge has been effectively transferred to Indonesian members. Though, it is necessary to implement those skills and knowledges for running the activities in Indonesia after the equipment are installed and the research system is established in Indonesian institutes.

Laboratory space that is compliance with Biosafety level 2 (BSL-2) was prepared in BTC-BPPT. Other laboratory spaces for active compound purification in BTC-BPPT were also prepared for conducting the research. Laboratory space in AU was also prepared for conducting anti-amebic screening.

Sampling of biological resources was conducted on July 27-30, 2016, at Ambon and Saparua Islands, Eastern Indonesia. From the expedition, 90 samples (soil, plant litter, insect, and medicinal plant) were taken and brought to BTC-BPPT for further microbial isolation. Until February 2016, more than 700 microbes were newly isolated, consisted from fungi and actinomycetes. All of the isolates were preserved in BTC-BPPT.

Extract of microbes for screening was prepared at BTC-BPPT. From the starting date of the project until February 2016, more than 1400 microbial extracts had newly

prepared for screening of compound for anti-malarial and anti-amebic activity. Extract preparation employed currently available microbe collection in BTC-BPPT. Each microbial isolate was cultivated in 2 different kind of medium before being extracted with butanol.

First screening of extract for anti-malarial and anti-amebic activities was mainly conducted at Japanese counterpart institutes (University of Tokyo, National Institute of Infectious Diseases, and Kitasato University) using extracts prepared in BTC-BPPT. More than 5000 currently available extracts, as well as more 800 newly prepared extracts were objected to enzyme- and cell-based screening for both anti-malarial and anti-amebic activities. The screening was conducted mainly by Indonesian researchers who were dispatched to Japan for training under supervision of Japanese experts from each institute. Secondary screening and purification of active compounds were also conducted mainly in Japan, since the equipment were not installed in Indonesian institute, yet.

Establishment of enzyme-based screening system for both anti-malarial and anti-amebic activity was started from end of January 2016, when the enzymes needed for screening the extract were prepared in BTC-BPPT under supervision of Japanese expert. Five (5) enzymes were being prepared using currently available equipment in BTC-BPPT. These enzymes will be used for enzyme-based screening in BTC-BPPT, as well as in AU for anti-amebic activity, soon after the equipment are installed.

1-3 Achievement of Output

1-3-1. Achievement of Output 1

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

Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	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. Chemical structure elucidation is completed for at least one (1)	The indicator has been achieved • The chemical structure of two (2) compounds

compound with anti-malarial activity by the time of the Terminal Evaluation.	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. 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. • According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.

1-3-2. Achievement of Output 2

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

Indicators	**Achievement**
2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	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. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.	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. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	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.

1-3-3. Achievement of Output 3

Output 3	
Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.	
Indicators	**Achievement**
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.	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 the in-house biological resource libraries.
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.	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. 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.	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 installed.
3-4. Technologies of isolation and purification of compounds are	The indicator is expected to be achieved by the time of the Terminal Evaluation.

introduced at the Indonesian research institute(s) 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 training.
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. • 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 NMR as one that owned by Kitasato University.
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 end of the project. • The symposium are expected to be held in 2017 and 2019.

1-4 Achievement of the Project Purpose

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.

Although 2015 was the first year of this project, some of indicators of output designed in PDM had already achieved. Two compounds with anti-malarial activities were purified from microbial extract, and the structure was also elucidated successfully (part of Output 1). Although not novel (as the chemical structure of the compounds had already been reported), the compounds showed high anti-malarial activity, which has not been reported before. Manuscript of paper related to this result is being prepared, and will be submitted to related scientific journal in this year (2016) with Indonesian researcher as the

first author (Project Purpose).

Indicators	Achievement
1. At least one (1) lead compound with anti-malarial 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. • 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. 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. • 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. 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 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)

1-5 Changes of Risks and Actions for Mitigation

1-6 Progress of Actions undertaken by JICA

1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)

1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2a-1 Provision of Equipment

The schedule of equipment which was planned to install within 2015 in Indonesian

institutes had delayed and it has not been installed yet.

2a-2 Cause

During the preparation of shipping to Indonesia in Dec 2015, the project was noticed about necessity of permissions of Min of Trade (MoT) and Min of Health (MoH) by the suppliers'-contracted forwarder just before shipping date. It took another month to apply for these permissions, however MoT finally refuse to issue permit to import the equipment with freezing function except special license issued by MoT according to a new decree issued in 2015.

2a-3 Action to be taken

To avoid consume time more, the project will import equipment excluding freezers firstly. Regarding freezers, JICA will accept importation without tax exemption specially for the freezers so that a licensed trader can deal it. To realize this solution, UT and JICA HDQ have rearranged the annual budget.

2a-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

BPPT prepared various documents (letters to the Indonesian authorities) in accordance to the requirement for exemption of custom duties and import permits.
JICA Indonesia Office gave various advices for the importation, and applied PP19 (exemption of custom duties' permit) to the SEKNEG (Office for Sate Secretary).

2b-1. Consumables for laboratory

The annual estimated cost of required consumables for experiments in the Indonesian laboratories exceeds the budget which is prepared by Indonesian institutes (BPPT and AU).

2b-2 Cause

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).
The proposed amount of both BPPT and AU to their own institutes for 2016 were not enough to realize the annual working plan**.**

2b-3 Action to be taken

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.

2b-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

As mentioned in article 2b-3 in the above.

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the first year, the amendments are agreed in the 1st JCC Meeting on Feb 02, 2016. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2016

Accordance to BPPT's organizational reforming, the role of research function of Biotech Center was transferred to the Center for Pharmaceutical and Medical Technologies (PTFM) in beginning of 2016. Then, the main institute to implement project activities in BPPT changed from Biotech Center-BPPT to PTFM. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of PTFM and Program Head of PTFM accordingly.

3-2-2. Amendments due to reviewing the role of LIPI's institutes

The Director of Research Center for Biotechnology-LIPI was stipulated as the Project Co-manager in the original R/D signed in Feb 2015. However, the main institute to implement the project activities in LIPI must be the Indonesian Culture Collection (InaCC) under the Research Center for Biology-LIPI. Therefore, the project decided to replace the Project Co-manager to Director of InaCC from the Director of Research Center for Biotechnology-LIPI.

3-2-3. Amendments due to the reformation among Japanese institutes

The role of Japan Science and Technology Agency (JST) for the project was handed over to Japan Agency for Medical Research and Development (AMED) which was newly established in April 2016.

3-2-4. Amendments due to mistyping in the original R/D

There were several corrections on mistyped words in original R/D.

4 Preparation of Gov. of the Republic of Indonesia toward after

completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.02 (Apr. 2016 – Sep. 2016)

Name: Prof. Tomoyoshi NOZAKI
Title: Chief Advisor

Submission Date: 01 Oct. 2016

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese : 6 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in this term (the cumulative number of dispatched researchers become 18 since the project start) A coordinator have been staying continuously in the project office

1-1-1-2. Indonesian: the Project Director, Project Manager and Project Co-managers were appointed in accordance with the R/D. Researchers were listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: One Indonesian researcher participated in trainings in a Japanese institute in this term. Another Indonesian researcher have started her Ph.D study in Univ. of Tsukuba from Apr. 2016. The cumulative number of participation of Indonesian researcher become 14 since the project start.

1-1-2-2. In-country Training : There were following trainings organized in the project with Japanese instructors.

a. Training on "Purification of Active Compounds" (by Dr. Takemoto)
b. Training on "Target Enzyme Preparation and High Throughout Screening" (by Dr. Daniel)
c. Training on "Mammalian Cell Culture and Screening System"(by Ms. Miyazaki)
d. Training on "Purification of Active Compounds" (by Dr. Mori)

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side : BPPT prepared BSL-2 level's laboratory spaces to install new equipment in BTC-BPPT and AU prepared the laboratory space as well. BPPT provided a room for Japanese experts. BPPT provided laboratory facilities in the LABTIAB during the preparation period of new equipment in the BTC. Bio-resources possessed in BPPT were provided to the project.

1-1-3-2. Provision by Japanese side: Required laboratory equipment for the 1st years' activities was procured in Japan and Indonesia in 2015. The disbursement for the procurement was around 63 million Japanese yen. Some equipment were procured locally by UT with the amount of 405 million rupiah in the 1st year as well.

As the 2nd years' program, 17 items of equipment were listed up in the annual plan and have been procured in Indonesia since Apr. 2016, the disbursement is estimated at 23.5 million Japanese yen.

Due to the new Indonesia regulation on the importing freezers, the delivery of imported equipment from Japan was delayed to Jun 2016, however those equipment was installed successfully in the BTC-BPPT and ITD-AU in Aug. 2016.

1-1-4. Local costs

1-1-4-1.Indonesian Side: BPPT allocated 450 million rupiah for employing personnel, travel and consumable. Due to BPPT Chairman direction as the implementation of the direction from Ministry of Finance, the budget was rationalized into around 390 million rupiah in August 2016. As October 1st 2016, The budget has been utilized as much as 81%, mainly for employing personnel and purchasing reagents/consumables. The remained budget will be utilized until end of 2016. For FY 2017, BPPT is tentatively allocating budget around as much as 500 million rupiah for employing personnel, travelling cost, and consumables. BPPT is also seeking funding from outside of BPPT for FY 2017. As October 2016, BPPT has submitted 5 proposals to Ministry of Research, Technology, and Higher Education, as well as local research funding organization, with total requested budget as much as 3.2 billion rupiah.

1-1-4-2.Japanese Side: JICA Indonesia Office provided running expenses for such as employing secretary, car rental and consumables with its amount around 210 million rupiah for the 1st year. For the 2nd year, JICA has provided those running cost with its amount around 305 million rupiah as of 1 Oct 2016.

UT locally procured and provided laboratory supplies (reagents and plastic wears) with the amount of 156 million rupiah in the 1st year. In the 2nd year, provision of laboratory supplies are built in as part of the provision of equipment as the mentioned above 1-1-3-2.

1-2 Progress of Activities

Sampling of biological resources was conducted on June 23-27, 2016, at Biak Island, Eastern Indonesia. From the expedition, 127 soil samples were collected from 24 sampling points. The samples were then brought to BTC-BPPT for further microbial isolation. Until end this semester, more than 1000 microbes consisted from fungi and

actinomyces were newly isolated and identified. All of these isolates were preserved in BTC-BPPT.

Enzymes that are needed for enzyme-based screening were prepared in Q1 of this year. The screening of 3200 extracts to search extract with inhibitory activity against pfDHODH enzyme (for antimalarial activity) was carried out from Q2 of this year using equipment that was available at that time, resulting in 21 active extracts (hit). Microbe that producing these extracts were then re-cultured, and extract of them were prepared. These extract were assayed against pfDHODH and hsDHODH enzymes to confirm their activities. Cytotoxicity of these extracts were also tested against 5 mammalian cells, resulting 9 non-toxic antimalarial active extracts. Five of these extracts were produced in larger scale (100 ~ 5000 ml) for purification of active compounds. Currently, 2 of these upscaled extracts are being purified, while the others will be purified in next semester.

In this semester, a new enzyme for screening of extract with antimalarial activity, pfMQO (malate-quinone oxidoreductase), was introduced. The enzymes were produced and purified in Q2 of this year in BPPT. Characterization of this enzyme was done in BPPT resulting a good quality of enzyme and ready to be used for screening.

To date, more than 1200 extracts were screened agains pfMQO, resulting a total of 118 active extracts. Microbe that producing 74 of these active extracts were re-cultured, but only 56 of them were grown. Extract of these microbes were objected to pfMQO assay, resulting 25 active extracts with inhibitory activity. Cytotoxicity test against 5 mammalian cells revealed that 9 of them remained non-toxic and ready for purification. Upscaled extracts were already prepared and will be purified in next semester.

Previously, first screening of microbial extract to search active extract that had inhibitory activity against CS3 enzyme were carried out by Indonesian researcher at Japanese counterpart institute (Kitasato University), resulted in several hit of extracts. Four of them were then produced in larger scale in this semester and used for further purification to isolate the active compound in BTC-BPPT by Indonesian researcher. Purification process of these extracts is currently undergone, and some of them will be finished before the end of this year.

In the other side, screening of extracts against CS3 and SAT1 enzymes was carried out in AU. More than 2000 extracts were screened, resulting 21 and 28 hits with inhibitory activity of CS3 and SAT1, respectively. These hits are currently being confirmed by re-culturing the producing microbes and checking their inhibitory activity.

Equipment were finally installed in BTC-BPPT and AU in August 15, 2016, after delayed from initial plan for about 8 months, due to change of import regulation from the government of Indonesia. Some of research activities that could not be done before, including human cell culture, enzyme- and cell-based screening, and purification, could be started in both laboratories. Training on using these equipment were also carried out by the vendor and attended by Indonesian researcher.

1-3 Achievement of Output

1-3-1. Achievement of Output 1

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

Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	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. 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 two (2) compounds with anti-malarial activity had been elucidated) • Purification of other 2 active extracts are currently being performed
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. • According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.

1-3-2. Achievement of Output 2

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

Indicators	Achievement
2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	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. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.	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. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	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.

1-3-3. Achievement of Output 3

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

Indicators	Achievement
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.	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. 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.	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. 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.	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. 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. • 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.	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.	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.

1-4 Achievement of the Project Purpose

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.

In this semester, a lot of progress was achieved, especially in screening of extracts and purification of active compound. Four enzymes used for enzyme-based screening had been prepared in BTC. More than 5000 extracts had been prepared during this semester, and more than 6000 extracts had been already objected for screening to search active extract with inhibitory activity against DHODH, MQO, CS3 and SAT1, and against parasite *Entamoeba histolytica*. Secondary screening of active extracts had also been conducted. Mammalian cell culture system was established in BTC, and cytotoxicity test using these cells of active extracts has been performed. Purification of 6 active compounds has been started, and some of them have been objected to structural analysis using NMR. A manuscript of paper related to these achievement is being prepared, and will be submitted to related scientific journal within this year with Indonesian researcher as the first author.

Indicators	Achievement
1. At least one (1) lead compound with anti-malarial 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 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. 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. • 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. 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 expected to be achieved by the time of the end of the Project. • 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)

1-5 Changes of Risks and Actions for Mitigation

1-6 Progress of Actions undertaken by JICA

1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)

1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2a-1 Provision of Equipment

The schedule of equipment which was planned to install within 2015 in Indonesian institutes had delayed and the equipment was installed in Aug. 2016.

2a-2 Cause

Due to consuming time for getting import permission in several Indonesian authorities, especially new Indonesian regulation on the restriction of importing freezers prevented the procedure.

2a-3 Action to be taken

To avoid consume time more, the project imported equipment excluding freezers firstly. Regarding freezers, JICA accepted importation without tax exemption specially for the freezers so that an Indonesian licensed trader could deal it. UT and JICA HDQ rearranged the annual budget for the extra cost for this treatment.

2a-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

BPPT prepared various documents (letters to the Indonesian authorities) in accordance to the requirement for exemption of custom duties and import permits.

JICA Indonesia Office gave various advices for the importation, and applied PP19 (exemption of custom duties' permit) to the SEKNEG (Office for State Secretary).

2b-1. Delay of project related documents

Some required documents to implement and manage project activities are not available yet. Those are as follows;

i. Material Transfer Agreement (MTA)
ii. Minutes of Meeting of the 1st Joint Coordinating Committee.

2b-2 Cause

i. The format of MTA is designed to be part of Implementation Arrangement (IA) that will be signed by BPPT and University of Tsukuba (UT). Initial draft of IA was prepared by

BPPT and sent to UT to be reviewed. Currently the draft is under reviewed by UT.

ii. BPPT needed to confirm some points in the minutes to LIPI. LIPI had been contacted by BPPT to discuss this issue; however, the meeting had not been performed due to unmatched schedule of both parties.

2b-3 Action to be taken

i. To remind the authorities to finalize the draft of document

ii. To seek a meeting schedule that is satisfied by both parties

2b-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

i. BPPT will ask UT about the status of IA review, and then follow up comments that might be sent by UT regarding to the draft.

ii. BPPT and LIPI will jointly seek a meeting schedule to discuss some points in minutes of the 1st JCC meeting, and then finalize and sign it.

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the first year, the amendments are agreed in the 1st JCC Meeting on Feb 02, 2016. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2016

Accordance to BPPT's organizational reforming, the role of research function of Biotech Center was transferred to the Center for Pharmaceutical and Medical Technologies (PTFM) in beginning of 2016. Then, the main institute to implement project activities in BPPT changed from Biotech Center-BPPT to PTFM. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of PTFM and Program Head of PTFM accordingly.

3-2-2. Amendments due to reviewing the role of LIPI's institutes

The Director of Research Center for Biotechnology-LIPI was stipulated as the Project Co-manager in the original R/D signed in Feb 2015. However, the main institute to implement the project activities in LIPI must be the Indonesian Culture Collection (InaCC) under the Research Center for Biology-LIPI. Therefore, the project decided to replace the Project Co-manager to Director of InaCC from the Director of Research Center for Biotechnology-LIPI.

3-2-3. Amendments due to the reformation among Japanese institutes
The role of Japan Science and Technology Agency (JST) for the project was handed over to Japan Agency for Medical Research and Development (AMED) which was newly established in April 2016.

3-2-4. Amendments due to mistyping in the original R/D
There were several corrections on mistyped words in original R/D.

4 Preparation of Gov. of the Republic of Indonesia toward after completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.03 (Oct. 2016 – Mar. 2017)

Name: Prof. Tomoyoshi NOZAKI
Title: Chief Advisor

Submission Date: 01 Apr. 2017

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese : 9 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in this term (the cumulative number of dispatched researchers become 33 since the project start) A coordinator have been staying continuously in the project office

1-1-1-2. Indonesian: the Project Director, Project Manager and Project Co-managers have been appointed in accordance with the R/D. Researchers have been listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: Eight Indonesian researchers participated in trainings in Japanese institutes in this term. Another Indonesian researcher have been studying in Ph.D course in Univ. of Tsukuba Since Apr. 2016. The cumulative number of participation of Indonesian researcher become 22 since the project start.

1-1-2-2. In-country Training : There were following trainings organized in the project with Japanese instructors.

a. Training on "Purification of Active Compounds" *(by Dr.Mori)*

b. Training on "Target Enzyme Preparation and High Throughout Screening" *(by Dr. Daniel)*

c. Training on "Mammalian Cell Culture and Screening System" *(by Ms. Miyazaki)*

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side : Continued from previous semester, BTC-BPPT provided its microbial collection as bio-resources for this project. Some of plant sample collection owned by PTFM-BPPT (Center for Pharmaceutical and Medical Technology) were also provided to this project.

1-1-3-2. Provision by Japanese side: Required laboratory equipment as the 1st years' input was procured in Japan and Indonesia in 2015. The disbursement for the procurement was around 63 million Japanese yen. Some equipment were procured locally by UT with the amount of 405 million rupiah in the 1st year as well. Due to the new Indonesia regulation on the importing freezers, the delivery of imported equipment from Japan was delayed to Jun 2016, however those equipment was installed successfully in the BTC-BPPT and ITD-AU in Aug. 2016.

As the 2nd years' program, 17 items of equipment were listed up in the annual plan. However, As of the end of 2nd years, finally 28 items of equipment and other consumables (reagents, plastic wears. And so on) have been procured in Indonesia since Apr. 2016, the disbursement in the 2nd year was around 30.5 million Japanese yen.

1-1-4. Local costs

1-1-4-1.Indonesian Side: Last fiscal year (2016), BPPT allocated 450 million rupiah for employing personnel, travel, meeting, and consumables. This budget was rationalized according to direction from Ministry of Finance to around 390 million rupiah in August 2016. In the end of December 2016, total expenses from BPPT was about 345 million rupiah. The remained 45 million rupiah could not be used due to further budget rationalization in November 2016. In fiscal year 2017, BPPT allocated 500 million rupiah for employing personnel, travel, meeting, and consumables. BPPT is also going to seek funding from outside of BPPT, especially from Ministry of Research, Technology and Higher Education (4 proposals are prepared to be submitted).

Meanwhile, AU prepared annual budget for running cost around 450 million rupiah for 2016 as well.

1-1-4-2.Japanese Side: JICA Indonesia Office provided running expenses for such as employing secretary, car rental and consumables with its amount around 210 million rupiah for the 1st year. In the 2nd year, JICA had provided those running cost with its amount around 475 million rupiah.

1-2 Progress of Activities

Isolation of microbial strain from sample taken at Biak Island on May 23-26, 2016 resulted in 883 newly isolated microbes composed from fungi and actinomycetes. These microbes were then registered in BTC-BPPT microbial collection, so total number of registered microbes at the end of Dec 2016 was 25,435 isolates.

Microbial extracts were prepared by cultivate microbes from the collection in appropriate medium. In 2016, more than 8,400 microbial extracts were prepared for 1st screening. Moreover, 341 reconfirmation extracts (30 mL culture) were prepared based on result of 1st screening. Furthermore, 19 scaled-up extracts (100 mL ~ 5 L culture) were also prepared for purification of active compound.

Extensive enzyme-based screening of microbial extracts for antimalarial and antiamebic activities were conducted in BTC and AU, respectively, after installation of equipment in both institutions (August 2016). More than 6,000 extracts were screened based enzymatic activity of anti-malarial target enzyme PfDHODH in 2016. From this screening, more than 110 hits (active extract) were obtained. After reculturing the producer, only 21 of them showed activity. Two of them were then further purified. Screening of 119 plant extracts had been done against this enzyme, resulting in 29 active extracts. About 1400 extracts were also objected to 1st screening against PfMQO enzyme, resulting in 89 hits. These hits are still under reculturing process. Starting from January 2017, cell-based screening against malarial parasite was established in BTC-BPPT. Currently, the screening is performed by BTC-BPPT using currently prepared microbial extracts.

Enzyme-based screening of microbial extract for searching inhibitory activity against CS3 and SAT1 enzymes, target enzyme for anti-amebic screening, were also done. More than 2200 extracts were objected to CS3 and SAT1 enzyme assay system, resulting in 21 and 28 hits, respectively. Currently these extracts are being recultured. Cell-based screening using *Entamoeba histolytica* cell was done by employing 1240 extracts, and resulting 49 hits. Four of them showed activities after reculturing the producer, and proposed as hits to be further purified.

Toxicity of the hits were done using DLD-1 cell (colon carcinoma cell). To date, 93 hits that showed inhibitory activity against PfDHODH and PfMQO were objected to toxicity assay resulting 77 of them remained not toxic.

Purification of active compound was done for 8 and 16 active extracts with anti-amebic and anti-malarial activity, respectively. Most of them are currently being fractionated by open column, and some of them were not continued due to low activity. Soybean meal, an ingredient in fungi medium, also showed inhibitory activity against both PfMQO and PfDHODH. Purification of active compound of this ingredient, as well as other plant originated active extracts was currently being performed.

1-3 Achievement of Output

1-3-1. Achievement of Output 1

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

Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	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. 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 • Currently, purification of active extract are being perfomed.
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. • According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.

1-3-2. Achievement of Output 2

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

Indicators	Achievement
2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	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. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.	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. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	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.

1-3-3. Achievement of Output 3

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

Indicators	Achievement
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.	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. 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.	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. 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.	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. 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. • 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.	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.	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.

1-4 Achievement of the Project Purpose

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.

In this semester, a lot of progress was achieved, especially in screening of extracts and purification of active compound. Four enzymes used for enzyme-based screening had been prepared in BTC. More than 8000 extracts had been prepared during this semester, and more than 6000 extracts had been already objected for screening to search active extract with inhibitory activity against DHODH, MQO, CS3 and SAT1, and against parasite *Entamoeba histolytica*. Cell-based screening system using *P.falciparum* has been introduced and established in BTC, and will be implemented for routine screening in next semester. Secondary screening of active extracts had also been conducted. Mammalian cell culture system was established in BTC, and cytotoxicity test using these cells of active extracts has been performed. Purification of 24 active compounds has been started, and some of them have been objected to structural analysis using NMR. A manuscript of paper related to these achievement is being prepared, and will be submitted to related scientific journal within this year with Indonesian researcher as the first author.

Indicators	Achievement
1. At least one (1) lead compound with anti-malarial 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 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. 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. • First screening of more than 2200 microbial extracts was 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 and proliferation of *E.histolytica* cell are currently conducting. • Efficacy test using animal experiment will be conducted in 2018
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 expected to be achieved by the time of the end of the Project. • 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)

1-5 Changes of Risks and Actions for Mitigation

1-6 Progress of Actions undertaken by JICA

1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)

1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2-1. Delay of project related documents

Some required documents to implement and manage project activities are not available yet. Those are as follows;

i. MoU between BPPT and Univ. of Tokyo
ii. “Implementation Arrangement” including Material Transfer Agreement (MTA)
iii. Minutes of Meeting of the 2nd Joint Coordinating Committee(JCC) Meeting.
iv. Handing over documents of provided equipment from JICA to BPPT
v. Submission of the last version of the Project Monitoring Sheets

2-2 Cause

i. Due to the alteration of Japanese Coordinating Research Institute in April 2017, MoU between BPPT and Univ. of Tokyo is necessary to succeed the project implementation.
ii. The format of MTA is designed to be part of Implementation Arrangement (IA) that will be signed by BPPT and the Japanese Coordinating Institute. Initial draft of IA was prepared by BPPT and sent to Japanese side to be reviewed. Currently the draft is under reviewed by BPPT HDQ
iii. After the 2nd JCC Meeting, the draft of minutes was prepared to exchange the signature. Now the draft has been reviewed by BPPT side.
iv. JICA needs to handover the equipment officially right after its provision, however BPPT side needs to confirm the required Indonesian official documents to handover.
v. After the edition of the initial draft made in the project site, the authority of BPPT have not endorse the document yet.

2-3 Action to be taken

i. To remind the authority to complete the MoU
ii. To remind the authorities to accelerate to finalize the document
iii. To remind personnel concerned frequently
iv. To remind personnel concerned frequently
v. To remind authority of BPPT to finalize

2-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

i. BPPT (Project Director, Project Manager, Project Co-manager)
ii. BPPT (Project Director, Project Manager, Project Co-manager) and U.Tokyo

iii. BPPT (Project Director, Project Manager, Project Co-manager) and JICA (JICA office and Coordinator)
iv. BPPT (Project Director, Project Manager, Project Co-manager) and JICA (JICA office and Coordinator)
v. BPPT (Project Manager, Project Co-manager)

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the 2nd year. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2017
The main institute to implement project activities in BPPT changed from PTFM-BPPT to the Biotech Center-BPPT. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of Biotech Center and Program Head of Biotech Center accordingly.

3-2-2. Amendments due to the alteration of Japanese Coordinating Research institute
The Japanese Coordinating Research Institute is required to change from University of Tsukuba U to University of Tokyo on 1st April 2017, the reason is the alteration of belonging university of the Japanese Chief Advisor.

4 Preparation of Gov. of the Republic of Indonesia toward after completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.04 (Apr. 2017 – Sep. 2017)

Name: Prof. Tomoyoshi NOZAKI
Title: Chief Advisor

Submission Date: 01 Oct. 2017

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese : 11 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in this term (the cumulative number of dispatched researchers become 44 since the project start). Other 2 researchers were invited as guest speakers of the International Symposium which the project organized. A coordinator have been staying continuously in the project office

1-1-1-2. Indonesian: the Project Director, Project Manager and Project Co-managers have been appointed in accordance with the R/D. Researchers have been listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: Three Indonesian researchers participated in short-term trainings in Japanese institutes in this term. Other three more Indonesian researchers have been studying in Ph.D course in Univ. of Tokyo and Kitasato Univ. since April 2017. The cumulative number of participation of Indonesian researcher become 27 since the project start.

1-1-2-2. In-country Training : There were following trainings organized in the project with Japanese instructors.

a. Training on "Purification of Active Compounds" *(by Dr.Mori, Dr.Dobashi & Dr.Yamashita)*

b. Training on "Target Enzyme Preparation and High Throughout Screening" *(by Dr. Daniel)*

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side : BPPT has been providing facility of BSL-2 level's laboratories and AU also has been doing as well. Bio-resources possessed in BPPT have been provided to the project.

1-1-3-2. Provision by Japanese side: Required laboratory equipment as the 1st years' input was procured in Japan and Indonesia in 2015. The disbursement for the procurement was around 63 million Japanese yen. Some equipment were procured locally by UT with the amount of 405 million rupiah in the 1st year as well. Due to the new Indonesia regulation on the importing freezers, the delivery of imported equipment from Japan was delayed to Jun 2016, however those equipment was installed successfully in the BTC-BPPT and ITD-AU in Aug. 2016.

As the 2nd years' program, 17 items of equipment were listed up in the annual plan. However, As of the end of 2nd years, finally 28 items of equipment and other consumables (reagents, plastic wears. And so on) have been procured in Indonesia since Apr. 2016, the disbursement in the 2nd year was around 30.5 million Japanese yen.

As the 3rd years' program, 10 items of equipment were listed up in the annual plan. However, As of the end of Sep 2017, 4 items of equipment and other consumables (reagents, plastic wears. And so on) have been procured in Indonesia since Apr. 2017, the disbursement in the 3rd year until Sep 2017 was around 400 million Indonesian Rupiah.

1-1-4. Local costs

1-1-4-1.Indonesian Side: In this fiscal year (2017), BPPT allocated budget as much as 500 million Rupiah for employing personnel, travel, meeting, and consumables. This budget was rationalized according to direction from Ministry of Finance to about 477 million Rupiah in September 2017. Total expense until end of September 2017 was about 341.6 million Rupiah (71.64%). BPPT also received external funding from Ministry of Research, Technology and Higher Education as much much as 258.175.000 Rupiah.

1-1-4-2.Japanese Side: JICA Indonesia Office provided running expenses for such as employing secretary, car rental and consumables with its amount around 210 million rupiah for the 1st year. In the 2nd year, JICA had provided those running cost with its amount around 475 million rupiah. For the 3rd year JICA estimates the annual cost provided with around 600 million rupiah due to additional expenses for the International Symposium

1-2 Progress of Activities

Field trip to Togean Island, Central Sulawesi, was held in May 15-19, 2017, to collect bioresource samples (soil, plant litter, etc.). More than 90 samples were obtained during this trip. Before that, sample collection was also done in Puspiptek Area (around Laboratory for Biotechnology, BPPT) together with an expert from Japan in May 10-13, 2017. During this activity, 9 plant litter samples and 3 soil samples were obtained.

Isolation of microbial strain from these samples was conducted. So far, more than 359 newly isolated microbes (fungi and actinomycetes) were obtained. In parallel, these microbial isolates, as well as those isolated from sample that was taken last year from Biak Island), were identified morphologically. So far, more than 410 fungi isolates were identified and registered into BTC-BPPT microbial collection.

Microbial extract for 1st screening was produced by cultivating the newly isolated microbes, as well as microbial isolates from microbial culture collection in BPPT, in 2 kinds of medium. Until end of September 2017, more than 1600 microbial extracts for reconfirmation had been produced.

Extensive screening of microbial extracts were done using 1st screening extract that had already been prepared. By the end of September 2017, more than 4400 and 3800 microbial extracts were screened for searching inhibitor of malaria parasite specific enzyme *Pf*DHODH and *Pf*MQO, respectively. Starting from 2017, extensive malarial cell-based screening was perfomed. So far, more than 3200 extracts had been screened.

Screening of microbial extract against amebic parasite specific enzyme EhCS3 and EhSAT1 were also performed. So far, more than 2800 and 480 microbial extracts were screened to search inhibitor of EhCS3 and EhSAT1, respectively. At the same time, more than 2800 extracts were screened by amebic cell-based assay.

Toxicity test of hit extracts was performed by using colon cancer cell line (DLD-1). So far, more than 110 active extracts were objected into toxicity test for hit from malaria cell-based screening result.

Experiencing loss of activity of microbial extract that was prepared by reculturing the microbe that showed activity last year, a reconfirmation extract was produced to make sure that the activity of extract produced from recultured microbe is still reminded. All of hit extracts were reproduced by reculturing the producing microbes in 2 kinds of medium, each 100 mL, for 5 days, and part of the culture was taken on day-3, 4, and 5. These culture samples were extracted and objected into appropriate assay system to know whether the extract was active or not. By the end of September, more than 400 reconfirmation extracts were produced.

To accelerate purification process of active compound, starting from 2017, characteristic of active extract is examined before the extract is objected into main purification process, by a process called Pre-Extraction Test (PET). PET was done by using the remained reconfirmation culture broth. Based on PET result, strategy for purification of active compound will be determined. Until end of September 2017, 27 extracts were objected into PET and continued to purification. Most of them are currently

being fractionated by open column, and some of them were not continued due to low activity.

In August 22-23, 2018, an international symposium on natural resources-based drug development was held in BPPT Main Office, Jakarta. This symposium was aimed to build a network and national research consortium on drug development, especially based on natural resources. Seventeen invited speakers from Indonesia and Japan delivered their recent achievements in drug development from natural resources. The symposium was attended by more than 140 participants from universities, research institutes, and related ministries. The Project team also received a letter from Prof. Satoshi Ohmura, Professor Emeretus of Kitasato University and Nobel Laurette in Physiology and Medical Field 2015. Prof. Ohmura stressed out the importance of drug discovery from natural products, especially from untapped Indonesian microbial resources, for the sake of humankind over the world.

In this occasion, BTC exchanged Material Transfer Agreement with Obihiro University of Agriculture and Veterinary Medicine (PI: Dr. Yasufumi Nishikawa). Under this agreement, BTW will share more than 3800 microbial samples to OUAVM to be screened against *Toxoplasma gondii*, parasite causing toxoplasmolysis in human. This would be a milestone of network development in microbial resources-based drug discovery originated from Indonesia with international partners.

1-3 Achievement of Output

1-3-1. Achievement of Output 1

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

Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	The indicator has been achieved (3 compounds with anti-malarial had been isolated and purified) • More than 11000 of microbial extracts were objected for 1st screening against PfDHODH and PfMQO.in cumulative • Confirmation of inhibitory activity about of 80 and 120 active extracts has been done resulting in about 20 active extracts with inhibitory activity against PfDHODH and PfMQO, respectively. • Cell based screening of more than 3000 extracts resulting more than 600 hits. • Toxicity assay was conducted for more than 450 extracts, resulting in near 380 extracts remained

	active. • Purification of 2 extracts with PfMQO inhibitory activity and 4 extracts with PfDHODH inhibitory activity 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.	The indicator has been achieved (The chemical structure of two (2) compounds with anti-malarial activity had been elucidated) • Purification of other 6 active extracts are currently being performed
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. • According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.

1-3-2. Achievement of Output 2

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

Indicators	**Achievement**
2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	The indicator is expected to be achieved by the Mid-term Review. • More than 3000 extracts were objected to enzyme- and cell-based screening for anti-amebic activity. The activity of twelve hits was remained after reconfirmation. • Confirmation of inhibitory activity of 30 active extracts from cell-based screening has been done. • Purification of active compound from 12 active extracts that have inhibitory activity against CS3 enzyme are currently conducting
2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.	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. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	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.

1-3-3. Achievement of Output 3

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

Indicators	**Achievement**
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.	The indicator is already achieved. More than 11000 extracts for first screening have been produced from newly-obtained and existing microorganisms and plants. All of them have been registered. • Currently, more than 4000 of microbial extracts were newly prepared from January 2017. About 500 microbes were newly isolated from soil sample that was taken from Togean Island in May 2017. All extracts and microbes were registered in the in-house biological resource libraries. • In cumulative, more than 11000 extracts for first screening had been produced.
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.	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) 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. 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.	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. • Establishment of culture and evaluation system using *P.falciparum* 3D7 are established at BTC. • Mammalian cell culture and evaluation system are established at BTC.
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. • Introduction of pre-extraction test to ensure the extract remained active was introduced.
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.
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 2019.

1-4 Achievement of the Project Purpose

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.

Malarial cell-based screening was initiated and accelerated from the beginning of 2017 in BTC. To date, more than 3200 extracts have been screened. Toxicity of active extracts as the result from this screening was examined using mammalian cells. Currently BTC is maintaining 5 mammalian cells that can be used for toxicity assay purpose.

In other side, dereplication method to avoid fatty acids as frequents hit of PfMQO was introduced into screening pipeline. This method was effective to select active extracts with active compound other than fatty acid for further purification.

Loss of activity of active extract after reculture became the main issues in this semester. To overcome this problem, reconfirmation extract was produced to ensure that the extract was still active after being reproduced using the same producer.

Introduction of PET as a step before main purification of active extract increased efficiency of purification process. From PET result, more precise purification step could be predicted, resulting in wasting valuable microbial extract during laborious purification process.

Indicators	Achievement
1. At least one (1) lead compound with anti-malarial 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 11000 of microbial extracts were objected for 1st screening against PfDHODH and PfMQO. • Confirmation of inhibitory activity about of 80 and 120 active extracts has been done resulting in about 20 active extracts with inhibitory activity against PfDHODH and PfMQO, respectively. • Cell based screening of more than 5000 extracts resulting more than 600 hits. • Toxicity assay was conducted for more than 3000 extracts, resulting in near 400 extracts remained active. • Purification of 2 extracts with PfMQO inhibitory activity and 4 extracts with PfDHODH inhibitory

	activity are being purified. • Efficacy test using animal experiment will be started in 2018
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 3000 extracts were objected to enzyme- and cell-based screening for anti-amebic activity. The activity of twelve hits was remained after reconfirmation. • Confirmation of inhibitory activity of 30 active extracts from cell-based screening has been done. • Purification of active compound from 12 active extracts that have inhibitory activity against CS3 enzyme are currently conducting. • Efficacy test using animal experiment will be conducted in 2018.
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 expected to be 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 submitted to peer-reviewed journal.

1-5 Changes of Risks and Actions for Mitigation
1-6 Progress of Actions undertaken by JICA
1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)
1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2-1. Delay of project related documents

Some required documents to implement and manage project activities are not available

yet. Those are as follows;

i. "Implementation Agreement " including Material Transfer Agreement (MTA)

ii. Handing over documents of provided equipment from JICA to BPPT

iii. Submission of the last two versions of the Project Monitoring Sheets

2-2 Cause

i. The format of MTA is designed to be part of Implementation Agreement (IA) that will be signed by BPPT and the Japanese Coordinating Institute. The draft of IA was prepared by BPPT and requested to Japanese side to review. Currently the draft is still under reviewing in Japan from the view point of the Convention of Biological Diversity (CBO).

ii. JICA needs to handover the equipment officially right after its provision, however BPPT side needs to confirm the required Indonesian official documents to handover.

iii. After the endorsement in BPPT side, the document have not been endorsed by the Japanese side yet.

2-3 Action to be taken

i. To remind the authorities in Japan to finalize the document

ii. To confirm the required transaction in Indonesian side

iii. To remind the Japanese authority to finalize

2-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

i. U.Tokyo, and Kitasato Univ.

ii. BPPT (Project Director, Project Manager, Project Co-manager)

iii. U.Tokyo (Chief Advisor)

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the 2nd year. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2017

The main institute to implement project activities in BPPT re-changed from PTFM-BPPT to the Biotech Center-BPPT. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of Biotech Center and Program Head of Biotech Center accordingly.

3-2-2. Amendments due to the alteration of Japanese Coordinating Research institute
The Japanese Coordinating Research Institute was changed from University of Tsukuba to University of Tokyo (UTokyo) on 1st April 2017, the reason was the alteration of belonging university of the Japanese Chief Advisor.

4 Preparation of Gov. of the Republic of Indonesia toward after completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*

Head of Laboratory for Biotechnology

Agung Eru Wibowo

SLeCAMA P.O. (Plan of Operation) version

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	Remarks	Monitoring: Issue	Monitoring: Solution
	Year	2015	2016	2017	2018	2019			
	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			
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					Detail plan for equipment of mass production is to be discussed			
	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								
Post Graduate Course (long term training)	Plan								
	Actual	(Ms.Retno) ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	2 more Ph.D students from Apr 2017			
	Actual	(Dr.Feni) ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■				
	Actual	(Ms.Amila) ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■				
In-country/Third country Training									
	Plan								
	Actual	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■				

Activities / Sub-Activities	JFY	1st Year	2nd Year	3rd Year	4th Year	Responsible Organization		Achievements	Issue & Countermeasures
	Year	2015	2016	2017	2018				
	Month	4 7 6 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	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					NU	BPPT	Screening against 2 targets enzymes were conducted.	
	Actual	■ ■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■					
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Plasmodium falciparum*	Plan					NU	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					NU	BPPT	Screening against proliferation of the parasites and toxicity against mammalian cell were conducted.	
	Actual	■		■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■					
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium	Plan					KU NU	BPPT		
	Actual	■ ■ ■		■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■					
1.5 Establishment of mass production system of the lead compound candidates	Plan					KU MBJ	BPPT	Reconfirmation extract production was introduced before large scale production	
	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					NU	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.)								Achievement	Issue & Countermeasures
2.1 Primary screening for inhibitory activity of extracts to the *Entamoeba histolytica* -derived site-specific recombinant enzyme	Plan					UTokyo	AU (BPPT)	2-1. 3000 extracts were screened by enzyme-based in Japan, 3000 extracts were screened in Indonesia 2-1. 320 extracts were screened by cell-based in Japan, 3000 extracts were screened in Indonesia	
	Actual	■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■					
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of *Entamoeba histolytica*	Plan					UTokyo	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					UTokyo	AU (BPPT)		
	Actual	■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■					
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against *Entamoeba histolytica*	Plan					KU Utokyo	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	2-6. The chemical structure of isolated and purified active compound will be elucidated	
	Actual								
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing	Plan					UTokyo	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
		1st Year	4 5 6 7 8 9 10 11 12				
		2nd Year	1 2 3 4 5 6 7 8 9 10 11 12				
		3rd Year	1 2 3 4 5 6 7 8 9 10 11 12				
		4th Year	1 2 3 4 5 6 7 8 9 10 11 12				
		2015					
		2016					
		2017					
		2018					
		2019	1 2 3				
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			KU MBU	BPPT LIPI	Field trip for collecting microbial samples were conducted at Togean Island, Central Sulawesi.	
	Actual						
3.2 Establishment of screening systems	Plan			U.Tokyo NU	BPPT AU	Enzyme production for screening has been done in BPPT	
	Actual						
3.3 Establishment of culture and evaluation systems	Plan			U.Tokyo NU	BPPT AU	Cell based culture and evaluation system have been established in BTC	
	Actual						
3.4 Introduction of technologies of isolation and purification	Plan			KU	BPPT	Introduction of dereplication technique	
	Actual						
3.5 Introduction of technologies of chemical structure elucidation	Plan			KU	BPPT		
	Actual						
3.6 Establishment and enhancement of a research network in Indonesia	Plan			ALL	BPPT,AU, LIPI	#1 International symposium was held, collaboration with a research institute in Japan was established	
	Actual						
Duration / Phrasing	Plan						
	Actual						

Monitoring Plan	JFY	Year	Month	Remarks	Issue	Solution
		1st Year	4 5 6 7 8 9 10 11 12			
		2nd Year	1 2 3 4 5 6 7 8 9 10 11 12			
		3rd Year	1 2 3 4 5 6 7 8 9 10 11 12			
		4th Year	1 2 3 4 5 6 7 8 9 10 11 12			
		2015				
		2016				
		2017				
		2018				
		2019	1 2 3			
Monitoring						
Joint Coordinating Committee	Plan			#2 JCC Meeting was held		
	Actual					
Scientific Meeting	Plan			Occasionally		
	Actual					
Set-up the Detailed Plan of Operation	Plan			Annual Working Plan		
	Actual					
Submission of Monitoring Sheet	Plan			2st Monitoring Sheet	2nd sheet submission was delayed	
	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				Establishment of website was delayed due to reorganization in BPPT	
	Actual					
International symposiums are held for drug discovery	Plan			#1 International Symposium was held		
	Actual					

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.05 (Oct. 2017 – Mar. 2018)

Name: Prof. Tomoyoshi NOZAKI

Title: Chief Advisor

Submission Date: 01 Apr. 2018

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese: 14 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in this term (the cumulative number of dispatched researchers become 58 since the project start). A coordinator have been staying continuously in the project office

1-1-1-2. Indonesian: The Project Director, Project Manager and Project Co-managers have been appointed in accordance with the R/D. Researchers have been listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: Five Indonesian researchers participated in short-term trainings in Japanese institutes in this term. Other three Indonesian researchers have been studying in Ph.D course in Univ. of Tokyo and Kitasato Univ. since April 2017. The cumulative number of participations of Indonesian researcher become 32 since the project start.

1-1-2-2. In-country Training: There were following trainings organized in the project with Japanese instructors.

a. Training on "Purification of Active Compounds" *(by Dr.Mori, Dr.Dobashi & Dr.Yamashita)*

b. Training on "Antimalarial Target Enzyme Preparation and High Throughout Screening" *(by Dr. Daniel)*

c. Training on "Antimalarial Cell Screening System" *(by Dr. Sakura)*

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side : BPPT has been providing facility of BSL-2 level's laboratories and AU also has been doing as well. Bio-resources possessed in BPPT have been provided to the project.

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1-1-3-2. Provision by Japanese side: Required laboratory equipment as the 1st years' input was procured in Japan and Indonesia in 2015. The disbursement for the procurement was around 63 million Japanese yen. Some equipment were procured locally by UT with the amount of 405 million rupiah in the 1st year as well. Due to the new Indonesia regulation on the importing freezers, the delivery of imported equipment from Japan was delayed to Jun 2016, however those equipment was installed successfully in the BTC-BPPT and ITD-AU in Aug. 2016.

As the 2nd years' program, 17 items of equipment were listed up in the annual plan. However, As of the end of 2nd years, finally 28 items of equipment and other consumables (reagents, plastic wears. And so on) have been procured in Indonesia since Apr. 2016, the disbursement in the 2nd year was around 30.5 million Japanese yen.

In the 3rd years, since Apr 2017 total 15 items of equipment and consumables have been procured in Indonesia and Japan, the disbursement for those procurement in the 3rd year were around 1.37 billion Indonesian Rupiah in Indonesia and approximate 6 million Japanese yen in Japan.

1-1-4. Local costs

1-1-4-1.Indonesian Side: Throughout fiscal year 2017, BPPT allocated 500 million rupiah for employing personnel, travel and consumable. This budget was then optimized according to direction from Ministry of Finance in the middle of fiscal year. At the end of 2017, BPPT has disbursed 477 million rupiah from allocated budget and another 151.5 million rupiah from other budget for this project,. BPPT also got funding from Ministry of Research, Technology, and Higher Education (MoRTHE) as much as 258 million rupiah. Total cost bared by BPPT for this project in FY 2017 was 822.6 million rupiah.

For fiscal year 2018, BPPT allocated 418.4 million rupiah. At the same time, BPPT is also seeking funding from other agency including MoRTHE.

Meanwhile, AU disbursed about 156 million rupiah in 2017, and prepared annual budget for running cost around 210 million rupiah for 2018.

1-1-4-2.Japanese Side: The annual disbursement for the local running expenses of such as employing assistants, car rental and consumables which JICA Indonesia Office supported in the 1st , 2nd and 3rd years were around 210 million, 475 million and 600 million Rupiah respectably. Especially, the disbursement of the 3rd year was included of the cost for the International Symposium held in Aug 2017.

1-2 Progress of Activities

Isolation of microbial strain taken from Togean Island on May 15-19, 2017, was conducted from 71 samples of soil, plant litter, and mushroom. To date, more than 374 fungi and 121 actinomycetes new isolates were obtained. Identification of these isolates, as well as revived microbes from frozen stock, was done based on observation of their morphology. In 2017, about 280 actinomycetes isolates and 46 fungi isolates were identified and registered in the microbial database.

About 4400 extracts for first screening were produced in 2017, and cumulative first screening extract production from the beginning of this project reached as much as 13500 extracts. More than 600 extracts for reconfirmation and 36 extracts for purification were produced in 2017.

More than 4500 and 6000 extracts were screened against anti-malarial target PfDHODH and PfMQO, respectively. Hit rate of these screening was 0.9% and 2.7% respectively. Since fatty acid is one of frequently obtained PfMQO inhibitor from microbial extracts, additional screening step was added to determine whether the activity comes from fatty acid or not. The extract was treated by α-cyclodextrin, which will form a complex with fatty acids, and its inhibitory activity was compared with that before treatment. About 20% of hit was excluded by this step, suggesting that this step is effective to reduce number of hit.

Antimalarial cell-based screening was extensively started from the beginning of 2017. Although a bit behind the schedule, more than 5700 extracts have been screening resulting more than 670 hits (11%). The hit were then examined their toxicity against mammalian cell (using DLD-1 colon cancer line), but most of them remained non-toxic under designed condition. Optimization of screening system was performed to obtain promising active extract, by raise the threshold value in parasite growth-inhibition calculation, as well as lowering the threshold value in mammalian cell growth-inhibition calculation and diluting extract amount in the assay system. Comparison of toxicity assay under hypoxia and nutrient free condition was also performed. In addition, dereplication method by testing active extract against gram-positive bacteria was also examined. Through this examination, hit rate of anti-malarial screening could be decreased more than 50%.

Screening of extracts against Entamebic EhCS3 and SAT1 were conducted using 3800 and 1500 extracts during 2017. Twelve hit extracts with EhCS3 inhibitory activity showed activity after reconfirmation, while 10 extracts with EhSAT1 inhibitory activity showed activity after reconfirmation and proceeded for purification. More than 5100 extracts were objected for Amebic cell-based screening resulting in 33 active extracts which were reconfirmed.

Development of target and assay system is important for sustainability of drug discovery activity. In last March, a target and assay system for screening of anti-tuberculosis agent was proposed by researcher from BTC. The system targeted enzymes involved in shikimate pathway, which is commonly present in bacteria and plants but not in Mammalia. The system will be further developed and verified before applied for

screening using bioresources extracts.

Purification of active compound with PfMQO inhibitory activity was done by adding α-cyclodextrin (α-CD) treatment into the protocol before further fractionated. This step is aimed to remove fatty acids from the extract, which is the frequently obtained common PfMQO inhibitor. As result, among 13 extracts proposed to be purified, 11 of them were stopped due to loss of activity after α-CD treatment. Purification of 11 active extracts with PfDHODH inhibitory activity was conducted during 2017. Purification of 2 of these extracts almost finished, while the remained extract are currently being produced in larger scale.

In November 14-15, 2017, BTC participated in the 9th International Seminar of Indonesian Society for Microbiology, held in Palembang. In this occasion, BTC presented 4 titles related to activities in this Project. The audiences highly appraised this Project, showed by enthusiast of some participants who were interested in drug discovery from bioresources. BTC also promoted its microbial collection to the participants who are willing to utilize them for drug discovery.

The first publication written by Indonesian researcher as first author was published on December 2017. The paper was published in BBA-Bioenergetics (impact factor=4.9). On 15-18 March 2018, part of progress of this project was also published in The 2018 Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry, held in Nagoya.

From February 2017 BTC added a new researcher in this project and assigned to join with enzyme-based screening.

1-3 Achievement of Output

1-3-1. Achievement of Output 1

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

Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	The indicator has been achieved (3 compounds with anti-malarial had been isolated and purified) • About 13000 of microbial extracts and 128 of plant extracts were objected for 1st screening against DHODH and MQO in cumulative. • More than 600 reconfirmation extracts and 36 extracts for purification were produced in 2017. • About 5700 extracts have been objected into malarial cell-based screening in cumulative. • Optimization of cell-based screening system was performed. • Purification of 2 active extracts with PfDHODH

	inhibitory activity are currently being performed
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 two (2) compounds with anti-malarial activity had been elucidated) • Purification of 2 active extracts with PfDHODH inhibitory activity are currently being performed
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. • According to PO, efficacy test will be tentatively conducted in the 4th year of the Project.

1-3-2. Achievement of Output 2

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

Indicators	**Achievement**
2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	The indicator is expected to be achieved by the Mid-term Review. • More than 4600 extract were screened against EhCS3, 2200 extracts against EhSAT1, and 6000 extracts against parasite in cumulative. • About 10 extracts with enzymatic inhibition activity and 30 extracts with cell proliferation inhibition activity were reconfirmed to be active. • Purification of active compound from 12 active extracts that have inhibitory activity against parasites are 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.	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. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of	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.

the project period.	

1-3-3. Achievement of Output 3

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

Indicators	**Achievement**
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.	The indicator is already achieved. More than 13500 extracts for first screening have been produced from newly-obtained and existing microorganisms and plants. All of them have been registered.
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.	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) 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.
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.	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. • Establishment of culture and evaluation system using *P.falciparum* 3D7 are established at BTC. • Mammalian cell culture and evaluation system are established at BTC.
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.
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.
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 2019.

1-4 Achievement of the Project Purpose

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.

Application of α-CD in dereplication of active extracts with PfMQO activity was effective to select active extracts to be further purified. Number of hit to be proposed for further purification could be reduced, and obtaining fatty acids as frequent hit could be avoided.

Optimization of malarial cell-based screening was performed to reduce the number of hit, which was reached >10%. Increasing threshold level of inhibition rate, lowering threshold for toxicity rate, examining toxicity against mammalian cell under hypoxia and nutrient free environment, and introducing dereplication method by examining antibiotic activity against gram positive bacteria effectively reduced hit rate of the screening to <2%.

New target for screening of anti-tuberculosis agents was proposed. This proposal will be further developed and examined before used for screening using bioresources extracts.

Indicators	**Achievement**
1. At least one (1) lead compound with anti-malarial 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 13000 of microbial extracts were objected for 1st screening against PfDHODH and PfMQO. • More than 600 reconfirmation extracts and 36 extracts for purification were produced in 2017. • About 5700 extracts have been objected into malarial cell-based screening in cumulative. • Optimization of cell-based screening system was performed. • Purification of 2 active extracts with PfDHODH inhibitory activity are currently being performed • Efficacy test using animal experiment will be started in 2018
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 4600 extract were screened against EhCS3, 2200 extracts against EhSAT1, and 6000 extracts against parasite in cumulative. • About 10 extracts with enzymatic inhibition activity and 30 extracts with cell proliferation inhibition activity were reconfirmed to be active. • Purification of active compound from 12 active extracts that have inhibitory activity against parasites are being purified

	• Efficacy test using animal experiment will be conducted in 2018
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.

1-5 Changes of Risks and Actions for Mitigation

1-6 Progress of Actions undertaken by JICA

1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)

1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2-1. Delay of project related documents

Some required documents to implement and manage project activities are not available timely yet. Those are as follows;

i. Handing over documents of provided equipment from JICA to BPPT

ii. Submission of the last two versions of the Project Monitoring Sheets(PMS)

2-2Causes

i. JICA needs to handover the equipment to BPPT officially right after its provision by the document signed between BPPT and JICA expert, however BTC has not been able to confirm how to proceed the handing over document according to the rule of BPPT so far. Therefore the transaction can not be proceeded yet.

ii. After making initial drafts by Japanese side, the document have not been edited by the BPPT side yet.

2-3 Action to be taken

i. To confirm the required transaction in BPPT side

ii. To edit the PMS punctually every 6 months by BTC

2-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

i. Biotech Center, BPPT (Project Manager, Project Co-manager)

ii. Biotech center, BPPT (Project Manager, Project Co-manager)

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the 2nd year. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2017

The main institute to implement project activities in BPPT re-changed from PTFM-BPPT to the Biotech Center-BPPT. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of Biotech Center and Program Head of Biotech Center accordingly.

3-2-2. Amendments due to the alteration of Japanese Coordinating Research institute

The Japanese Coordinating Research Institute was changed from University of Tsukuba to University of Tokyo (UTokyo) on 1st April 2017, the reason was the alteration of belonging university of the Japanese Chief Advisor.

4 Preparation of Gov. of the Republic of Indonesia toward after completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*

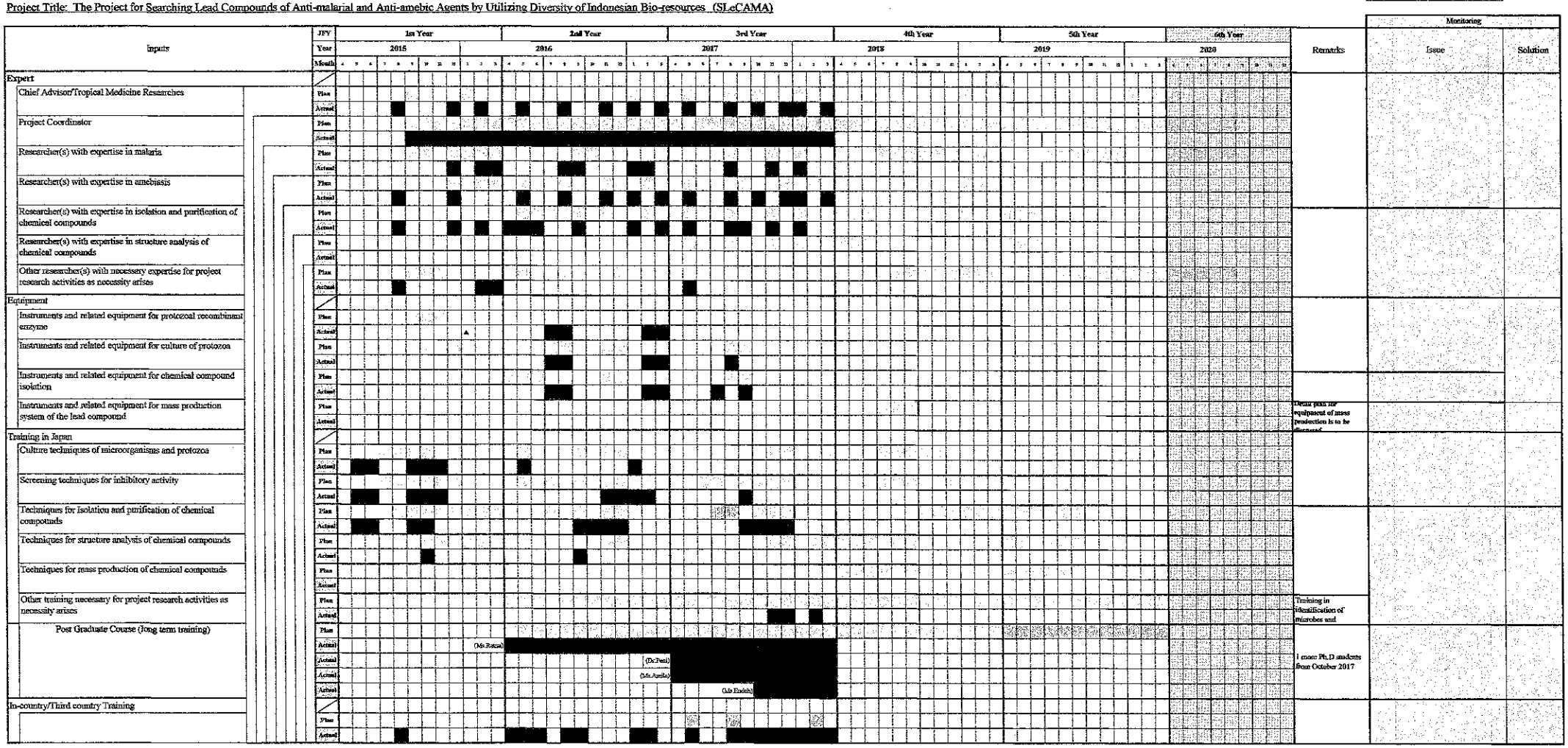
Head of Laboratory for Biotechnology

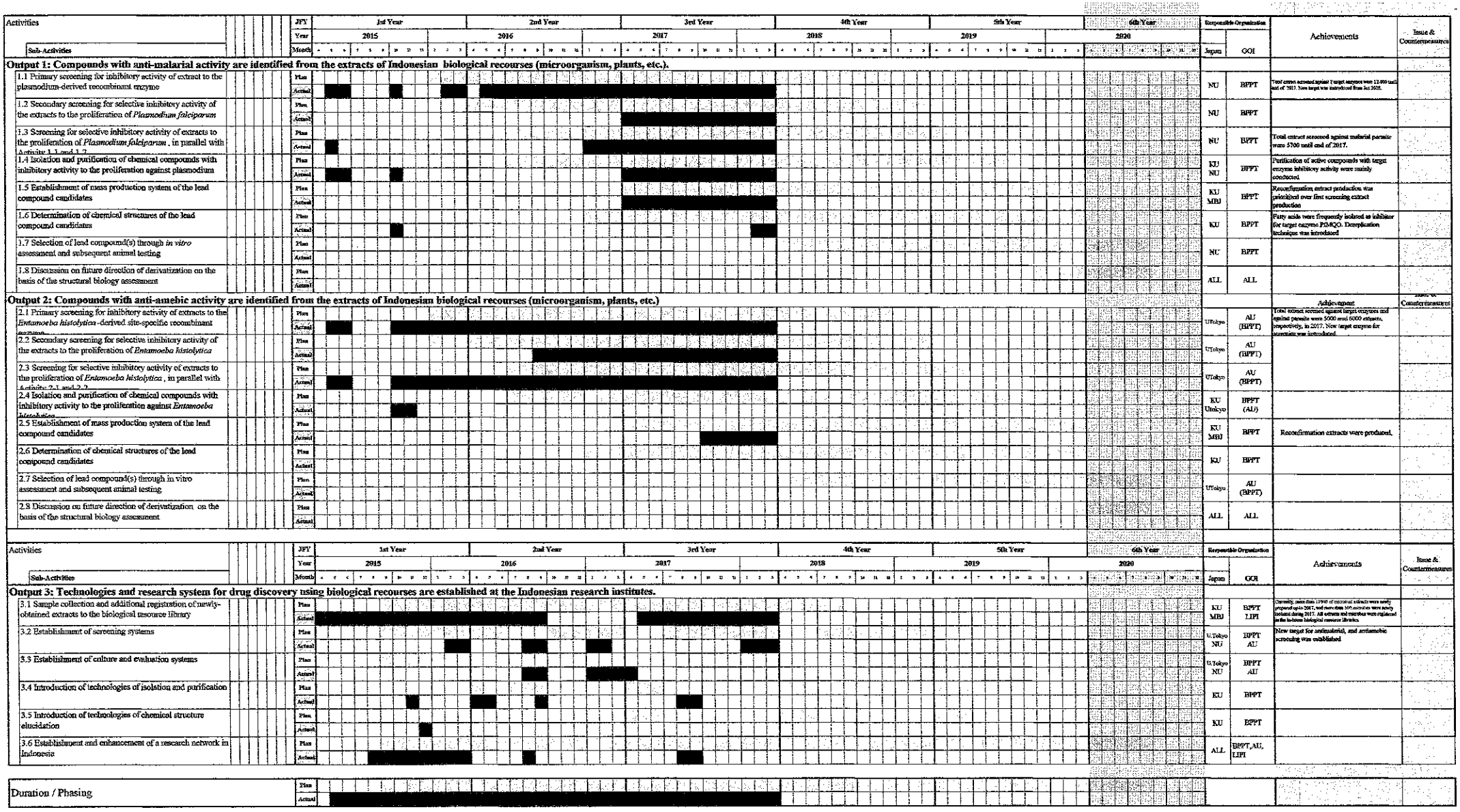
Agung Eka Wibowo

SLeCAMA P.O. (Plan of Operation) version 5

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)




Activities
JFY
Year
Sub-Activities
Month
1st Year
2nd Year
3rd Year
4th Year
5th Year
6th Year
2015
2016
2017
2018
2019
2020
Responsible Organization
Japan
GOI
Achievements
Issue & Countermeasures
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
1.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of Plasmodium falciparum
1.3 Screening for selective inhibitory activity of extracts to the proliferation of Plasmodium falciparum , in parallel with Activity 1.1 and 1.2
1.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against plasmodium
1.5 Establishment of mass production system of the lead compound candidates
1.6 Determination of chemical structures of the lead compound candidates
1.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing
1.8 Discussion on future direction of derivatization on the basis of the structural biology assessment
Plan
Actual
NU
BPPT
Total extract screened against 7 target enzymes were 12,000 until end of 2017. New target was introduced from Jul 2018.
KU
NU
MRJ
ALL
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
2.2 Secondary screening for selective inhibitory activity of the extracts to the proliferation of Entamoeba histolytica
2.3 Screening for selective inhibitory activity of extracts to the proliferation of Entamoeba histolytica , in parallel with Activity 2.1 and 2.2
2.4 Isolation and purification of chemical compounds with inhibitory activity to the proliferation against Entamoeba histolytica
2.5 Establishment of mass production system of the lead compound candidates
2.6 Determination of chemical structures of the lead compound candidates
2.7 Selection of lead compound(s) through in vitro assessment and subsequent animal testing
2.8 Discussion on future direction of derivatization on the basis of the structural biology assessment
UTokyo
AU (BPPT)
KU
Utokyo
BPPT (AU)
Reconfirmation extracts were produced.
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
3.2 Establishment of screening systems
3.3 Establishment of culture and evaluation systems
3.4 Introduction of technologies of isolation and purification
3.5 Introduction of technologies of chemical structure elucidation
3.6 Establishment and enhancement of a research network in Indonesia
U.Tokyo
NU
BPPT
AU
LIPI
Currently, more than 11900 of microbial extracts were newly prepared upto 2017, and more than 300 microbes were newly isolated during 2017. All extracts and microbes were registered in the in-house biological resource libraries.
New target for antimalarial, and antiamebic screening was established
Duration / Phasing

Monitoring Plan	JFY	1st Year	2nd Year	3rd Year	4th Year	5th Year	6th Year	Remarks	Issue	Solution
	Year	2015	2016	2017	2018	2019	2020			
	Months	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			
Monitoring										
Joint Coordinating Committee	Plan									
	Actual	●	●	●				JCC Meeting was held		
Scientific Meeting	Plan			△ △				Occasionally		
	Actual	● ● ● ●	● ● ● ●	● ● ● ● ● ●						
Set-up the Detailed Plan of Operation	Plan							Annual Working Plan		
	Actual	● ●	● ●	● ●						
Submission of Monitoring Sheet	Plan							1st Monitoring Sheet	3rd sheet submission was delayed	
	Actual	● ●								
Monitoring Mission from Japan	Plan								Monitoring mission was postpone due to limiting budget	
	Actual									
Post Monitoring	Plan									
	Actual									
Reports/Documents										
Project Completion Report	Plan									
	Actual									
Public Relations										
Establishment and Operation of Web Site	Plan								Establishment of website was delayed due to reorganization in BPPT.	
	Actual									
International symposiums are held for drug discovery	Plan									
	Actual			●						

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.06 (Apr. 2018 – Sep. 2018)

Name: Prof. Tomoyoshi NOZAKI
Title: Chief Advisor

Submission Date: 01 Oct. 2018

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese: 16 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in this term (the cumulative number of dispatched researchers become 74 since the project start). A coordinator have been staying continuously in the project office

1-1-1-2. Indonesian: the Project Director, Project Manager and Project Co-managers have been appointed in accordance with the R/D. Researchers have been listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: Four (4) Indonesian researchers participated in short-term trainings in Japanese institutes in this term. Other three Indonesian researchers have been studying in Ph.D course in Univ. of Tokyo and Kitasato Univ. since April 2017. The cumulative number of participation of Indonesian researcher become 36 since the project start.

1-1-2-2. In-country Training : There were following trainings organized in the project with Japanese instructors.

a. Training on "Purification of Active Compounds" *(by Dr.Mori, Dr.Dobashi & Dr.Yamashita)*

b. Training on "Antimalarial Target Enzyme Preparation and High Throughout Screening" *(by Dr. Daniel)*

c. Training on "Antimalarial Cell Screening System" *(by Dr. Sakura)*

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side : BPPT has been providing facility of BSL-2 level's laboratories and AU also has been doing as well. Bio-resources possessed in BPPT have been provided to the project. To boost purification performance, BPPT also installed a new lab desk for purification room in July.

1-1-3-2. Provision by Japanese side: According to the plan of project's 4th year, several of equipment and consumables are going to procure. Terms of half period of 4th year, one equipment, necessary reagents and consumables have been provided for technical transfer between Japanese scientists and Indonesian scientists and well organized. However, some of installed and handed over equipment have the timing of maintenance for keep performance.

1-1-4. Local costs

1-1-4-1.Indonesian Side: In fiscal year 2018, BPPT allocated 418.4 million rupiah for employing personnel, equipment, travel and consumable. BPPT also got another budget from Ministry of Research, Technology, and Higher Education as much as 175 million rupiah. So total budget for 2018 is as much as 593.4 million rupiah. Until September 2018, BPPT has disimbursed 83% of the budget.

1-1-4-2.Japanese Side: In Japanese fiscal year 2018 means start from April 2018 to March 2019, the annual budget as local cost are allocated approximate 550 million rupiah through JICA Indonesia Office for employing personal, transportation, equipment and consumables. Then roughly 175 million rupiah (30% of total annual budget) were already expended by the end of September 2018.

Progress of Activities

This year, field trip to collect microbial sample was done in Puspiptek Area, near to BTC. In the vast site with 460 hectares area, there are 2 wide botanical gardens with highly diverse unique plants from all provinces in Indonesia. Guided with leading microbiologist from Japan, 42 soil samples and 28 plant litters samples, as well as 12 unique samples (including bird's feather, mushroom, dead insects, and bee's nest) were collected. Microbial isolation, especially isolation of fungi, was closely guided by the experts, resulting near to 500 isolates. Using the same sample, isolation of actinomycetes was also conducted, and more than 160 isolates were obtained.

One of important issues in current project is the reproducibility of active extract. Following up the result of both enzymatic and cell-based screening, active extracts would be produced using their produces. As already in its 4th year, this project put more emphasize on purification of active compound from active extract. Thus, it is necessary to ensure the extracts for purification to be remained active. For this purpose, in addition to reconfirmation extract production, a pre-scale up extract (PSU extract) production was introduced prior to large scale extract production. This extract was prepared similarly to that of reconfirmation extract, but the fermentation was done only in one kind of medium and the activity was monitored up to day 7th of fermentation (instead of day 5th of fermentation for reconfirmation extract production). This step is important, since large scale extract production will be done immediately (within one week, if possible) after PSU extract production, so the condition of the producer could be considerably similar when it

is used for both extract production and resulting in reproducible large scale extract with considerable similar activity. In other side, about 4000 extracts for first screening have been produced from the beginning of 2018 up to September 2018.

Starting from 2018, a new target for antimalarial screening (pfNDH2) was introduced. The enzyme was prepared and characterized in 2017 at BTC. Until September 2018, more than 3000 microbial extracts were screened. One of active extract had been prepared in larger scale for purification of active compound, which is currently being conducted. Screening against other antimalarial targets (pfDHODH and pfMQO) was also conducted against more than 3000 microbial extracts. Some of active extracts were also produced in larger scale for purification of active compounds.

Continuing from 2017, cell-based antimalarial screening was performed against microbial extracts. Since number of active extract was higher than that of enzymatic screening, although active extracts with low toxicity against mammalian cell (DLD-1) had been selected with high selectivity (100x), a new dereplication method was introduced into screening pipeline. Peptibols and polyethers are two groups of compound those are frequently obtained as active compound produced by fungi and actinomycetes, respectively, against malarial parasite. Since these compounds are also active against gram positive bacteria, dereplication of hit obtained from screening process were perfomed by selecting the hit with less activity against the bacteria. About 50% of hit number can be reduced by this step before continuing to the next step (producing reconfirmation extract). Some of active extracts from this type of screening is currently under purified.

Enzymatic screening of 7000 microbial extract was done in AU using newly added EhNAD Kinase/NO1 as target resulting more than 90 hit. Reconfirmation extract of these hit are being produced. At the same time, cell-based screening against *Entamoeba* cell was conducted against more than 7000 extracts, resulting 326 hit. After testing their toxicity against mammalian cells and reproducibility of their activity, 4 of them were currently recultured in larger scale for purification of active compounds. The other 3 active extracts were being purified at UTo and KU.

Purification of active compound was conducted extensively against active extracts with target inhibitory, as well as parasite proliferation inhibitory activity. More than 20 large scale microbial extracts (1 to 5 L) were produced. Purification of 2 plant extract with activity against PfMQO were also conducted, following up screening result performed in 2017. Four active compounds from microbial extracts and another 1 active compound were isolated and structure elucidated from microbial and plant extract, respectively. More than 5 other extracts are currently being purified. In addition to BTC, purification was also being conducted in AU, UTo, and KU. This will help the project to widening bottle neck of laborious work in purification step.

Optimization of work flow in purification room could also increase the efficiency of the project in achieving the target. In Mid of 2018, new lab desks were installed in purification room of BTC. Rearrangement of purification room layout was also performed, so the researchers could work efficiently. Some new equipment (such as pH meter, heat

block, and electric stabilizers) were also newly installed.

Following up collaborative research between BTC-BPPT and Obihiro University of Agriculture and Veterinary Medicine (OUAVM) started from last August 2017, BTC produced and delivered reconfirmation extracts those are requested by OUAVM based on their result in screening of microbial extracts against *Toxoplasma gondii*, parasites causing toxoplasmosis in human. Both BTC and OUAVM agreed to seek funding for continuing this collaborative research.

BTC also shared 2000 microbial extracts to University of Tokyo to be used for phenotypic screening against *Mycobacterium tuberculosis* under MTA signed by both parties in frame of collaborative research on development of anti-tuberculosis agents, funded by TB Alliance, USA. This would be the second spin-out microbial resources-based drug discovery topic from this project, after toxoplasmolysis drug discovery project with OUAVM.

1-2 Achievement of Output

1-3-1. Achievement of Output 1

Output 1	
Compounds with anti-malarial activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.).	
Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	The indicator has been achieved (8 compounds with anti-malarial had been isolated and purified) • About 17000 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 10000 extracts have been objected into malarial cell-based screening in cumulative. • Optimization of cell-based screening system was performed. • Five active compounds with antimalarial activity were isolated and structure elucidated.
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 7 compounds with anti-malarial activity had been elucidated) • Five active compounds with antimalarial activity were isolated and structure 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.	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.

1-3-2. Achievement of Output 2	
Output 2	
Compounds with anti-amebic activity are identified from the extracts of Indonesian biological recourses (microorganism, plants, etc.)	
Indicators	**Achievement**
2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	The indicator is expected to be achieved by the Mid-term Review. • 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.
2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.	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. Efficacy testing using experimental animal is completed for at least one (1) compound with anti-amebic activity by the end of the project period.	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.

1-3-3. Achievement of Output 3	
Output 3	
Technologies and research system for drug discovery using biological recourses are established at the Indonesian research institutes.	
Indicators	**Achievement**
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.	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.
3-2. Screening systems for inhibitory activity of the extracts	The indicator has been achieved. Enzyme- and cell-based screening systems have been

from biological resources are established at the Indonesian research institutes by the end of the 2nd year of the Project.	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.
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.	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. • Establishment of culture and evaluation system using *P.falciparum* 3D7 are established at BTC. • Mammalian cell culture and evaluation system are established at BTC.
3-4. Technologies of isolation and purification of compounds are introduced at the Indonesian	The indicator is expected to be achieved by the time of the Terminal Evaluation. • Equipment needed for isolation and purification

research institute(s) by the time of the Terminal Evaluation.	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.
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.
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 2019.

1-3 Achievement of the Project Purpose

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.

Increasing the diversity of microbial strains used for this project is one of successful key. In this semester, leading mycology experts from Japan were invited to Indonesia to deliver training on isolation and identification of fungi. Researchers from microbial team were learning a lot about identification of fungi based on their morphology. Microbial handling and preservation procedures in order to increase reproducibility of its activity were also discussed. In parallel, a researcher from BTC was dispatched to KU to have training on identification of actinomycete for 4 months.

Starting from 2018, two new targets for screening were introduced: PfNDH2 and EhNADKinase/NO1. Optimization of malarial cell-based screening resulted in decreased number of hit to be followed up for reconfirmation and purification.

To ensure the activity of large scale extract before purification, a new step of extract production, so-called pre-scale up (PSU) extract production, was introduced.

Indicators	**Achievement**
1. At least one (1) lead compound with anti-malarial activity are	This indicator is expected to be achieved by the time of the end of the Project.

determined on the basis of animal experiments for efficacy.	• About 17000 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 10000 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. • Efficacy test using animal experiment will be started in 2018
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 2018
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.

1-5 Changes of Risks and Actions for Mitigation

1-6 Progress of Actions undertaken by JICA

1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)

1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2-1. Delay of project related documents

Some required documents to implement and manage project activities are not available timely yet. Those are as follows;

i. Handing over documents of provided equipment from JICA to BPPT
ii. Submission of the last two versions of the Project Monitoring Sheets(PMS)

2-2Causes

i. JICA needs to handover the equipment to BPPT officially right after its provision by the document signed between BPPT and JICA expert, however BTC has not been able to confirm how to proceed the handing over document according to the rule of BPPT so far. Therefore the transaction can not be proceeded yet.
ii. After making initial drafts by Japanese side, the document have not been edited by the BPPT side yet.

2-3 Action to be taken

i. To confirm the required transaction in BPPT side
ii. To edit the PMS punctually every 6 months by BTC

2-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

i. Biotech Center, BPPT (Project Manager, Project Co-manager)
ii. Biotech center, BPPT (Project Manager, Project Co-manager)

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the 2nd year. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2017

The main institute to implement project activities in BPPT re-changed from PTFM-BPPT to the Biotech Center-BPPT. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of Biotech Center and Program Head of Biotech Center accordingly.

3-2-2. Amendments due to the alteration of Japanese Coordinating Research institute

The Japanese Coordinating Research Institute was changed from University of Tsukuba to University of Tokyo (UTokyo) on 1st April 2017, the reason was the alteration of belonging university of the Japanese Chief Advisor.

4 Preparation of Gov. of the Republic of Indonesia toward after completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.07 (Oct. 2018 – Mar. 2019)

Name: Prof. Tomoyoshi NOZAKI
Title: Chief Advisor

Submission Date: 01 Apr. 2019

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese : 13 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in this term (the cumulative number of dispatched researchers become 87 since the project start). A coordinator have been staying continuously in the project office

1-1-1-2. Indonesian: the Project Director, Project Manager and Project Co-managers have been appointed in accordance with the R/D. Researchers have been listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: Four Indonesian researchers participated in short-term trainings in Japanese institutes in this term. Other three Indonesian researchers have been studying in Ph.D course in Univ. of Tokyo and Kitasato Univ. since April 2017. The cumulative number of participation of Indonesian researcher become 40 since the project start.

1-1-2-2. In-country Training : There were following trainings organized in the project with Japanese instructors.

a. Training on "Purification of Active Compounds" *(by Dr.Mori, Dr.Dobashi & Dr.Yamashita)*

b. Training on " Antimalarial Cell Screening System" *(by Dr. Sakura)*

c. Training and workshop on "Identification of Mitosporic Fungi" *(by Dr. Ando & Dr. Mori)*

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side : BPPT has been providing facility of BSL-2 level's laboratories and AU also has been doing as well. Bio-resources possessed in BPPT have been provided to the project. To boost purification

performance, BPPT also installed a new lab desk for purification room in July 2018.

1-1-3-2. Provision by Japanese side: According to the plan of project's 4th year, required equipment and consumables were procured and delivered to each institutes. Several of equipment, necessary reagent and consumables which provided in project's 4th year are well utilized for technical transfer between Japanese scientists and Indonesian scientists.

1-1-4. Local costs

1-1-4-1.Indonesian Side: In fiscal year 2018, BPPT allocated 418.4 million rupiah for employing personnel, equipment, travel and consumable. BPPT also got another budget from Ministry of Research, Technology, and Higher Education as much as 175 million rupiah. Total budget for this project in 2018 was as much as 593.4 million rupiah.

Total disimbursment from BPPT to this project for 2018 was as much as 582.9 million rupiah. For 2019, BPPT allocated about 700 million rupiah for employing personnel, equipment, travel and consumable.

1-1-4-2.Japanese Side: In Japanese fiscal year 2018 means start from April 2018 to March 2019, the annual budget as local cost are allocated approximately 550 million rupiah through JICA Indonesia Office. Then 522 million 250 thousand rupiah were expended for employing personal, transportation, equipment, reagents and consumables by the end of this fiscal year.

1-2 Progress of Activities

In 2018, under supervision of expert, more than 1200 microbes were isolated from sample taken from Puspiptek (2018) and remained sample taken from Togean Island (2017), composed from 700 isolates of fungi and 500 isolates of actinomycete. Identification of microbes was also performed. Under supervision of experts, more than 2000 microbes were identified based on their morphology. Capacity of fungi identification was increased during 2018. In 2017, 13 fungal genus could be identified from 220 isolates based on their morphology. In 2018, number of fungal genus that able to be identified was tremendously increased to 29 (from 459 isolates). Detail identification of interesting microbes, which showed lower DNA sequence similarity to currently known isolates, was also conducted. A fungi and actinomycete isolates were successfully identified and are being proposed as newly found species under known genera. This indicated that capability of Indonesian researcher on morphological identification of microbial isolate was increased significantly.

During 2018, more than 5000 microbial extracts were produced, composed from 2800 actinomycetes extracts and more than 2200 fungi extracts. From mid of 2018, a new protocol on large scale extract production, so-called PSU extract production, was

introduced. This modified protocol was aimed to increase reproducibility of large-scale extract production. Compared to first half of 2018, reproducibility of active large-scale extracts was significantly increased from 17% to 41% in the second half of 2018. This indicated that newly introduced protocol could increase the reproducibility of extract production.

To anticipate increased number of active extracts as resulted from screening process, an extract production schedule was created and shared among project members. This schedule is useful to monitor the progress of extract production, so each team could arrange their activities based on this schedule. This also could minimalize unnecessary waiting time until the extract has been prepared.

During 2018, more than 3600 extracts were screened against PfMQO and 5000 extracts against PfDHODH and PfNDH2, and followed up until large scale extract production as much as 5, 5, and 1 extract, respectively. Total cumulative number of extract tested against PfMQO and PfDHODH since the beginning of this project reached more than 17.000 extracts. In parallel, total cumulative number of microbial extract that was tested against plasmodial cell since 2017 up to 2018 are more than 10.000 extracts. More than 400 of them showed antiplasmodial activity with low toxicity against mammalian cell. After dereplication step by testing their toxicity against gram positive bacteria, total hit achieved so far was 83 (hit rate=0.82%). At the end of 2018, 6 of them were continued into purification step. Meanwhile, screening to search inhibitor against target PfNDH2 was stopped due necessity to improve selectivity of assay system to measure enzyme activity.

Antiamebic screening was conducted against amebic cell and several targets (enzyme based screening). Total cumulative number of extract tested against amebic cell up to 2018 was more than 12.000 extracts. Screening against target enzyme CS3, SAT1, and NADK/NO1 was conducted by employing more than 5000, 6000, and 7000 microbial extracts, respectively. Currently, 1 extract with antiamebic activity and 1 extract with CS3 inhibitory activity are being purified, where 2 extracts with SAT1 inhibitory activity are being produced in larger scale for purification purpose. Fifty hits were obtained from screening against NADK/NO1 enzyme, and currently being reconfirmed.

A screening system for antituberculosis drug discovery was proposed and started to develop by BTC. Determination and construction of expression system of target enzyme had been conducted. The enzyme had also been overexpressed and purified, and the activity is currently being measured. Another target had been proposed, and will be developed within next semester. Production of diaphorase, an enzyme required for LDH assay (in antiplasmodial activity measurement) was also performed. The activity had been confirmed and ready to be used for assay.

More than 40 extracts with antimalarial were proceeded into purification step. Half of them were aborted due to loss of activity during purification. Six compounds were isolated and structurally elucidated. Two of them showed same structure, although they were produced from 2 different microbes. One of them caused false positive result on PfMQO assay system. All of them are known compound, unfortunately. In other side, an active

compound with antiamebic activity was also elucidated. This compound, citrinin, is also known compound and member of mycotoxin, so it could not be regarded as promising drug candidate. Citrinin was isolated from several active extracts, so dereplication procedure to avoid frequently obtained compound is urgently required.

To widen research network in drug discovery field in Indonesia, BTC visited Cancer Chemopreventive Research Center (CCRC) in Gadjah Mada University (UGM), Yogyakarta on November 2, 2018. This visit aimed to initiate a collaborative research on anti-cancer drug discovery by utilizing Indonesia microbial bioresources. During this visit, CCRC agreed to collaborate with BTC on anti-cancer drug discovery. Second visit to CCRC was held on March 28, 2019. BTC shared some of 800 microbial extracts to CCRC as an initial set of resources for screening. CCRC also agreed with BTC to develop an enzymatic screening system for anticancer screening based. BTC also visited InaCC LIPI on October 31, 2019 to discuss about microbial preservation and sharing of microbial isolates for screening. Following up collaboration with Obihiro University of Agriculture and Veterinary Medicine (OUAVM), a meeting was held on December 6th, 2018 to discuss progress of screening against toxoplasma parasite and future plan on large scale extract production and purification of active compound.

1-3 Achievement of Output

1-3-1. Achievement of Output 1

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

Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	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.
1-2. 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.
1-3. Efficacy testing using experimental animal is completed for at least one (1) compound with	The indicator is expected to be achieved by the end of the project period. • Large scale production of antimalarial active

anti-malarial activity by the end of the project period.	compound for efficacy test is being prepared

1-3-2. Achievement of Output 2

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

Indicators	**Achievement**
2-1. At least one (1) compound with anti-amebic activity is isolated and purified by the time of the Mid-term Review.	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.
2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.	The indicator has been achieved (1 compound with antiamebic activity was structurally elucidated) • 1 compound with antiamebic activity was structurally elucidated
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.	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.

1-3-3. Achievement of Output 3

Output 3
Technologies and research system for drug discovery using biological recourses are

established at the Indonesian research institutes.

Indicators	Achievement
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.	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.
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.	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.
3-3. Culture and evaluation systems for each research objective of *Plasmodium falciparum* and *Entamoeba*	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.

histolytica are established at the Indonesian research institute 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. • 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.
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.
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.

1-4 Achievement of the Project Purpose

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.

Increasing the diversity of microbial strains used for this project is one of successful key. In this semester, leading mycology experts from Japan were invited to Indonesia to deliver training on isolation and identification of fungi. Researchers from microbial team were learning a lot about identification of fungi based on their morphology. Microbial handling and preservation procedures in order to increase reproducibility of its activity were also discussed. In parallel, a researcher from BTC was dispatched to KU to have detail identification of an interesting fungi isolate that is predicted to be a new species of fungi.

Building capacity on development of a screening system using bio-resources is essential for drug development. To improve the capability in this area, a researcher from BTC was dispatched to UTo to have training in determination of target for development of drug for infectious diseases.

Indicators	**Achievement**
1. At least one (1) lead compound with anti-malarial 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. • 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.

1-5 Changes of Risks and Actions for Mitigation
1-6 Progress of Actions undertaken by JICA
1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)
1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2-1. Delay of project related documents
(None)

2-2Causes
(None)

2-3 Action to be taken
(None)

2-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)

(None)

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the 2nd year. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2017

The main institute to implement project activities in BPPT re-changed from PTFM-BPPT to the Biotech Center-BPPT. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of Biotech Center and Program Head of Biotech Center accordingly.

3-2-2. Amendments due to the alteration of Japanese Coordinating Research institute

The Japanese Coordinating Research Institute was changed from University of Tsukuba to University of Tokyo (UTokyo) on 1st April 2017, the reason was the alteration of belonging university of the Japanese Chief Advisor.

4 Preparation of Gov. of the Republic of Indonesia toward after completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*

TO CR of JICA INDONESIA OFFICE

PROJECT MONITORING SHEET

Project Title :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

Version of the Sheet: Ver.08 (Apr. 2019 – Sep. 2019)

Name: Prof. Tomoyoshi NOZAKI

Title: Chief Advisor

Submission Date: 01 Oct. 2019

I. Summary

1 Progress

1-1 Progress of Inputs

1-1-1. Personnel

1-1-1-1. Japanese : 12 turns of short-term (less than 1 year) researchers were dispatched to Indonesia in this term (the cumulative number of dispatched researchers become 99 since the project start). A coordinator have been staying continuously in the project office

1-1-1-2. Indonesian: the Project Director, Project Manager and Project Co-managers have been appointed in accordance with the R/D. Researchers have been listed as project researchers in each field.

1-1-2. Capacity Development

1-1-2-1. Training in Japan: Seven Indonesian researchers participated in short-term trainings in Japanese institutes in this term. Other three Indonesian researchers have been studying in Ph.D course in Univ. of Tokyo and Kitasato Univ. since April 2017. The cumulative number of participations of Indonesian researcher become 47 since the project start.

1-1-2-2. In-country Training: There were following trainings organized in the project with Japanese instructors.

a. Training on "Purification of Active Compounds" *(by Dr.Mori, Dr.Dobashi & Dr.Yamashita)*

b. Training and workshop on "Sample Collection from Indonesian Nature", "Cultivation and Identification of Mitosporic Fungi" *(by Dr. Ando, Dr. Okuda & Dr. Mori)*

1-1-3. Facilities, equipment and materials.

1-1-3-1. Provision by Indonesian side: BPPT has been providing facility of BSL-2 level's laboratories and AU also has been doing as well. Bio-resources possessed in BPPT have been provided to the project. To boost purification performance, BPPT also installed a new lab desk for purification room in July 2018.

TO CR of JICA INDONESIA OFFICE

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1-1-3-2. Provision by Japanese side: According to the plan of project's final year, required equipment and consumables were procured and delivered to each institutes. Several of equipment, necessary reagent and consumables which provided in project's 4th year are well utilized for technical transfer between Japanese scientists and Indonesian scientists. However, it seems to set up problem on HPLC which were already handover to Indonesian Institute and replacement parts were provided from Japanese side.

1-1-4. Local costs

1-1-4-1.Indonesian Side: In fiscal year 2019, BPPT allocated 700 million rupiah for employing personnel, equipment, travel and consumable.
Total disimbursment from BPPT to this project for 2019 until Sep 2019 was 510 million rupiah (72% of total budget).

1-1-4-2.Japanese Side: In Japanese fiscal year 2019 means start from April 2019 to March 2020, the annual budget as local cost will approximate 875 million rupiah through JICA Indonesia Office. Then roughly 300 million rupiah were allocated and expended around 250 million rupiah (28% of total annual budget) for employing personal, transportation, equipment, reagents and consumables by the end of September 2019.

1-2 Progress of Activities

In April 23-24, 2019, field trip for collecting samples as resources for microbial isolation was conducted at East Jawa Province (Gresik, Sidoarjo, Mojokerto). Microbiologist expert from Japan joined to the trip to give technical supervision related to method of sample collection to the researcher from BTC. About 60 samples composed from soil, plant litter, sea sand, and disease-infected leaf were taken from the sites. Isolation of microbes was conducted accordingly in BTC. To date, 378 fungi and 178 actinomycetes were isolated and identified morphologically. All of them were registered in BTC's microbial collection database. Analysis of most frequent isolated fungi during last 5 years revealed that diversity of fungi from each location was highly varied. Number of unknown fungi was decreased from 49% in 2017 to 30% in 2019, indicated improved capability of BTC's researchers in microbial identification and significant impact of training by experts from Japan. Total number of microbes in the collection reached 27 thousands isolates, among them more than 3500 microbes was newly isolated by this project.

Production of microbial extract was focused on production of extract in larger scale for active compound purification process. To date, about 100 pre-scale up (PSU) extracts and 29 large scale (LS) extracts were produced (65 PSU and 46 LS extracts were produced in 2018). In contrast, number of extracts produced for first screening was decreased from 5000 extracts in 2018 to 2000 in 2019, indicating that management of extract production was performed as expectation. With the increased number of request

for extracts due to increased number of target used for screening, good management system for extract production should be established. Application of schedule and calendar to manage the production of extracts since end of last year helped the team to respond and communicate the progress to the requester and helped the requester to arrange their activities while the extracts they requested were being produced.

To date, more than 18 thousands and 17 thousands extracts were screened for searching active extracts with antimalarial activity against plasmodial target enzyme PfDHODH and PfMQO, and resulting 44 and 104 active extracts that had been proposed to be further investigated, respectively. Screening against PfNDH2 was also conducted using more than 5000 extracts, but currently the screening is being hold due to low selectivity of the assay system. Along with this, More than 12 thousands extracts were screened against malarial cell *in-vitro*, and resulting 97 active extracts tested all hit from first screening against mammalian cell for excluding toxic extracts and against gram positive bacteria cell for excluding extracts that may contains frequently obtained active compounds. In other side, more than 16 thousands extracts were used for screening against amebic target enzyme EhSAT1, EhSAT1/CS3, and EhNADK/NO1, and against *E.histolytica* cell.

Establishment of a screening system for searching anti-TB drug, continuing last semester, was conducted by introducing a new target, MtSK. Expression system for this enzyme was established, and MtSK enzyme was obtained with sufficient purity and activity. Initial trial of screening using about 500 extracts showed good performance with wide window of assay, indicates that the screening system is ready to be used for searching anti-TB agents using microbial extracts. The developed system employed 384-well plate platform, which is new to be conducted in this project. Moreover, the assay is based on fluorescence monitoring, which is also new to be applied in this project.

Tens of anti-malarial compounds were isolated from active extracts. Some of them were same compounds but isolated from different resources, and total kind of antimalarial compounds isolated from Indonesian microbes (as well as plants) was 8 compounds. One of them, Borrelidin, showed very strong activity against malarial parasites (IC50=1.8 nM). This is even stronger than currently available antimalarial drug (atovaquone IC50=6 nM, chloroquine IC50=9.7 nM). Other tens of active extracts with antimalarial activity were prepared and ready to be objected for active compound purification.

Two kinds of antiamebic compound were also isolated from Indonesian microbial extracts, i.e. citrinin and fumagilin. Currently, 3 extracts with antiamebic activity, which are not one of citrinin or fumagilin, are being processed to obtain the compounds those are responsible for the activity.

During 2019, 31 large scale extracts with antimalarial activity were processed for purification of active compound. In this semester, some active compounds were isolated from microbial extracts. Structure elucidation of these compounds revealed some known compounds including Altenusin and Borrelidin. Borrelidin is an anti-malarial compound that showed very potent activity against the parasite, even more active compared to currently available known drugs such as atovaquone and chloroquinine. Selectivity of this

compound is also very high (>1000) indicated that this compound is promising to be an anti-malarial drug candidate.

Efficacy test of an antimalarial compound, gentisyl alcohol, was also conducted with cooperation of Brawijaya University, Malang. The sampe was prepared by BTC, and as much as 200 mg of pure gentisyl alcohol was isolated from approximately 15 L microbial cultures. Preliminary test indicated that the compound could decrease parasitemia of *P.berghei* in infected mice, indicated that the compound also showed antimalarial activity *in-vivo.* Another antimalarial active compound, borrelidin, is currently being prepared by BTC using jar fermentor. This compound will be objected for structure modification and followed by efficacy test *in vivo*.

During 4 years, this project has successfully improved the capacity of Indonesian counterparts on developing antimalarial and antiamebic drug from Indonesian bio-resources. Some of leads had been isolated and tested in animal model for confirming its efficacy. To further develop these leads into a drug candidate, some activities should be done including structure modification and pre-clinical trial. These activities are indispensable in drug development pipe line, and building the capacity for these expertise is required. A proposal for requesting technical assistance through SATREPS project (second phase) was submitted to Japanese Government through Ministry for Research Technology and Higher Education. For the next term, several research institutes from 3 countries will be involved including BPPT, LIPI and IPB from Indonesia side, and The University of Tokyo, Kitasato University, Nagoya Institute of Technology, and Bozo Research Center from Japan Side, and University of Malaya, University Putra Malaya, and Universiti Teknologi Mara from Malaysia Side. This proposal was focused in capacity building on lead modification and pre-clinical trial for anti-malaria, anti-amebiasis, anti-tuberculosis, and anti-dengue agents. Letter of Intent document was signed between all of involved Indonesian side, as well as between representative institutes from 3 countries.

BPPT also submitted a grant proposal to National Institute of Health Research and Development, Ministry of Health with topic of purification and structure elucidation of anti-malarial agents from microbial resources. Another grant proposal is also being prepared to be submitted to Ministry of Research Technology and Higher Education with topic of screening for anti-tuberculosis agents from microbial resources.

BPPT is currently preparing an international symposium, which will be held in Jakarta. Together with AMED, BPPT will jointly co-organized an international symposium to promote social implementation of health technology, particularly in infectious diseases field, in Asia. The symposium will be held in on October 9, 2019, and will be attended by leading researchers from Asian countries. This symposium will be followed by the 2nd international symposium on natural resources-based drug development, which will be held on October 10, 2019 and co-organized with JICA. Several leading researchers in drug developments from both Indonesia and Japan will joint to the symposium.

BPPT opened a website for publishing its activities in this project. The site is managed and maintained by BTC. It can be accessed though the followed address:

https://balaibiotek.bppt.go.id/info-publik/inovasi/satreps-new.

1-3 Achievement of Output

1-3-1. Achievement of Output 1

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

Indicators	**Achievement**
1-1. At least one (1) compound with anti-malarial activity is isolated and purified by the time of the Mid-term Review.	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.
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 11 compounds with anti-malarial activity had been elucidated). • Two active compounds with antiplasmodial activity were isolated and structure 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.	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.

1-3-2. Achievement of Output 2

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

Indicators	**Achievement**
2-1. At least one (1) compound with anti-amebic activity is isolated	The indicator has been achieved. (2 compound with antiamebic activity was isolated and purified)

and purified by the time of the Mid-term Review.	• More than 16000 extract were screened against amebic target enzyme EhSAT1, EhSAT1/CS3, and EhNADK/NO1, and against E.histolytica cell in cumulative.
2-2. Chemical structure elucidation is completed for at least one (1) compound with anti-amebic activity by the time of the Terminal Evaluation.	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.
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.	The indicator is expected to be achieved by the end of the project period. • Efficacy test using animal experiment will be conducted in 2020.

1-3-3. Achievement of Output 3

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

Indicators	**Achievement**
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.	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.
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.	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.
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.	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 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.

the Terminal Evaluation.	• 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.

1-4 Achievement of the Project Purpose

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.

Increasing the diversity of microbial strains used for this project is one of successful key. In this semester, leading mycology experts from Japan were invited to Indonesia to deliver training on isolation and identification of fungi. Researchers from microbial team were learning a lot about identification of fungi based on their morphology. Microbial

handling and preservation procedures in order to increase reproducibility of its activity were also discussed. In parallel, a researcher from BTC was dispatched to KU to have detail identification of an interesting fungi isolate that is predicted to be a new species of fungi.

Building capacity on development of a screening system using bio-resources is essential for drug development. To improve the capability in this area, a researcher from BTC was dispatched to UTo to have training in determination of target for development of drug for infectious diseases.

Indicators	**Achievement**
1. At least one (1) lead compound with anti-malarial 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 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. • Another antimalarial active compound will be objected for structure modification and followed by efficacy test *in vivo*.
2. At least one (1) lead compound	This indicator is expected to be achieved by the time

with anti-amebic activity are determined on the basis of animal experiments for efficacy.	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.
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. • Another scientific paper about the use of Indonesian microbes as resource for antimalarial drug discovery was submitted and being reviewed.

1-5 Changes of Risks and Actions for Mitigation
1-6 Progress of Actions undertaken by JICA
1-7 Progress of Actions undertaken by Gov. of the Republic of Indonesia

1-8 Progress of Environmental and Social Considerations (if applicable)
1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

1-10 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

2 Delay of Work Schedule and/or Problems (if any)

2-1. Delay of project related documents
(None)

2-2Causes
(None)

2-3 Action to be taken
(None)

2-4 Roles of Responsible Persons/Organization (JICA, Gov. of the Republic of Indonesia, etc.)
(None)

3 Modification of the Project Implementation Plan

3-1 PO

There is no modification on the P.O.

3-2 Other modifications on detailed implementation plan

(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HDQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)

There are some amendments in the R/D due to some reasons in the 2nd year. Those amendments are as follows:

3-2-1. Amendments due to organizational reforming in BPPT 2017
The main institute to implement project activities in BPPT re-changed from PTFM-BPPT to the Biotech Center-BPPT. Therefore, the Project Manager and the Project Co-manager in BPPT replace to Director of Biotech Center and Program Head of Biotech Center accordingly.

3-2-2. Amendments due to the alteration of Japanese Coordinating Research institute
The Japanese Coordinating Research Institute was changed from University of Tsukuba to University of Tokyo (UTokyo) on 1st April 2017, the reason was the alteration of belonging university of the Japanese Chief Advisor.

4 Preparation of Gov. of the Republic of Indonesia toward after completion of the Project

There is not information available yet

II. Project Monitoring Sheet I & II *as Attached*