BACTERIAL LEAF BLIGHT OF RICE PLANT IN SOUTHEASTERN ASIA

January 1970

OVERSEAS TECHNICAL COOPERATION AGENCY

FOREWORD

It has recently been recognized that bacterial leaf blight of rice, one of the most serious diseases to rice plant in Japan has also causing considerable damages to the plants in Southeast Asia. In this region, the disease has made little harm as far as local rice varieties had been cultured without any fertilization. As all countries in the region, however, are implementing their own programs for increased production of rice, they are actively breeding and extending high yielding varieties and introducing new method of rice culture accompanied by fertilizer application, along with the improvement of irrigation system. But almost all high yielding varieties which these countries have adopted are very susceptible to bacterial leaf blight. Besides, they require a large amount of fertilizer. As a result, they become easily a prey to the disease, which, therefore, is considered to be a big barrier to the implementation of increased production of rice.

In Japan, occurrence of the disease was formerly limited to warmer area in southwestern Japan. But it began to spread gradually almost all over the country along with the improved method of rice culture by dense sowing and heavy application of fertilizer and is now seen notably in central to northern regions such as Kanto, Hokuriku, and Tohoku. The disease is now listed among the most disastrous such as blast and sheath blight.

In Japan, there is a long history of the study on bacterial leaf blight of rice from its discovery. Many results of study have accumulated so much as to make a remarkable progress in the control of the disease.

The Overseas Technical Cooperation Agency (OTCA) focussed its attention to the fact that the control of the disease is very important to the project for increasing rice production in Southeast Asian countries and entrusted Professor Hideo Mukoo of the Tokyo Agricultural University who is noted as an authority of the disease, to make a "Study on Bacterial Leaf Blight of Rice Plant in Southeast Asia" for three years from 1967 to 1969. This study was actually carried out mainly by Satoshi Wakimoto, Chief of the First Laboratory of Bacterial Diseases, Plant Pathology and Entomology Division, National Institute of Agricultural Sciences, under the guidance of Dr. Takeyuki Mizukami, Head of the Division, and achieved splendid results as expected in the collection of pathogenic bacteria and phages from many parts of Southeast Asia, investigation on resistance of various kinds of Indica rice varieties, and research on the characteristics of both bacterial and phage strains.

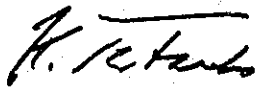
This book was prepared based on the studies on bacterial leaf blight of rice in Japan as well as in southeastern Asia, including of course those made during the three year period as mentioned above, with a view to serving as a general introduction of the disease.

I am assured, therefore, that it will give a right and proper direction to technical guidance, research and investigation on bacterial leaf blight disease in Southeast Asia.

In sending this book out to the public, my sincere gratitude is due: Dr. Satoshi Wakimoto, who actually wrote up the whole book; Professor Hideo Mukoo, who accepted willingly the general responsibility for the study on my request; Dr. Takeyuki Mizukami who played an active role in the study group; and Mr. Tsutomu Uematsu, Mr. Toshiki Shiomi, and Mr. Yukio Tsuchiya, all of whom made every

possible cooperation for this project. My gratitude should also be extended to various organizations concerned in Thailand, Cambodia, Malaysia, India, Ceylon, Taiwan and the Philippines (International Rice Research Institute). All of these organizations were generous enough to help the promotion and progress of the study in all possible ways.

January 1971



Keiichi Tatsuke
Director General
Overseas Technical Cooperation Agency

Photographs (by Wakimoto)

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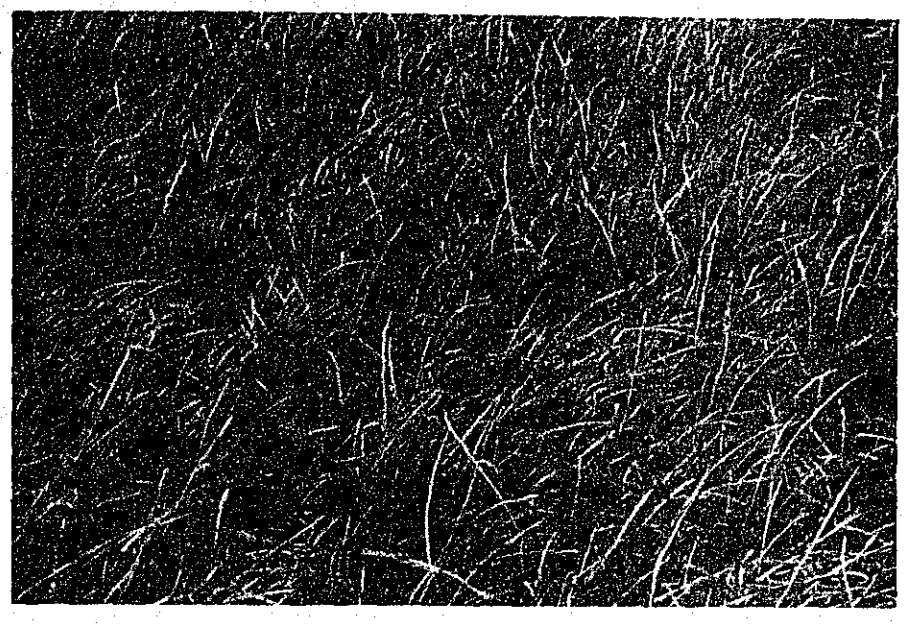

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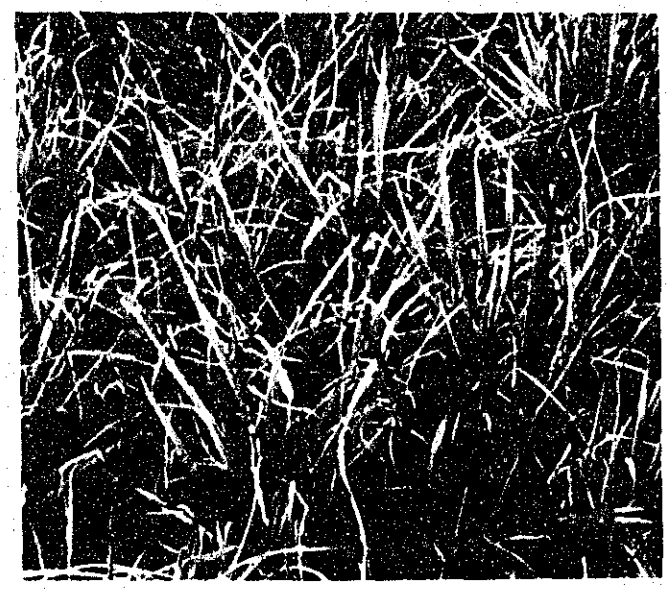

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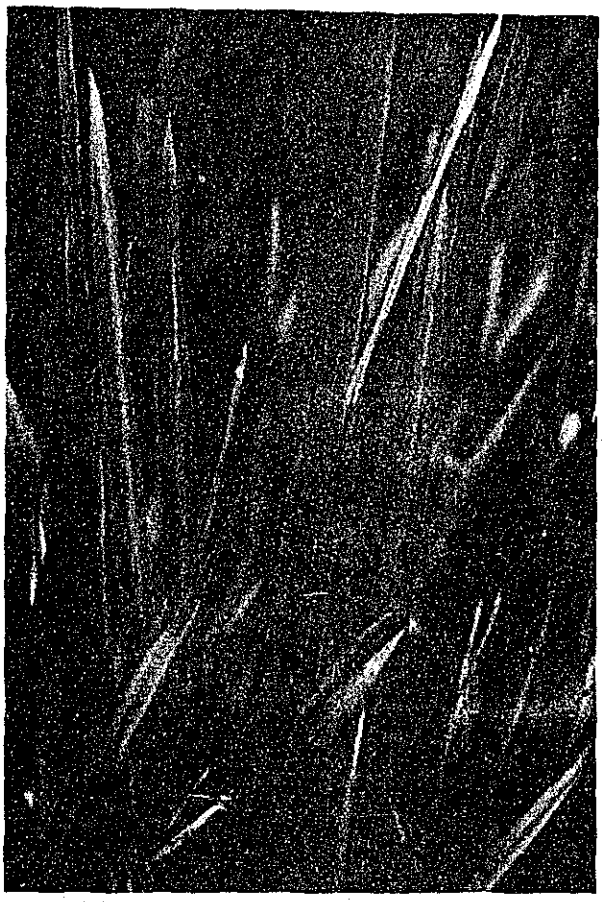

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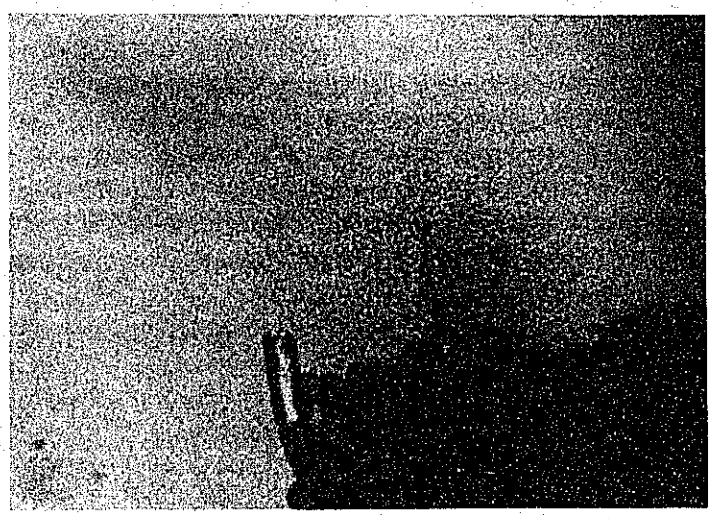

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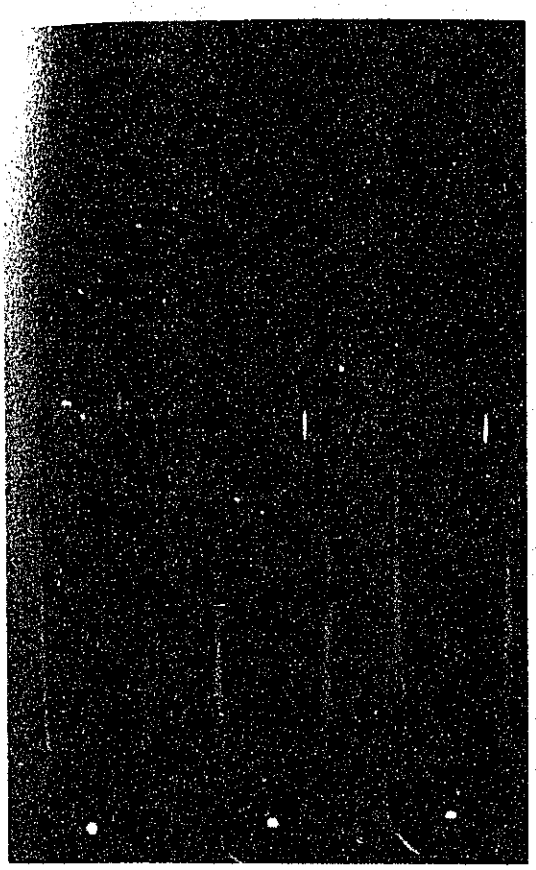

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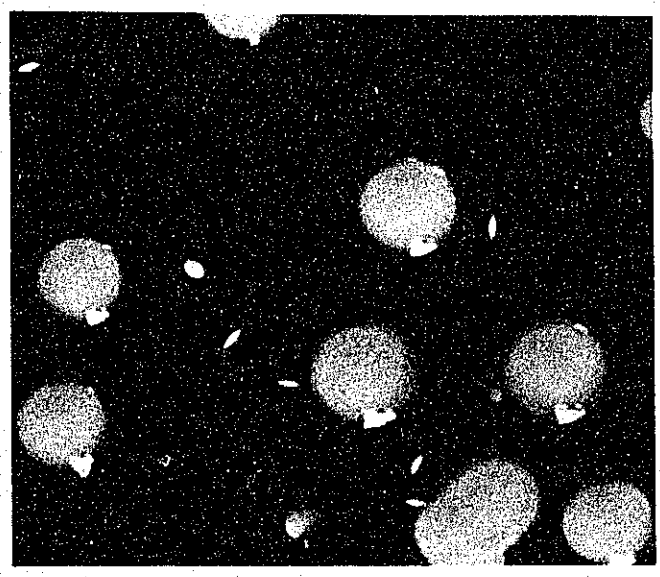

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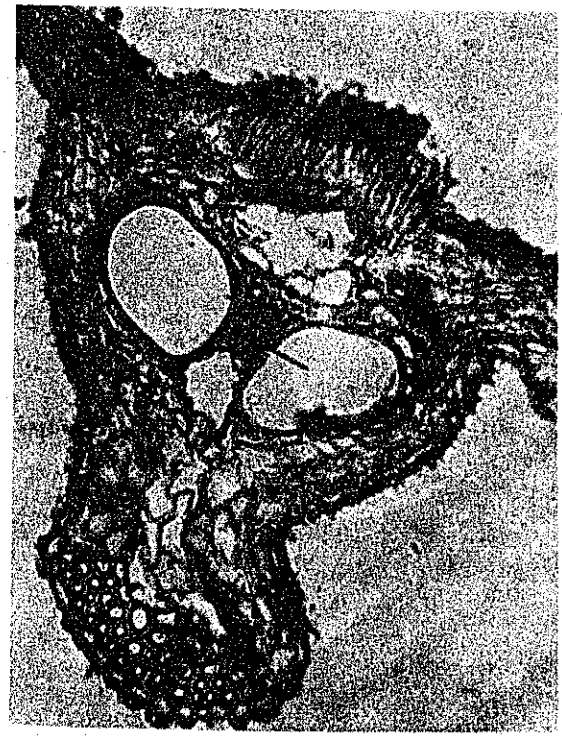

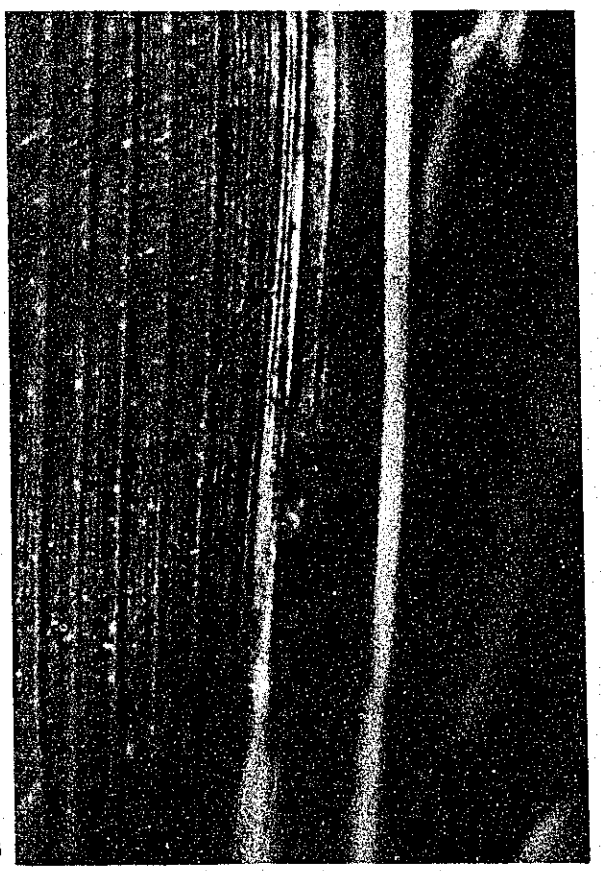

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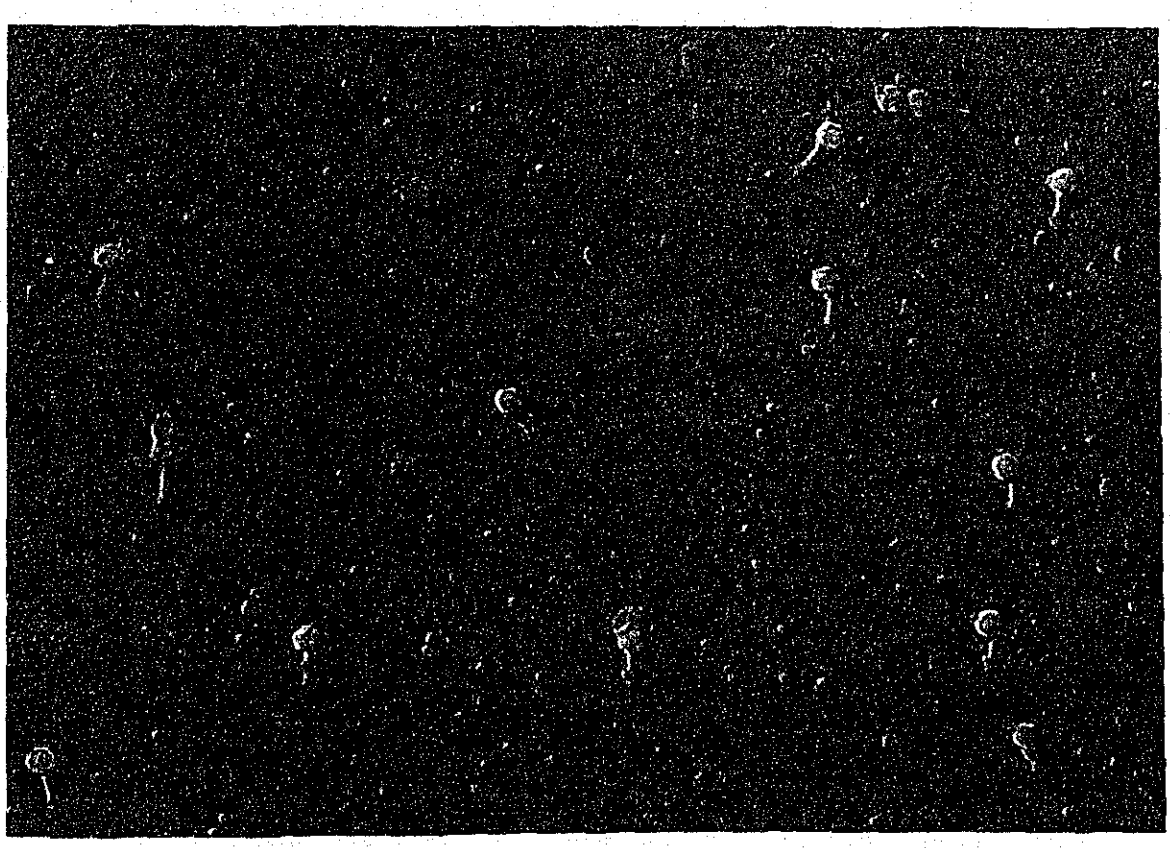

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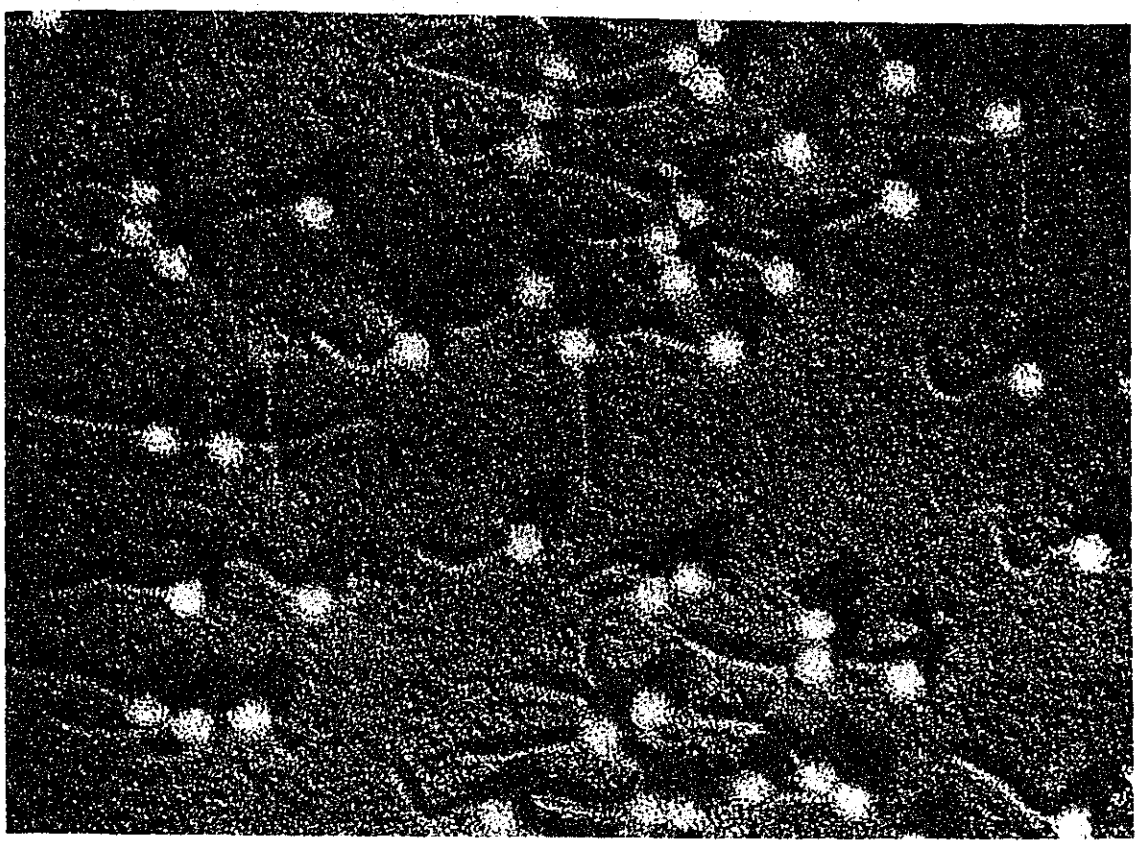

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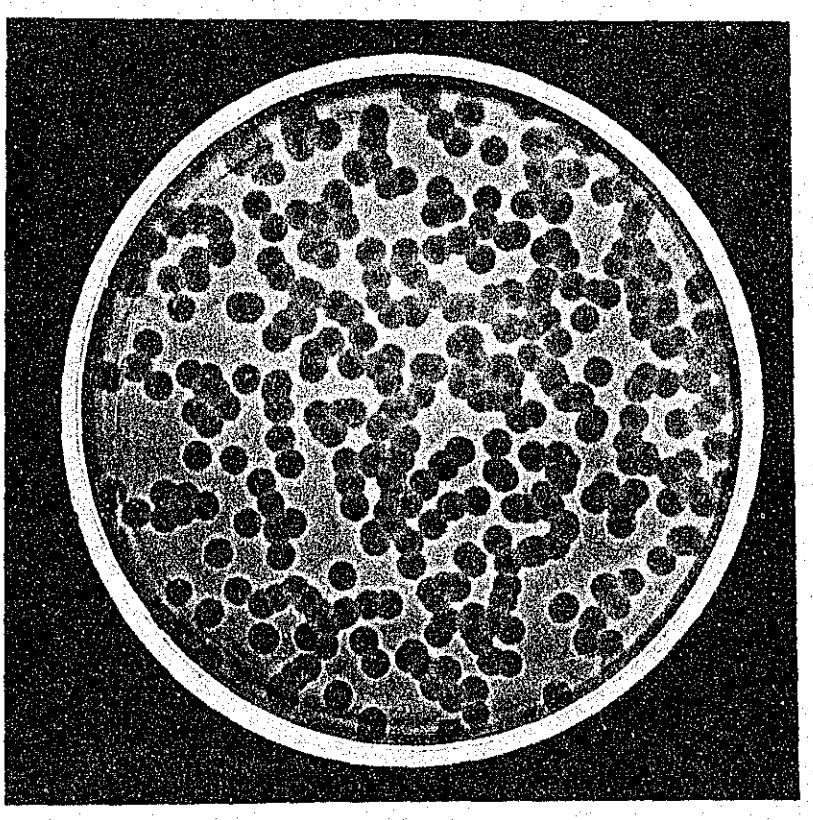

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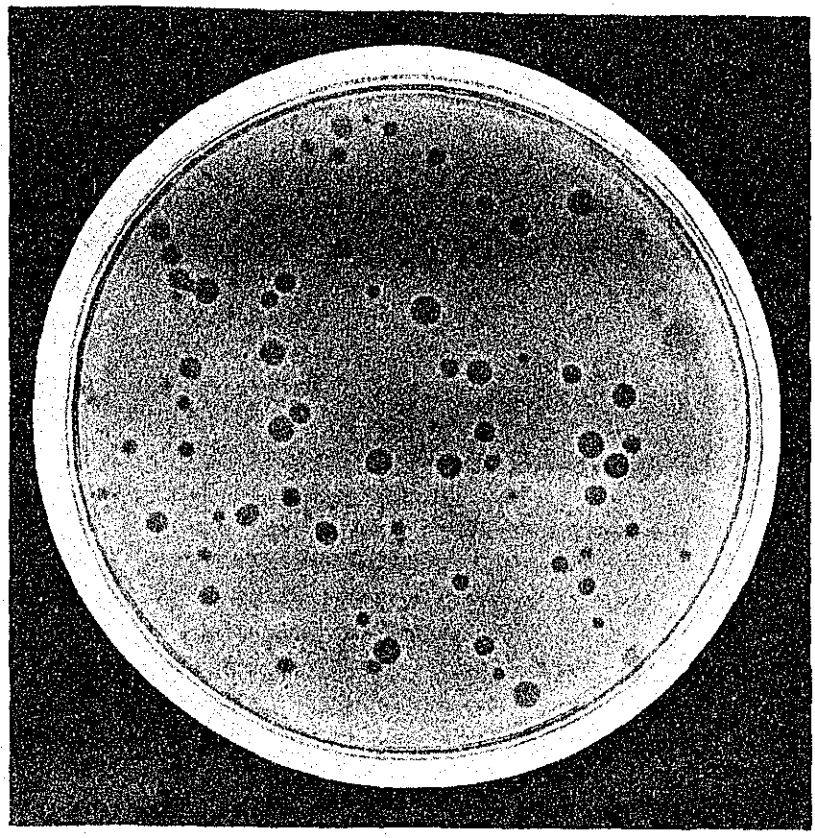

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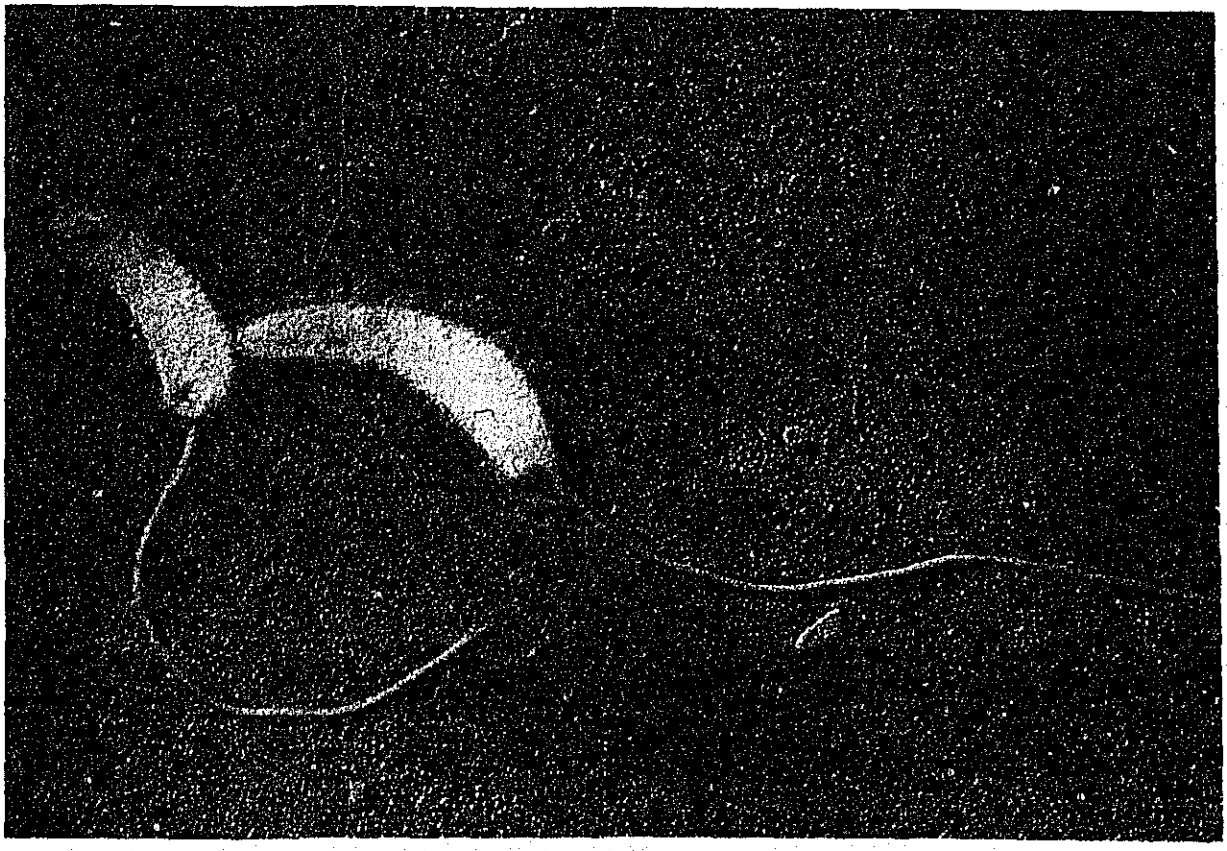

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BACTERIAL LEAF BLIGHT OF RICE IN SOUTHEAST ASIA

I. INTRODUCTION

1. Bacterial leaf blight of rice was discovered already in the latter part of the 19th century in Japan. Its occurrence was however limited only to the warm area in southwestern Japan. As agricultural techniques advanced and rice varieties shifted from one to another for higher yield, the disease began to spread over wider area and its occurrence is now seen not only in western Japan but also in northern regions such as Kanto, Hokuriku and Tohoku. Although it caused only a slight damage, it occurred in a part of Hokkaido in 1962. The rice area affected by bacterial leaf blight came to rank next to that affected by blast or sheath blight, drawing general attention to the disease as one of the most disastrous to rice plant, Under the situation, there is a long history of study on bacterial leaf blight and the results thus accumulated have contributed much to the technical advancement in controlling the disease in Japan.

Bacterial leaf blight of rice is also distributed in other parts of the world, especially in Southeast Asian countries where it has only recently been found out to be one of the most important diseases of rice. These countries have been making a drive for food production increase and actively improving irrigation system, breeding and extending high yielding varieties, and spreading advanced fertilization and farming techniques. Along with these efforts for higher level of agricultural techniques, however, damages from disease and insect pest are gradually aggrandizing so much that some control measures are now vitally needed to achieve the target of increased food production.

Many of the important diseases that attack rice plant in Southeast Asia are also seen in various parts of Japan. But their order of importance varies with different countries and regions. Although the acreage of paddy affected by bacterial leaf blight and according severity of damage cannot surpass those by blast and sheath blight in Japan, those are by far the largest in Southeast Asian countries. In these countries this disease is distributed as a kind of endemic in almost all rice producing areas. As far as the rice culture is exclusively made with some indigenous varieties without applying any manure or fertilizer, the disease can cause little damage to the plant. The damage will surely expand if new varieties susceptible to the disease are adopted and cultured with fertilization. As all of the high yielding varieties or rice with shorter stem which are now spreading wide in Southeast Asian countries are very susceptible to bacterial leaf blight and require heavy application of fertilizer, it is quite natural that they help aggrandize damage from the disease more and more.

OTCA thus came to have a firm belief that the control of bacterial leaf blight would be one of the most important problems in executing the project for increased food production in Southeast Asian countries, and then mapped out a program to promote the study on the disease in these countries and to remove a great hitch to the project by establishing a satisfactory control measure.

This general treatise was edited based not only on various results of studies performed on the disease in Japan but also on the researches on it conducted under the consideration of the disease in Southeast Asia between 1967 and 1969 under the sponsorship of OTCA. It was prepared with due expectation that it would serve as a useful reference for the establishment of effective measure to control disastrous bacterial leaf blight in that region.

II. HISTORY OF OCCURRENCE AND STUDY OF THE DISEASE

In Japan the occurrence of bacterial leaf blight was noted in Fukuoka, Chiba, Shimane, and Ehime prefectures towards the end of the 19th century when soybean cake and green manure began to be used for lowland rice fields. The area affected by the disease was gradually increasing from the beginning of the present century. Around 1910, it was reported that the disease broke out in almost all prefectures in the Kanto region. According to an investigation made in 1929, it extended over the Tohoku region. The occurrence began to be seen in the Hokuriku region around 1940 and the affected area spread over some parts of Hokkaido in 1962 although it was still very slight. Thus it is clear that the disease has been distributed virtually in every prefecture in Japan. The size of affected area shows considerable ups and downs from year to year. However it occupies almost 10 per cent of the total area of paddy fields on an average.

If the development of study on bacterial leaf blight of rice is historically pursued, it may be divided into three stages: The first period from 1900 to 1922 when the study was centered around the cause of disease as far as to succeed in the isolation and identification of pathogen; the second period from then to 1953 when the study on ecology of pathogenic bacteria, resistance of rice varieties, and control method of the disease were carried out through observations in the fields; and the third period from 1954 to date when the ecological study on bacterial leaf blight rapidly advanced through utilization of bacteriophage discovered in 1954, and at the same time a tremendous effort is now being rendered for the development of effective chemicals for disease control.

In the first period when the cause of the disease was unknown, there were a variety of views on it. For instance, a view referred it to the acidity of soil while the other to an attack made by some insects. In 1909, however, Takaishi attributed the cause to a certain bacteria. Then in 1911, Bokura isolated and identified a kind of bacteria from some affected leaves of rice plant. And in 1922 at last, Ishiyama pointed out that the actual pathogen was different from those described by Bokura as a result of a thorough study on its morphology, characteristics and pathogenicity, and named it *Pseudomonas oryzae* Ueda *et* Ishiyama[10]. This bacteria was later shifted into genus *Xanthomonas* by Dowson who renamed it *X. oryzae* (Ueda *et* Ishiyama) Dowson[1].

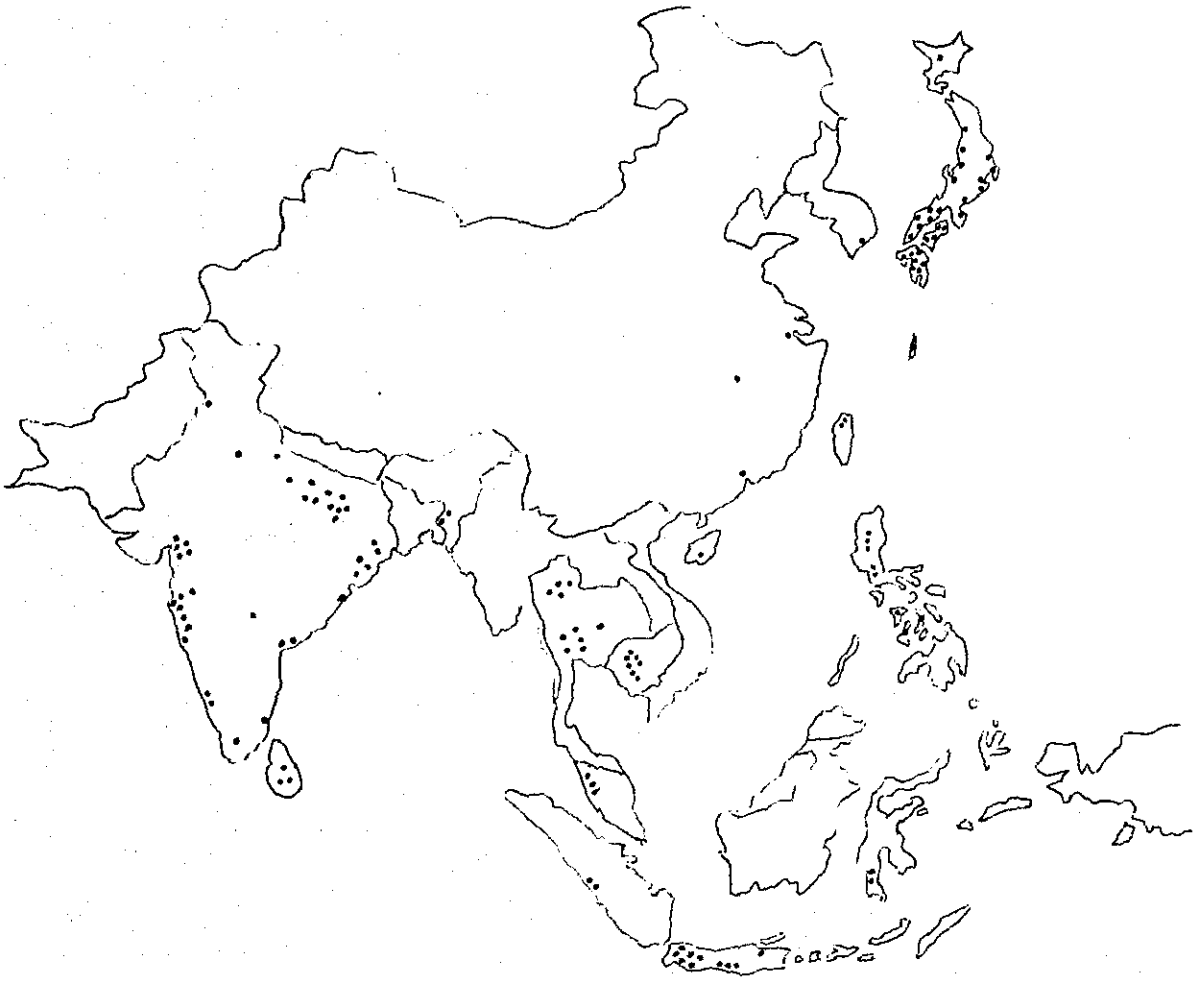
In the second period to 1953, the study was pushed forward in regard to resistance of rice varieties distributed in Japan, relation between environmental conditions and occurrence of the disease, and control method. Many research reports as a result were made public in detail in respect to the relations between the occurrence of the disease and climatic conditions such as precipitation during the rice culture season, amount of sunlight, temperature, and frequency or strength of wind and typhoon, between kinds and amount of applied fertilizers and the occurrence, and between the time and severity of occurrence and degree of damage[15].

In 1953, K. Goto and his coworker brought to light the important role of "Sayanukagusa" (*Leersia oryzoides* Sw. var. japonica Hack) as an intermediate host of leaf blight bacteria[4]. This wild grass was commonly found in levees

and footpaths of paddy fields. Mukoo and his coworkers developed the multi-needle prick inoculation method for study of the disease[47]. In the third period starting from about 1954 when Yoshii and others discovered a phage[48] which is host specific to and reproductive on leaf blight bacteria, new phage-techniques developed by Wakimoto and his coworkers contributed much to promote the ecological studies of the bacteria[37 41]. This progress in the study gave impetus to the development and utilization of controlling chemicals, some of which have been widely used to date.

The occurrence of bacterial leaf blight of rice has so far been recognized in Korea, Formosa, Mainland China, the Philippines, Indonesia, Malaysia, Thailand, Cambodia, India and Ceylon other than Japan (Fig. 1). It is very likely, however, that the disease has already been distributed in Viet Nam, Laos, Burma, and East Pakistan although there has been no report delivered telling about the disease. As mentioned above, almost all rice producing areas in Asia especially in its southeastern region seem to be contaminated by the disease, which will surely spread hazardously if any susceptible rice variety is introduced in company with application of fertilizers. In the light of this situation, Southeast Asian countries have actively started studies on the breeding of resistant rice varieties, on ecology and virulence of pathogenic bacteria and method of controling the disease. There are no statistical data available on the area affected and the amount of crop damaged by the disease in these countries. It is assumed, however, that the disease is spreading gradually along with expanding use of short stem high yielding varieties of rice.

Fig. 1 Distribution of Bacterial Leaf Blight in Southeast Asia



Note: ROK and China ..... According to literatures

Other countries ..... Acoording to studies of:
Dr. M. Goto - in the Philippines
Dr. S. Wakimoto - in Taiwan, Cambodia, Thailand and Malaysia
Dr. T. Mizukami, Dr. S. Wakimoto, Dr. S. Yoshimura, Dr. D. N. Srivastava and Dr. Y. P. Rao - in India
Dr. S. Matsumoto & Dr. H. Tabei - in Ceylon
Dr. M. Yamada - in Indonesia
For Viet Nam, Laos, Burma, Pakistan, etc. no information available.

In any regions other than Asia, any information has not been reported about the disease in their rice producing areas. Although it is not certain whether the disease actually exists or not, it may be safe to conclude that it has never caused any serious damages of economic importance.

III. SYMPTOMS OF THE DISEASE

Symptoms of bacterial leaf blight of rice appear on rice plant and host plants such as "Sayanukagusa" (*Leersia oryzoides* Sw. var. Japonica Hack.), "Ezosayanukagusa" (*Leersia oryzoides* (Linn.) Sw.), and "Makomo" (*Zizania latifolia* Turcz.).

1. Symptoms on Rice Plant

Leaves, leaf sheaths, and paddy grains of rice plant are affected by the leaf blight bacteria. Although the symptoms rarely observed as soaked lesions on lower leaves of the plant in the latter part of the nursery stage, the typical blight symptom generally begin to appear in the highest tillering stage and become severe in the heading stage. As pathogenic bacteria are carried mainly by water, symptoms of the disease develop gradually from lower leaves to the upper. Besides, as the bacteria invade into the plant mainly through hydatodes on leaf margin and wounds made by wind on leaves, symptom on a leaf develops first on its margin especially at the leaf tip. As hydatodes are more frequently distributed around the upper part of the leaf, it is often affected first. Soaked spots appear first at a hydatode and gradually turning yellow extending downwards (Photo 1.). If they develop further, they reach the sheath of leaf and the yellow lesions change into grayish white and the leaf withers completely. At the harvesting time, therefore, almost all leaves of rice plants will die and the whole paddy field turns grayish white. If the rice variety is susceptible to the disease, pathogenic bacteria are highly virulent, and all circumstances favor the disease, the plant may quickly wilt and die even before the lesions have turned yellow. In some cases during the period from immediately after transplanting to tillering, the bacteria from the leaf margin or a wound at the root propagate and remove rapidly through vascular bundle, bring forth the death of the whole or a part of a hill (Photo 2). These symptoms are specifically called "Kresek symptoms". They are not a kind generally seen in Japan but only in some parts of Niigata, Nagano, and Fukuoka prefectures up to the present. In a paddy field attacked hard by bacterial leaf blight there are recognized lesions of faded yellow color even at the glume of paddy grains (Photo 3). In this case the plant often becomes sterile. These lesions are especially remarkable while paddy grains are young and green, and the lesion turns into vague grey or yellowish white as they grow on to maturity.

Symptoms of bacterial leaf blight seen in the tropical areas in Southeast Asia are not different substantially from those seen in Japan. It may be pointed out, however, that the symptoms in Southeast Asia appear much severe (Photos 4-7), and are accompanied more frequently by the "Kresek symptoms" (Photo 8). In the tropical areas, sterility of rice plant is often observed as a result of bacterial leaf blight (Photo 9), and damages caused by it are by far the greater than in Japan. A symptom of the so-called yellowing in the Philippins had been reported as one of the symptoms of bacterial leaf blight[5].

2. Symptoms on Grasses

In Japan there are observed affected grasses of Gramineae such as "Sayanukagusa", "Ezosayanukagusa" and "Makomo", all of which are distributed on leaves of irrigation canals and paddy field borders. The first two, if affected, often produce soaked lesions at the leaf margin near the apex at first and these lesions extend rapidly downward and develop into the form of wedge or a larger spot covering a half of the leaf (Photo 10). The lesion may further extends to the

leaf sheath. As for these grasses, symptoms on their seeds have not yet been observed. In the case of "Makomo", symptoms also appear first on leaf margin near the leaf apex, and a yellow wavy line of larger lesion bordering healthy part of the leaf appears.

In the tropical regions, wild rice seems to be playing an important role as intermediate host of bacterial leaf blight. In some areas in India at least, they grow wildly on levees and banks of canals commonly and suffer from hard attack by the disease[42]. Symptoms in this case are rapid withering, similar to those usually seen in rice varieties with high susceptibility to the disease (Photos 11-12). *Leersia hexandra* is also known to be an intermidiate host in the tropics.

3. Discrimination between Bacterial Leaf Blight and Other Diseases

Symptoms resembled to those of bacterial leaf blight may be brought forth by yellow orange leaf (so-called Tungro, a kind of virus diseases) (Photo 13), yellowing of part of a leaf due to node blast, bacterial stripe (Photo 14), leaf spot (Photo 15), and by some physiological causes (Photo 16). But the symptoms of these diseases do not represent a wavy circumference of the lesion so as to make indistinctive the border line between healthy and affected parts of the leaf. Rice plant attacked by stem borer immediately after transplanting shows symptoms very much like the Kresek symptoms. In this case, however, affected leaf can easily be pull off and identified as its base has been bitten by the insect.

As for paddy grain, blast or brown spot also produces lesions similar to that of bacterial leaf blight. It is difficult to discriminate symptoms between the two. The discrimination, therefore, should be made in respect to symptoms on leaves of rice plant as they appear on them most conspicuously.

When it is difficult to identify the disease from its symptoms on leaf or paddy grain, a method as mentioned below may be helpful[9]. The affected part is cut in the size of ca. 0.5 x 0.5 cm and put it between slide and cover glasses. The sample is examined under a microscope of 100 magnification while dropping distilled water on it. If the affection is from bacterial leaf blight, a large amount of bacteria is observed to effuse from the cut section of the vascular bundle (Photos 17-18). If it is not, such effusion will not be observed.

IV. ENVIRONMENTAL CONDITIONS RELATED TO THE DISEASE OCCURRENCE

Occurrence of bacterial leaf blight of rice depends on circumstances surrounding the plant. They include conditions rather constant such as topography and soil as well as rather variable conditions such as climate changing annually and seasonally. In addition there are other conditions such as farming practices which may be changed artificially. Each of these conditions, independently affects rice plant and leaf blight bacteria, eventually determining frequency and severity of the disease.

1. Topographical and Soil Conditions

There are some regions where bacterial leaf blight of rice breaks out habitually. Southeast Asia is one of such regions. For each country and area in the region, location of habitual occurrence of the disease is rather limited within them respectively. The location can be decided whether topographical and soil conditions as well as climatic conditions are favorable for the disease to break out or not.

In Japan, bacterial leaf blight of rice is apt to occur in ill-drained areas with a high level of underground water in the neighborhood of river or lake and suffering from frequent flood. It affects also considerably in basins in mountainous area where heavy fogs often attacks and morning dews do not dry up soon.

Although it was formerly believed that the disease tended to break out in paddy field of acidic soil, it has not been positively proved if there is some relation between them. Some report says that the disease breaks out more on sandy loam originated from andesite and granite while the other tells that it is observed more on soil from shale than that from granite. But it may be generally concluded that occurrence of the disease is much more affected by topographic and climatic conditions and application of fertilizer than by soil conditions.

2. Climatic Conditions

Frequency and severity of occurrence of bacterial leaf blight of rice is closely related with climatic conditions. The occurrence is most significantly influenced by precipitation, moisture, temperature, wind and typhoon during rice culture season.

Pathogenic bacteria distributed on the surface of rice plant are easily killed by sunlight and dry air. Therefore, bacterial leaf blight usually cannot rage in any year or any area favored with a long fine and dry weather. On the contrary, a cloudy or rainy weather not only extends the life of the bacteria but also helps them to propagate actively and spread over wider area. A heavy rain submerging rice plants is contributive to spread pathogenic bacteria. Plants thus submerged in their nursery period or immediately after transplanting suffer seriously from the disease in the latter part of their growth period.

Occurrence of the disease is also expedited by temperature. The optimum temperature for propagation of the bacteria on a culture medium is between 25° C and 28° C. Symptoms of the disease in an inoculated rice plant develop rapidly above 25°C. The development slackens below 25° C and symptoms never appear below 20° C.

A strong wind also helps make pathogenic bacteria rampant as it gives wounds on rice leaves to open door for their easy invasion. If wind is accompanied by a heavy rain, it is most favorable for spreading the disease. After a typhoon, a rapid rise in the force of disease is usually observed.

In most of Japan, rice is cropped only once a year due to the condition of temperature which is so high in summer as to favor the growth of the plant. Although bacterial leaf blight has recently made inroads gradually into the northern regions, the affected area is comparatively small and correspondingly with lesser damage as the disease is generally controlled by cool weather.

In Southeast Asia, rice cultivation is controlled not by temperature but by water. In most of the region, a year is divided sharply into dry and wet seasons and rice culture is performed with water abundantly supplied in the wet season. In some areas where irrigation system is well furnished, rice is grown all the year round. In the region, therefore, the condition that induces bacterial leaf blight is not the temperature but chiefly rainfall. As a result, the disease generally rages more in areas with a high level of annual rainfall and rice plants grown in the wet season suffer from the disease more than those grown in the dry season.

3. *Cultural Practices*

Cultural practices closely related with the occurrence of bacterial leaf blight are: variety of rice, type of nursery, and application of fertilizer. In addition, they may include time of culture, density of planting, depth of soil plowed, depth of irrigation water, and time of draining the water.

a. Rice Varieties

Resistance of rice plant to bacterial leaf blight varies considerably with varieties. Therefore, if a rice variety susceptible to the disease is introduced in an area where all conditions are favorable for the disease, it will certainly bring about a rage of disease. Although climatic conditions seem to be important in contributing to changes in the size of area affected by bacterial leaf blight, rice varieties adopted are no less important. In the first half of the 1960s, when "Kinmaze", a high yielding rice variety susceptible to the disease was grown widely in Jpan, the area affected by the disease has increased remarkably. One of the reasons why the disease has recently drawn much attention in Southeast Asia, is that short stem rice varieties such as IR-5, IR-8 and TN-1 with high yielding ability have been extended wide according to the program for increased rice production despite of their high susceptibility to the disease. Resistance of rice varieties of Indica and Japanica types will be described later. At this moment, however, it is very urgent to breed and spread improved varieties of rice with higher resistance to bacterial leaf blight.

b. Types of Nursery

A nursery set up in ill-drained low and wet land is easily flooded and rice seedlings transplanted from it to paddy field are apt to fall a prey to bacterial leaf blight. Upland nursery is improved type to minimize damages from the disease. A nursery bed irrigated with river water has a danger of outbreak of the disease more than that using water from well. Especially in the tropical region rice plants in various stages of growth are grown at the same time and river water is contaminated with pathogenic bacteria to a higher degree and use of this water for irrigation will promote the infection in nurseries, making the disease rampant.

In such region where nurseries are supplied with water through other infected paddy field, seedlings catch the disease easily and to a higher degree and cause a serious outbreak of the disease. Therefore it will be most desirable to set up nurseries in upland field and irrigate them with water from wells in order to prevent the seedlings from infection by bacterial leaf blight in the tropical region.

c. Fertilization

Of the three plant nutrients, N, P and K, N is most closely related with the occurrence of bacterial leaf blight of rice. It is generally seen that the more the nitrogen fertilizer is used, the more the disease breaks out. The fertilizer applied heavily at once affects the outbreak much more than that applied separately from time to time. Top dressing at the later stage of plants' growth also promotes the occurrence of disease. Although P and K are not so effective to the occurrence as N, the disease is apt to take place when their application is inadequate. Silicic acid and magnesia also seem to help the disease occurrence if they are applied heavily. In Japan the disease breaks out more on shale soil and dressing of such soil is believed to bring about a more serious outbreak.

High yielding varieties of rice with shorter stem are being introduced wide in the tropical region. They are not only susceptible to bacterial leaf blight but also require more amount of fertilizer. It is clear that such varieties are assisting the disease to spread wider.

The kind and amount of fertilizer have a direct relation with resistance of rice to the disease, consequently with the outbreak of disease. But the mechanism has not been clarified yet. Biochemical studies should be necessary.

d. Cultural Season

There are seasonally different three kinds of rice cultures in Japan. They are early, medium and late cultures, which, however, are unexceptionally performed in summer. In some limited area in warm southwestern part of Japan, double cropping is practicable. The relation between each of these three kinds of cultures and outbreak of bacterial leaf blight is based on climatic conditions such as temperature, rainfall or typhoon and resistance of rice varieties adopted for each culture. Generally, rice varieties suitable for early culture are affected by the disease less than those for medium and later culture. This can be explained from the fact that the population of the pathogenic bacteria is not propagated enough to cause severe infection and the plants are harvested from August to September before the attack from typhoons. In some tropical regions, however, cultures of rice plants on different stages of growth are often carried out at the same time in many fields. Although the performance of culture is generally divided into the wet and dry seasons, it is done all the year round in some areas where irrigation system is completely furnished. The outbreak of disease is observed more on rice grown in the wet season. The reason may be traced back to high humidity and heavy rainfalls which have submerged rice plants in the field.

e. Cultural Methods

Rice plants are classified into upland and lowland rice by types of culture. As leaf blight bacteria are carried by water, they of course affect lowland rice more than the upland. This partially explains that nonexistence of the disease in Latin America. But if upland rice is irrigated with river water through sprinkling, it often becomes to suffer from the disease.

Of lowland rice, floating rice is often grown in some countries (Cambodia and Thailand) in Southeast Asia. It cannot be immune from bacterial leaf blight, which, however, do not generally give a serious damage to the plants[42]. It has not been proved yet whether the floating rice has some resistance or it is favored environmental conditions for its growth.

In Southeast Asia, rice harvesting is often made by cutting only the heads of rice plants. Infected parts of the plants are thus left in the field as a feed for buffaloes and other animals or most of them are plowed in under soil. Where leaf blight bacteria surviving in these leftovers will go and how the pathogenic bacteria will stand and live on through the dry weather under the tropical sun are very important problems relating each other and to be taken into consideration in the foreseeable future.

f. Other Cultural Conditions

Density of planting, depth of plowing, depth of irrigation water, time of draining, etc., all of these farming conditions may more or less have a relation with the outbreak of bacterial leaf blight of rice. But the relation is not so close as to draw general attention.

V. DAMAGE BY THE DISEASE

The damage caused by a plant disease may be most reasonably indicated by the reduction in plant crop. It has been long evidenced that bacterial leaf blight of rice give a tremendous damage to yield of the crop. But the method of assessment of damage has been established only since 1950.

On the other hand, in assessing the resistance of rice to the disease, virulence of bacteria, and efficacy of agricultural chemicals, appearance of symptoms or extent of affection on the plant has been employed instead of reduction in yield. And the relation between the degrees of infection and damage at a selected time has been made clear.

1. Assessment of Degree of Affection

Assessment of virulence of bacteria, rice plant's resistance to disease, and efficacy of agricultural chemicals often make use of young rice seedlings. A single-needle prick inoculation is given to the central part of each of three full-fledged upper leaves so as not to prick their main vein when the plant has had 5 or 6 leaves. Thus the length of lesions is measured to assess the virulence, resistance, and efficacy of chemicals as mentioned above. In this case the Affection Index is shown by the following equation (Mukoo et al, 1952):

$$\text{Affection Index} = \frac{10a + 5b + 2c}{\text{Total no. of leaves inoculated}} \times 100$$

a: No. of leaves with lesions more than 5 cm in length

b: No. of leaves with lesions 1 - 5 cm in length

c: No. of leaves with lesions less than 1 cm in length

Young seedling of Indica type rice shows wider variation in resistance according to different varieties compared to Japonica rice varieties in such tests. The International Rice Research Institute in the Philippines uses the standards as shown in Table 1.

Table 1. Standard of Scales for Indicating Degree of *Resistance to Bacterial Leaf Blight*

(Ann. Rept., IRRI., 1955)

Scale	Seedling Stage	Flowering Stage (Flag Leaf)
0	No lesion observable (immune)	Same
1	Lesions restricted to 1-2 mm around the points of inoculation	Same
2	Lesions more or less elliptical not more than 2-3 cm long	Same
3	Lesions elongated, extend to about 1/2 length of leaf blade or less.	Lesions elongated, less than 1/4 of leaf length
4	Lesions extend to any destroy 3/4 of leaf blade.	Lesions broad and coalesce, the upper portion of the leaves often dead, lesions extend to about 1/4 of the lower half of leaf surface below the points of inoculation.
5	Lesions extend to and destroy the entire leaf blade.	Lesions coalesce and upper portion of leaves dead, lesions extend to about 1/2 of the lower half of leaf surface.
6	Lesions extend to and destroy the entire leaf blade and less than 1/2 of leaf sheath.	Lesions extend to about 3/4 of the lower half of the leaf blade.
7	Entire leaf blade and more than 1/2 leaf sheath destroyed and a a few pale yellow symptoms appear.	Lesions extend to the base and destroy the entire leaf blade.
8	Leaf blade and leaf sheath destroyed, many pale yellow plants, and less than 25 seedlings completely killed (kresek).	Lesions destroy the entire leaf blade and extend to about 1/2 of leaf sheath.
9	About 50% or more seedlings entirely killed (kresek).	Lesions completely destroy the leaf blade and sheath.

The National Institute of Agricultural Sciences in Japan adopts the following standards for varieties of Indica type rice and high virulent pathogenic bacteria of foreign origin (Wakimoto et al).

$$\text{Affection Index} = \frac{9a + 7b + 5c + 3d + e}{a + b + c + d + e + f} \times 100$$

a: No. of leaves completely withered

b: No. of leaves with lesions longer than 10 cm but not completely withered

c: No. of leaves with lesions 5 - 10 cm in length

d: No. of leaves with lesions 1 - 5 cm in length

e: No. of leaves with lesion less than 1 cm in length

f: No. of leaves without lesion

A number of testing methods are used for assessing the degree of affection of rice plants grown up in the field. When affection is slight, 100 hills of the plants selected at random are often tested to obtain percentage of affected hills.

Several kinds of methods have been adopted to indicate the degree of infection on grown up rice plants in open field. Then the degree is low, the size of lesions on the upper leaves (e.g. the flag leaf, second and third leaves) is measured to show the degree by the following quation (Kyushu Agr. Expt. Sta.):

$$\text{Affection degree (per cent)} = \frac{a + 3b + 5c + 6d}{6N} \times 100$$

a: No. of hills in which the total area of lesions accounts for less than one third of the total area of leaves investigated.

b: No. of hills in which the total area of lesions accounts for from one third to two thirds of the total area of leaves investigated.

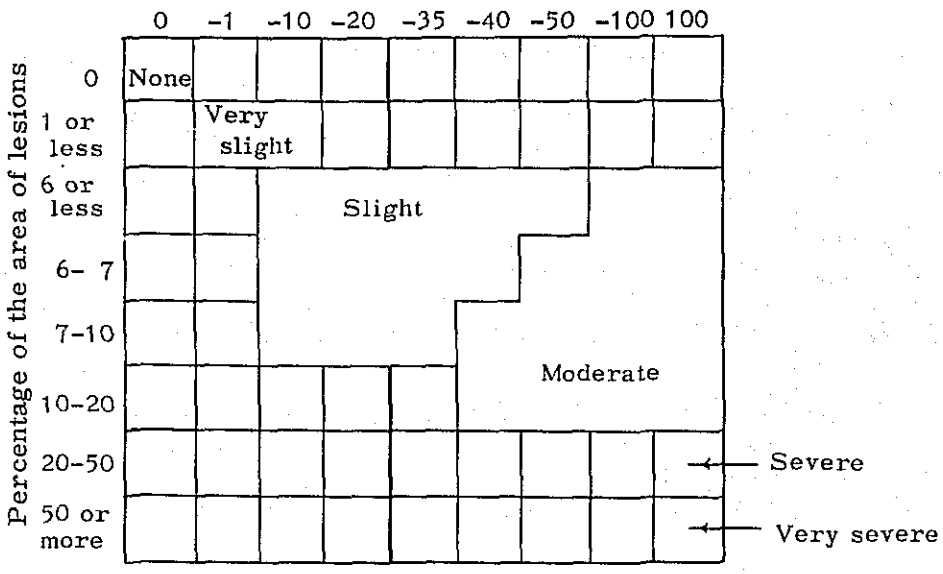
c: No. of hills in which the total area of lesions accounts for more than two thirds of total area of leaves investigated.

d: No. of hills all leaves of which are affected

N: No. of hills investigated

In case that the extent of affection is investigated, it is classified into 6 grades: non, very slight, slight, moderate, severe and very severe. These grades in relation with the number of hills affected and the rate of lesion area are shown in Fig. 2.

Fig. 2. Standard for Indicating Degree of Disease Affection in Fields
(Kyushu Agr. Expt. Sta.)

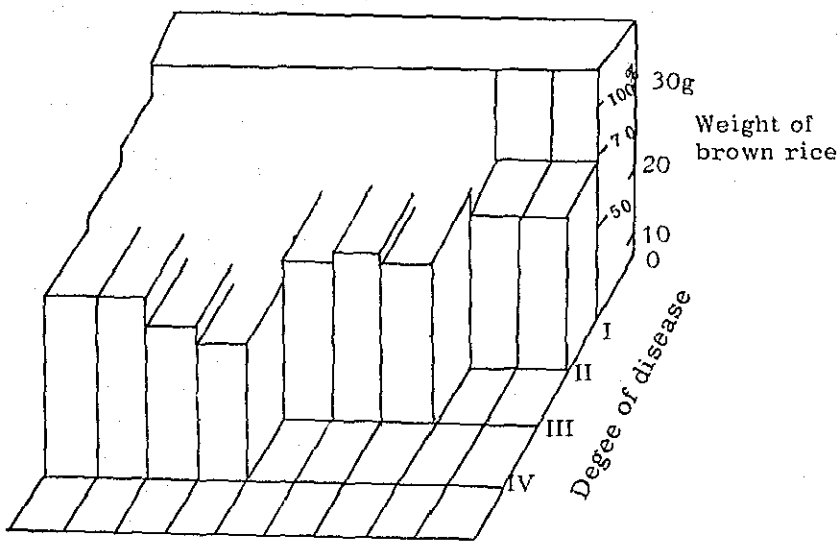


There are many different kinds of grades applied by various research workers. But they are fundamentally common as they are graded by index numbers derived from the rate of lesion area.

2. Assessment of Damages

Bacterial leaf blight reduces weight of straw, weight of brown rice per unit capacity, weight of brown rice grains and milling rate while increasing the number of sterile, immatured and broken grains and amount of cast-off rice. The size of such damage depends on the extent of infection and time of occurrence of the disease. Generally, the earlier the disease breaks out, the greater is the damage. In a year when the disease spreads comparatively late in the growth stage of rice plants, the damage is smaller compared to a wider affected area. Fig. 3 shows the relation between the time of disease occurrence and yield of brown rice.

Fig. 3. Retation between Time of Disease Occurrence and Yields (Weight of Brown Rice) (Kiryu et al., 1962)



X. 18	X. 10	X. 2	IX. 24	IX. 16	IX. 8	VIII. 31	VIII. 23	VIII. 15
-20	-12	-4	-26	-18	-10	-IX. 2	-25	-17

The Figure illustrates a result of experiment made in Kyushu. When the disease broke out in August, the damage was most serious and reduced yield of brown rice by almost 35 per cent. The later the time of occurrence, the smaller the reduction in yield. The Figure shows also that the occurrence in October did not affect the yield so seriously.

3. Damages in Tropical Areas

Damages made by bacterial leaf blight of rice in Southeast Asia are much different from those in Japan. Therefore, the methods applied in Japan for measuring the extension of affection and degree of damages may not be effectively used in this region. In the region, affection by the disease is observed in the very early stage of rice plants' growth, and it usually spreads wide and makes serious damage until the harvesting time of rice crop. As a result, damages caused by the disease there must be of course more serious than those seen in Japan. With a view to establishing the method of assessment of such damages, a study should be started anew on the relation between the time or extent of the occurrence of disease in Southeast Asia, where the relation between the kresek symptoms frequently seen on transplanted rice plants and yield of rice in particular should be clarified as soon as possible.

VI. CHARACTERISTICS OF PATHOGENIC BACTERIA

Leaf blight bacteria was first named *Pseudomonas oryzae* Ueda et Ishiyama by Ishiyama[10] according to Migula's classification. Then *Bacterium oryzas* (Ueda et Ishiyama) Nakata was adopted for a short period according to E. F. Smith. At present, however, *Xanthomonas oryzae* (Ueda et Ishiyama) Dowson is commonly used[1].

1. Morphological Characteristics

Pathogenic bacterium of leaf blight of rice is typically in rod-shaped, has a long polar flagellum, and is mortile. Photo 19 shows a typical bacterium of the disease under electronmicroscope. The size of a bacterium is reported to the different according to different workers. According to Ishiyama's first report (1922), it is 1.0 - 2.0 μ long and 0.8 - 1.0 μ thick, with a flagellum 6 - 8 μ long[10]. Yoshimura et al. (1961) discovered a difference in the size between a bacterium grown on a culture medium and that grown in host and reported that the former were 1.35 - 2.17 μ in length and 0.55 - 0.75 μ in thickness while the latter respectively 0.65 - 1.40 μ and 0.45 - 0.60 μ, being a little smaller[49]. The surface of a bacterium is covered with slime of polysaccharoid consisting of galactose, xylose, glucose, and others with color reaction to ethanol solution added with 1 per cent aniline acid[16]. It seems to protect the bacterium from change in surrounding condition such as humidity.

2. Physiological Characteristics

Physiological characteristics of the leaf blight bacteria were defined by Ishiyama et al. as follows[10]. The bacterium is aerobic and does not liquefy gelatine, does not reduce nitrate, nor produces ammonia. It produces a little of hydrogen sulfide and not indole. It digests milk without coagulation, reddens litmus milk and does not make gases and acids from carbohydrates.

It was later found out that physiological characteristics of the bacterium varies much with different strains. Mukoo et al. (1960) reported that some of them were quite different from those reported by their senior bacteriologists in respect to production of ammonia, liquefaction of gelatine and production of acids from carbohydrates[20].

Many isolates collected from various parts of Southeast Asia are compared for their physiological characteristics as shown in Table 2. All of the characteristics studied so far are different for each isolate especially in respect to liquefaction of gelatine and production of acid from carbohydrates.

Table 2 Difference in Physiological Characteristics between Isolates of Leaf Blight Bacteria (Wakimoto & Tsuchiya)

Physiological character \ Grade	—	±	⊥	+	++	+++
Reduction of Nitrates	75(10				1	
Production of Ammonia					48 (2)	25 (8)
Production of H_2S		3(1)	32 (7)	27(2)	13	1
Reduction of Methylene Blue				42(7)	34 (3)	
Liquefaction of Gelatin			1 (1)	23(5)	42 (2)	10
Digestion of Milk	76(10)					
Decomposition of Starch				28	43(10)	5
Production of Acids from Carbohydrates: Glucose				3(1)	13 (4)	60((5)
Levulose		1	24 (5)	14(4)	11 (1)	2
Arabinose		2	27 (3)	19(1)	21 (3)	7 (3)
Xylose			27 (4)	23(2)	4 (1)	22 (3)
Ramnose	67 (8)	4(1)	5 (1)			
Mannose	37 (4)		11	2	3	9 (3)
Galactose		1(1)	2	7(1)	16 (4)	39 (4)
Maltose	55	10	3	4	4	
Lactose	76(10)					
Sucrose			35 (4)	14(2)	13 (1)	8 (3)
Raffinose	30 (5)		7	3(1)		
Dextrin	71(10)	1	2		2	
Starch	76(10)					
Sorbit			71(10)	5		
Mannit	70 (8)	2		2(2)		2
Glycerin	74 (9)		2 (1)			
Salicin	22 (5)	2	37 (5)	9	5	1
Pectin						76(10)
Cellulose	76(10)					

Figures show number of isolates, these in parentheses showing number of Japanese isolates.

3. Nutrients, Kinds of Culture Media, and Cultural Conditions

Leaf blight bacteria essentially require carbon and nitrogen sources for their growth while for their propagation a number of inorganic salts[44].

As the supply source of carbon, sucrose is considered best, followed by glucose, mannose, galactose, and maltose. Among organic acids, succinic acid is used for an effective source. Although starch is not an excellent source of supply, it is also decomposed by bacteria to some extent[44].

Glutamic acid is most desirable source of nitrogen for bacterial growth, followed by cystine. Inorganic nitrogen is difficult to be used but ammonium phosphate is only slightly used[44]. SII

A trace of magnesium sulfate, iron sulfate or magnesium chloride among inorganic salts in the culture medium promote the propagation of bacteria[44]. If 0.2 per cent of potassium phosphate monobasic or potassium phosphate is added, the medium will also accelerate the propagation.

Although the bacteria may grow on a culture medium of pH 4.0 - 8.8, they can grow best at pH 6.0 - 7.0.

The kind of culture medium most commonly applied consists of the following ingredients (Wakimoto, 1955):

a. Potato semi-synthetic Medium (PSA medium)[41]

Potato	300 g (decoction)
$Ca(NO_3)_2 \cdot 4H_2O$	0.5 g
$Na_2HPO_4 \cdot 12H_2O$	2.0 g
Peptone	5.0 g
Sucrose	15.0 g
Agar	15.0 - 20.0 g
Water	1 liter
pH	6.8 - 7.0

Although this culture medium is very suitable for the growth of *X. oryzae*, it is not for the colony formation from a single cell.

b. Synthetic Medium[44]

Sodium glutamate	2.0 g
KH_2PO_4	2.0 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$MgCl_2 \cdot 6H_2O$	0.05 g
Sucrose	20.0 g
(Agar)	15 - 20 g
Water	1 liter
pH	6.8 - 7.0

This medium is not so good for propagation of bacteria as the PSA medium. But it is often employed for an experiment for a specific purpose.

c. Medium for Single Cell Culture [31]

Sodium glutamate	2.0 g
KH_2PO_4	0.1 g
$MgCl_2 \cdot 6H_2O$	1.0 g
Sucrose	5.0 g
Fe (as EDTA-Fe)	1.0 mg
Agar	10.0 g
Water	1 liter
pH	7.0 - 8.0

As this medium is very favorable for colony formation of bacteria from a single cell, it is used for isolation of single cells.

d. Enriched Liquid Treatment for Single Cell Culture [16]

MnC12 40 ppm aquous solution or $FeO_6H_5O_7 \cdot 5H_2O$ 40 ppm aquous solution $\pm$ MnC $_2$ 40 ppm aquous solution

After suspending leaf blight bacteria on the above solution, they are cultured on a medium. In this case, about 50 per cent of single cells forms a colony even on the PSA.

The optimum temperature for culture is between 26° C and 30° C. The bacteria can hardly propagate below 5° C or above 40°C. They are killed if put in water of 53° C to 54° C for 10 minutes.

4. Variation of Pathogenic Bacteria

Variation of leaf blight bacteria is seen in their physiological characteristics, type of colony, virulence, resistance to chemicals, amino acids requirement, and susceptibility to bacteriophages.

a. Variation in Physiological Characteristics

As already referred to, there is a remarkable variation in physiological characteristics of leaf blight bacteria collected in Southeast Asia (Table 2).

b. Variation in Type of Colony

Yellow color of a colony of bacteria varies from light to dark according to different isolates. Even if isolates are of the same kind, the higher the concentration of sugar or agar in the culture medium, the darker the yellow color of the colony. The type of colony also varies with different isolates. The fluidal type is, it is said, more virulent than the smooth type [8].

c. Variation in Virulence

Bacterial isolates collected from the natural field show a remarkable variation in their virulence. The virulence is assessed in the following way: A certain number of rice varieties are taken out from each of highly resistant, moderately resistant and susceptible varieties. Leaves of seedlings or grown up plants thus chosen are inoculated with pathogenic bacteria. Thus the degree of symptoms appearing on these leaves is assessed. In this case, the kinds and number of rice varieties, age and concentration of bacterial culture, growth stage of rice inoculated, and many other factors affect the results. These factors are not always uniform because each tester employes his own method of testing. Methods for classification of bacteria by virulence reported upto the present are as shown in Table 3.

Table 3. Methods for Classification of Leaf Blight Bacteria According to Their Pathogenicity (From Recent Studies)

Author	Year	Varieties	Time of Inoculation	Leaves Inoculated	Inoculation Method	Measuring	Classification standard
Kariya et al.	1959	13 Japonica rice varieties	Flowering stage	Upper 7 leaves	Multi-needle	Extension of lesions	
Tagami et al.	1960	10 Japonica rice varieties	Flowering stage	Flat leaf	Multi-needle	Area of lesuons	Group I: No variety resistant " II: Only the most resistant varieties prove resistant. " III: Moderately resistant varieties also show resistance.
Yoshimura et al.	1960	11 Japonica rice varieties	Flowering stage	Uppermost leaf	Multi-needle	Area of lesions	Group I: Resistant " II: Intermediate " III: Susceptible
Yoshimura et al.	1960	6 wild grasses of Gramineae and 4 varieties of Japonica rice	Flowering stage	Flag leaf, young leaves of wild plants	Multi-needle	Area of lesions	Group I: Pathogenic to *Isachne globosa* O. Kuntze. " II: Pathogenic to "Kogyoku", "Yachikogane", and Canary reed (*Phalaris arundinacea* L). " III: Pathogenic to "Shin-" and Canary reed " IV: Pathogenic to "Kinmaze", "Ginmasari", *Leersia oryzoides* (L.) Shwartz and *L. Oryzoides*, Sw. var. japonica Hack.
Goto	1965	1 Foreign rice variety	Seedling stage		Needle	Degree of symptoms	0 (No symptom) - 4 (More than half killed by "Kresek" symptoms)
Kusaba et al.	1966	19 Japonica rice varieties	Seedling stage	Upper 3 leaves	Single-needle	Length of lesion	Group A: Affect almost all varieties " B: Affect only moderately resistant and susceptible varieties
Washio et al.	1966	44 Japonica and foreign varieties	Flowering stage	Flag leaf	Multi-needle		Group A, B and C
Sakaguchi et al.	1968	3 groups of Japonica and foreign rice varieties	Seedling stage	Upper 1-2 leaves	Multi-needle	Outbreak	Group I: Affect only "Kinmaze" group " II: Only "Rantaj-emas" group is not affected. " III: Affect all groups

Many isolates of *X. oryzae* collected from various parts of Southeast Asia show higher virulence compared with those from Japan[40]. Especially in India, Cambodia and Thailand, bacteria with higher virulence seem to be distributed with high frequency as shown in Table 4.

Table 4-A. Comparison of Virulence of Southeast Asian Isolates of Leaf Blight Bacteria (Wakimoto, 1967)

Isolates	Mean length value of lesions	Isolates	Mean length value of lesions
○ B 69	3.90	○ No. 3	2.17
△ N 6303	3.84	○ No. 1	1.99
△ N 6302	3.82	○ B 70	1.68
△ N 6309	3.33	○ No. 2	1.39
△ N 6307	3.00	○ B 42	1.33
△ N 6304	2.96	× N 5863	1.33
△ N 6602	2.87	○ No. 4	1.22
△ N 6306	2.57	△ N 6601	1.14
× H 5809	2.54	△ N 6301	0.88
△ N 6305	2.39	× N 5861	0.63
△ N 6308	2.38		

Note: ○.....Philippine △..... Indian ×.....Japanese

Table 4-B Comparison of Virulence of Southeast Asian Isolates of Leaf Blight Bacteria (Wakimoto et al.)

Isolate	Provisional nomenclature	Place of collection	Mean length value of lesion
N 6805-1	(i)	Cambodia	3.36
N 6804-1	(h)	do	3.31
N 6803-1	(g)	do	3.22
N 6815-1	(s)	Thailand	3.13
N 6806-1	(j)	Cambodia	2.83
N 6816-1	(t)	Thailand	2.67
N 6814-1	(r)	do	2.38
N 6804-2	(c)	Cambodia	2.31
N 5809-1	(b)	Japan	2.22
N 6811-1	(o)	Malaysia	1.73
N 6810-1	(n)	do	1.68
B 69	(d)	Philippines	1.50
N 6801-1	(e)	Cambodia	1.18
N 6802-1	(f)	do	0.80
N 6809-1	(m)	Malaysia	0.74
N 6808-1	(l)	do	0.51
N 6807-1	(k)	do	0.42
N 5861	(a)	Japan	0.35

If the bacteria are transferred on some culture medium from generation to generation, their virulence is reduced. But the degree and speed of this reduction vary with different kinds of isolates.

d. Variation in Resistance to Chemicals

Leaf blight bacteria isolated from diseased leaves contains some population of the bacteria resistant to streptomycin. The resistant bacteria also come out from the bacteria originated from single cells on a culture medium at a mutation rate of about 10^{-8}. Therefore, if a large amount of wild isolates is cultured on a medium containing the chemical (at 10 ppm - 100 ppm), several colonies of resistant bacteria grow on it. If they are then cultured on a medium free from streptomycin generation after generation, their resistance will remain constant.

These bacteria thus obtained are very useful for physiological and ecological studies on leaf blight bacteria.

e. Variation in Susceptibility to Phages

Variation is also observed in susceptibility to phages (referred to later) of *X. oryzae*. The bacteria common in Japan are divided into 5 groups, A, B, C, D, and E, according to their susceptibility to 4 kinds of phages which have different hosts of their own, as shown on Table 5[39].

Table 5. Strain Classification of *X. oryzae* According to Their Reaction to Phages (Wakimoto, 1960)

Bacterial strain \ Phage	OP_1	OP_1h	OP_1h_2	OP_2
A	+	-	+	+
B	-	+	+	+
C	-	-	-	-
D	-	-	+	+
E	-	-	-	+

Of these 5 groups, A is distributed most widely, followed by B, while strains belonging to C, D and E are very few in Japan[39]. Leaf blight bacteria commonly seen in Southeast Asia are completely different from those in Japan in their susceptibility to phages[40]. If they are classified according to different kinds of phages in the same way as applied in Japan, most strains will belong to C, D and E groups, leaving only a few belonging to A and B as shown in Table 6.

Table 6. Reaction of Southeast Asian Isolates of Leaf Blight Bacteria to Phages (Wakimoto et al.)

	Phage / Bacterial Isolate	Japanese				Indian	Philippine						Group
		OP_1	OP_1h	OP_1h_2	OP_2	OP_1h_3	Bp_1	Bp_3	Bp_4	Bp_5	Bp_6	Bp_7	
Indian	N 6301	-	-	-	+	+	+	-	-	-	-	-	E
	N 6302	-	-	-	+	+	+	-	-	-	-	-	E
	N 6303	-	-	-	+	+	+	-	-	-	-	-	E
	N 6304	-	-	-	+	-	-	-	-	-	-	-	C ?
	N 6305	?	?	?	+	?	?	?	?	?	?	?	?
	N 6306	-	-	-	+	?	+	-	-	-	-	-	E
	N 6307	-	-	-	-	-	-	-	-	-	-	-	C
	N 6308	-	-	-	+	+	+	-	+	-	-	-	E
	N 6309	-	-	-	+	+	+	-	+	-	-	-	E
	N 6601	-	-	-	+	+	-	-	-	-	-	-	E
	N 6602	-	-	-	-	+	-	-	-	-	-	-	C
	TNL 1	-	-	+	+	+	+	-	-	-	-	-	D
	TNL 2	-	-	+	+	+	+	?	+	-	-	-	D
	TNL 4	-	-	+	+	+	+	-	-	-	-	-	D
	L 1	-	-	-	-	-	-	-	-	-	-	-	C
	S 1	-	-	-	-	-	-	-	-	-	-	-	C
	WKL	+	-	+	+	+	-	?	+	-	-	-	A
Tahiland	No. 1	-	+	+	+	+	+	-	+	+	+	-	B
	No. 3	+	-	+	+	+	+	?	+	-	-	-	A
	No. 6	+	+	+	+	+	+	-	+	+	+	+	?
	No. 7	-	-	+	+	+	+	-	+	-	-	-	D
Philippine	No. 1	-	-	-	+	+	+	-	-	-	-	-	E
	No. 2	-	+	+	+	+	+	-	+	+	+	-	B
	No. 3	+	-	+	+	+	+	-	-	-	-	-	A
	No. 4	-	-	+	+	+	+	?	+	-	-	-	D
	B 42	-	?	+	+	+	+	-	+	+	+	+	D ?
	B 69	-	-	+	?	+	+	?	+	-	-	-	D ?
	B 70	-	-	-	+	+	-	?	-	-	-	-	E

Note: + ... susceptible
- ..... resistant
? ..... needs further test

As stated above, there are many groups of leaf blight bacteria in the world, which have different susceptibility to phages.

f. Variation in Amino Acid Requirement

If pathogenic bacteria are exposed to ultra-violet ray, strains requiring different kinds of amino acids are obtained. These bacteria thus obtained are used for genetical studies on the phathogenic bacteria.

VII. ECOLOGY OF PATHOGENIC BACTERIA

Up to the beginning of the 1950s, few studies had been performed on the ecology of leaf blight bacteria in the natural field. Therefore, it had been believed that the pathogenic bacteria overwintered in soil to become the source of contamination in the next season. In 1953, however, a case of "Sayanukagusa" (*Leersia oryzoides* Schwoihz), a grass belonging to the family of Gramineae was brought under light. Then the ecological study was technically advanced rapidly, which contributed much to clarify the ecology of pathogenic bacteria of leaf blight under natural condition.

1. Methods for Ecological Study

One of the most important matters concerning the ecological study on pathogenic bacteria is how to detect and /or quantitatively assay the expected bacteria in a sample taken from open field. The most basic method for this detection is to isolate and identify the bacteria. Since the middle of the 1950s, however, multi-needle prick inoculation, application of streptomycin resistant bacteria, inoculation of centrifugally concentrated bacteria, phage method, microscopic examination of bacterial effusion, and others have been developed. They were applied singly or in combination and contributed to rapidly clarify the ecological situations of pathogenic bacteria under natural conditions.

a. Methods for Isolation and Identification

Leaf blight bacteria can easily be isolated from fresh lesion of a rice plant. The lesion is cut in the size of 5 x 5 mm^2 and dipped in a 70% solution of ethanol for a moment and then in a 1/1000 corrosive sublimate solution for one or two minutes to disinfect surface of the sample. As a next step, they are washed by 5 ml of sterilized water for about 5 minutes, transferred to the central part of a slant of PSA medium and incubated at a temperature between 26° C and 28° C. In a few days there appears a yellow colony on the section of leaf (Photo 20). In most cases, the bacteria are obtained without any contamination. But some kinds of fungi sometimes grow up. In this case and when bacteria originated from a single cell are needed, they are diluted and cultured again by plating method. In order to identify the bacteria thus isolated, they are inoculated to a rice plant again to confirm their pathogenicity. In addition, a number of experiments must be performed on them to prove their physiological characteristics.

b. Multi-needle Prick Inoculation

This method is to detect the presence of leaf blight bacteria by the symptom on a rice leaf which has been inoculated by an inoculator with single or several of needles. A rice seedling of susceptible variety at 5 ~ 6 leaf stage is more susceptible to the disease than a grown up plant and the inoculated plant is easily affected at so high a temperature as around 30° C.

c. Inoculation by Centrifugally Concentrated Bacterial Suspension

A sample of leaf blight bacteria taken from open field is often in a concentration so low as not to cause the disease to rice plant if it is inoculated. In order to concentrate the bacteria sufficiently for inoculation, the sample suspended in a sufficient amount of water is differentially centrifuged at 1,000rpm for 5 minutes and 10,000 rpm for 10 minutes. The precipitation thus obtained are suspended in a small quantity of water and then used for inoculation.

A process taken after the inoculation is the same as in the case of multi-needle prick inoculation. The rate of affected leaves will serve to the rough estimate of concentration of pathogenic bacteria.

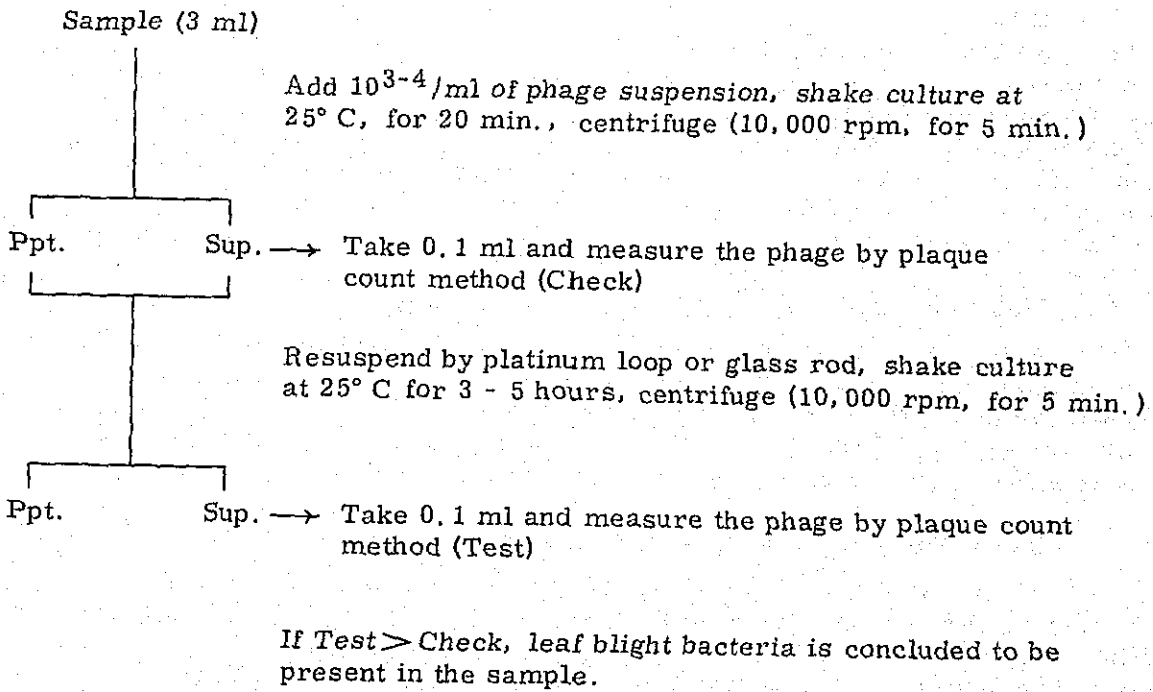
d. Inoculation by Streptomycin Resistant Bacteria

This method is to prepare resistant bacteria to streptomycin, to inoculate them to plants or soil, and to follow up how the pathogenic bacteria will increase or decrease in population by means of culture on the medium containing streptomycin. Although this method is useful for ecological study of the bacteria under artificial environment such as in laboratories and glass houses, it is not suitable for ecological studies in a natural fields.

e. Phage Method

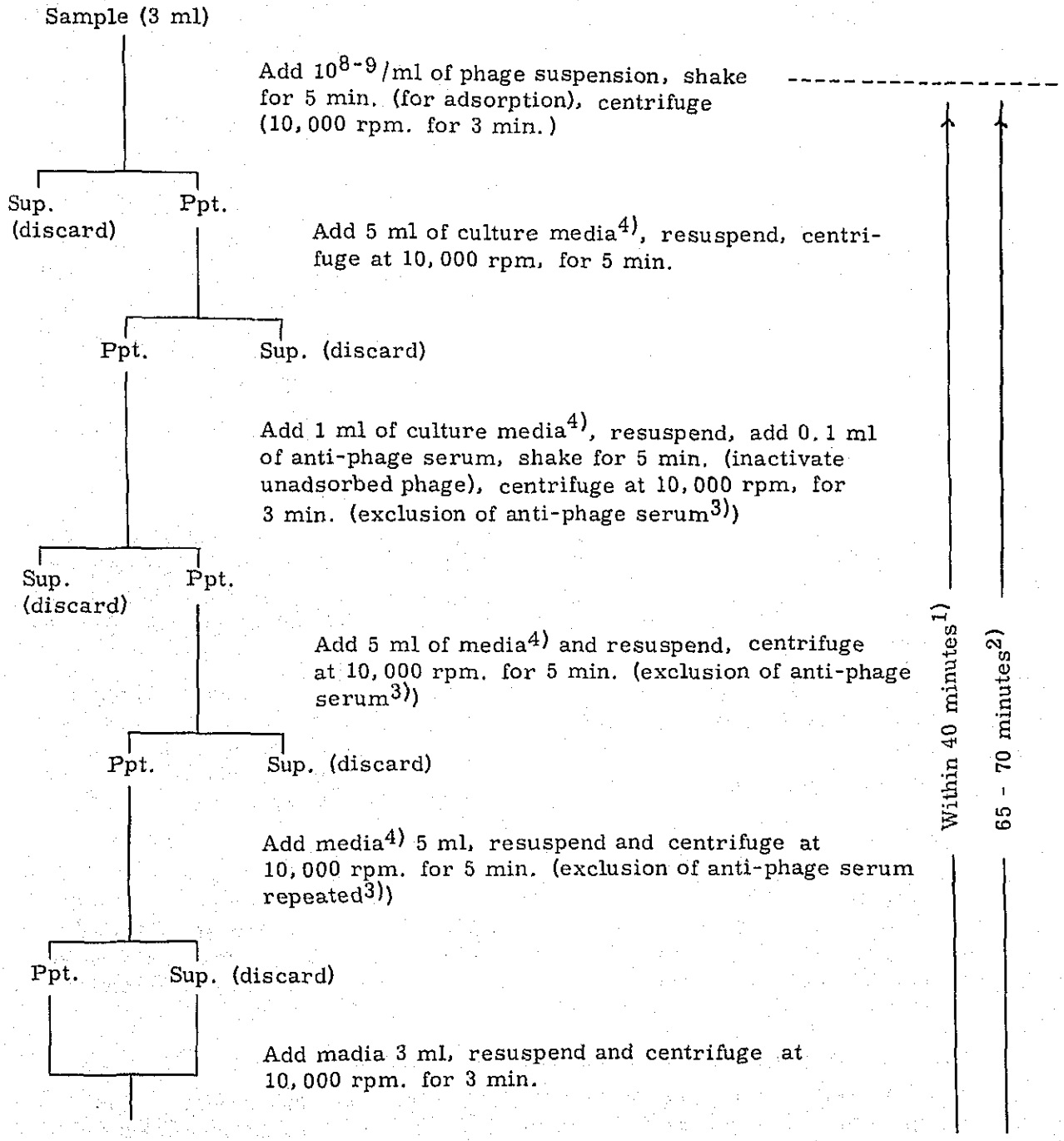
This method is based on a notable characteristics of the phages that is parasitic only on living *X. oryzae* to propagate. A sample suspended in CaVfCh medium (0.5 g or $CaCl_2$, 5 ml of a 10% solution of vitamin free, casein hydrolysate, 1 liter of distilled water, and pH of 6.8 - 7.0) or in PS medium (potato semi-synthetic medium is added with ca. $10^{3\sim4}$/ml of phages, and shake-cultured at an optimum temperature between 25°C and 30°C for five to ten hours. Changes in the number of phages before and after the cultural process are quantitatively assayed by plaque count method to learn whether the phages have propagated during the process or not. When the propagation is observed, it is learned that the leaf blight bacteria necessary for it existed in the sample37). The actual process of this investigation is shown in Fig. 4.

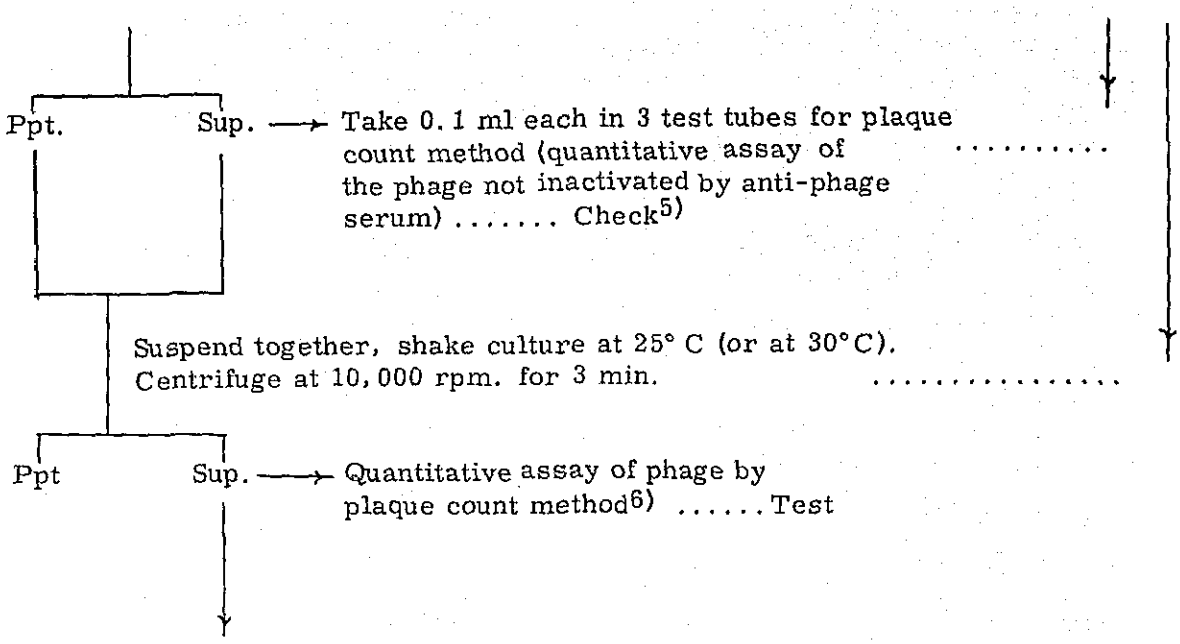
Fig. 4. Detection of Leaf Blight Bacteria by Phage (Wakimoto, 1955)



A more complicated method[41] has been devised to quantitatively detect leaf blight bacteria in a sample. It employs an anti-phage serum. Fig. 5 illustrates a practical method of making use of OP1 phage (referred to later). If OP2 phage (also referred to later) is to be applied, the time factor and the average burst size should be changed[49].

Fig. 5 Quantitative Identification of Leaf Blight Bacteria by Phage (Wakimoto, 1955)





Note: 1) Within the length of latent period of phage

2) Until the end of rise period of phage propagation

3) Remaining anti-phage serum may inactivate propagated phage.

4) Use the same culture media throughout the test. Temperature of the media is to be adjusted at 25° C (or 30° C) before addition.

5) The results comes to zero, if anti-phage serum acts sufficiently.

6) Anticipating the number of leaf blight bacteria in the sample, dilution at 1/100 or 1/10,000 is recommended before plating in some cases.

7) To secure sample for the test to be repeated next day, if tests might fail.

$$\text{Number of leaf blight bacteria} = \frac{\text{Total number of phages produced}}{\text{Average burst size}}$$

In utilizing phage, pre-treatment such as centrifugal concentration of the bacteria or selective propagation by inoculating to host will be useful for detection of bacteria in case that the concentration of bacteria seem to be too low in the original sample.

f. Microscopic Examination of Bacterial Effusion

A sample is inoculated to the central part of the fifth or sixth leaf of rice seedling of a variety susceptible to the disease by an inoculator with 200 needles which are bundled together with their all points in uniform height and kept at a temperature around 30° C optimum for infection. After 5 or 6 days,

it is cut about 5 mm wide at 1 cm above the inoculated point. The cut is then keep between a slide and a cover glasses and added with distilled water under a microscope to detect bacterial effusion if any (Photos 17 - 18). Even though the symptom of the disease is not observed on the leaf, if the effusion is detected, the existence of *X. oryzae* in sample is proved[9]. This method makes use of a phenomenon peculiar to leaf blight bacteria and is so sensitive as to detect 10^2- 10^3/ml of the bacteria in a sample.

2. Hibernation of Leaf Blight Bacteria

In Japan, a country in the temperate zone, overwintering of pathogenic bacteria in rice producing area is a very serious problem. Various methods for detection of the bacteria as mentioned above have contributed to bring to light the overwintering of leaf blight bacteria as follows:

a. Hibernation in Grasses

It was discovered in 1953[4] that grasses of the family of Cramineae such as "Sayanukagusa", "Makomo", "Kusayoshi" (*Phalaris arundinacea* L), "Yoshi" (*Phragmites communis* Trin.), and "Chigosasa" (*Isachne globosa* O. Kuntze) produced affected lesions on leaves if the pathogenic bacteria were inoculated with a needle-inoculator. Of these grasses, "Sayanukagusa" was often found to be infected naturally by the disease in areas where the disease had broken out habitually (Photo 10). The disease used to occur on this grass earlier than on rice plants. In the Tokai-Kinki regions it is observed already toward the end of June. In the light of these facts, "Sayanukagusa" was believed to play an important role in the overwintering of the pathogen. It was later made clear that the underground part or rhizosphere in particular of these grasses had a closer relation with the hibernation[38] of the bacteria).

Leaf blight bacteria are inclined to crowd together on the surface of roots of plants of the Gramineae family and others. In the case of rice plants, they like to swarm at the apex and hairs of root or any part with higher metabloic activity or higher oxidation-reduction potential (Eh). It was confirmed that several kinds of amino acids and saccharides secreted from these parts of the root[16]. Plant's roots thus provide not only the place of hibernation for pathogen but also activation to the bacteria that have lost their capacity of propagation on an ordinary culture medium or pathogenicity. This function of the root is therefore called activation and plays an important role in early stage of infection of rice plant by leaf blight bacteria[16].

b. Hibernation in Rice Straw

Pathogenic bacteria also survive in affected rice straws stored dry under roof or in the open air during winter[38] (Photo 22). But they cannot overwinter if the affectd straw is plowed in under soil from autumn to winter[38]. This bacterial death may be attributed to the fact that they are provided with water under a lower temperature without any supply of nutrients necessary for their propagation. It may also be caused by some influences from phages and competitive microorganisms.

c. Hibernation in Stubbles of Rice Plants

A large amount of pathogenic bacteria is detected in stubbles of rice plants after harvest. But they diminish rapidly from autumn to winter[33, 38].

No bacteria can be found out in stubbles that have withered during the winter season. In warm areas of Japan such as Kyushu, however, stubbles often live through winter and generate sprouts in spring. Pathogenic bacteria can winter in such live stubbles[33].

d. Hibernation in Rice Seed Grains

Pathogenic bacteria can easily winter in affected seed grains of rice stored under roof the same as in the case of affected straws[38]. The bacteria are crowded full in the vascular bundle of glume of rice plant and winter in a dry state. Those that survived through winter decrease rapidly in June. Although it has not been evidenced in Japan that they survived in brown rice, it was reported from China that when rice was seriously attacked, the bacteria were found out even in endosperm of grain (Fang et al. 1956).

e. Hibernation in Soil

It has long been said that leaf blight bacteria winter in soil. But a recent study has proved that they die rapidly in paddy field soil during the winter months[33, 38]. Those artificially mixed with soil die out soon and any of them cannot already be detected one or two months after and even in a field suffering from the attack every year no pathogenic bacteria can be found out in winter. The time length for survival of the bacteria in soil depends much on the degrees of pH and water content. They can live on only for several days at pH 4.0 - 5.0 while for about two months at pH 6.8. A report states that they can be alive for more than three months in dry soil. As for their life expectancy in soil, influences from other microorganisms there cannot be looked over. Although there has not been any evidence that they can safely live through winter in a pure soil (not containing live plants' roots), the possibility of their survival cannot at all be denied.

f. Overwintering Forms of Pathogenic Bacteria

From all ways of wintering as mentioned above, it may be concluded that there are two forms of overwintering. The one is in a dry form while the other in a growing form. The former is the wintering made by the bacteria in the tissues of affected straw and seed grain in a dry state. The bacteria need to be in a dry form as a mass if they can winter. Those which have survived in such form die as they absorb water under a low temperature in winter. In the wintering in a growing form, pathogenic bacteria swarming in the roots of "Sayanukagusa", winter crops and other biennial wild grasses, and live stubbles in warm areas hibernate feeding on nutrients available from their host while propagating themselves.

When bacteria in dry form are carried to a field under a higher temperature in spring, they reach to the roots of rice plants and other grasses where they are activated, changing their form to the growing. Thus they become the primary source of infection. The bacteria which have hibernated in growing form speed up their multiplication according to rise in temperature to become also the primary source of infection.

3. Invasion of Pathogenic Bacteria into Rice Plant

Pathogenic bacteria activated on the surface of plant's root move along the surface up to the ground part, enter into the plant through stomata always open for entrance on sheath and on coleoptile, and propagate rapidly[32]. At this time, however, no symptoms are observed on the seedling. Pathogenic bacteria which

have multiplied at such stomata are released from them, climb up the surface of rice plant's body and invade into the plant through hydatodes distibuted on the margin of upper leaves and through wounds made anew. Inside the tissues, they reach to the vascular bundle through vascular path[32], while they are propagating more and more. Thus they finally produce symptoms of disease. The bacterial concentration above 10^4/ml is said to be required to cause successful infection.

Water pores, the entrance for bacteria, are distributed on hydatode at the end of vascular bundle[16]. There are 50 - 70 pores per hydatode.

When a rice seedling is affected by pathogenic bacteria which are under favorable condition to propagate so much as to cause the disease to the plant, they move down rapidly through vascular bundle while continuing their propagation, reach the base of stem through leaf sheath, and then affect new leaves and tiller stems, producing the "kresek" symptom.

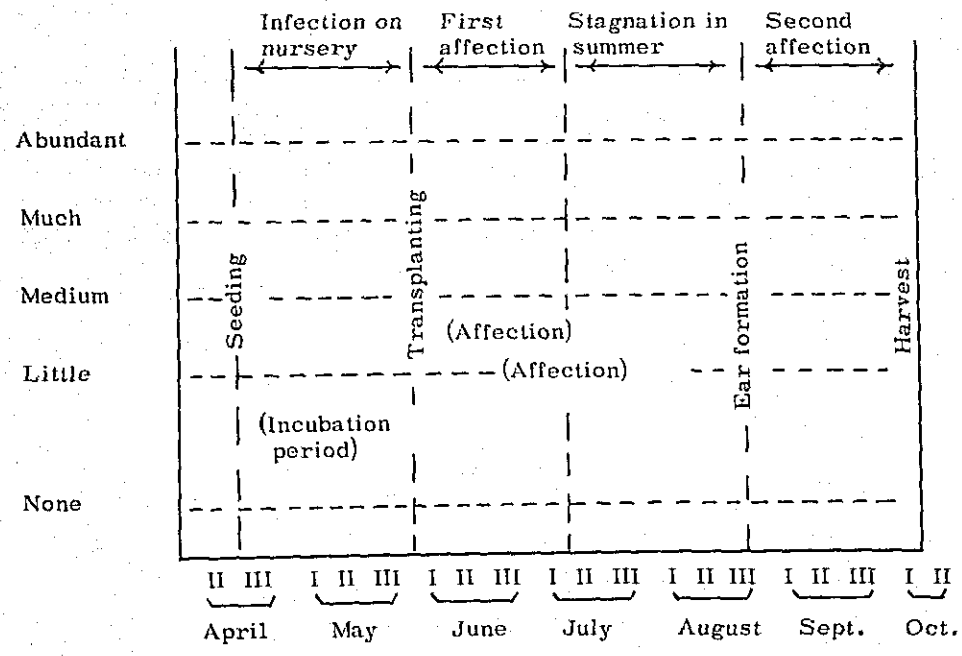
The pathogenic bacteria invaded through wounds on the roots also produce the same symptom.

4. Occurrence and Expansion of the Disease

In an area where bacterial leaf blight occurs annually, even if the disease does not occurred yet, the rice plants and wild grasses are contaminated with the pathogenic bacteria. The bacteria on leaves of these plants propagate if favored with environmental conditions and when their population reaches 10^4 per leaf, symptoms of the disease appear.

The phage method as mentioned before has traced the relation between seasonal changes in this number of bacteria and occurrence of disease in Japan as shown in Fig. 6[49].

Fig. 6. Occurrence of Bacterial Leaf Blight in Japan (on late varieties of rice) (Yoshimura, 1963)



Note: I. II. & III denote 1st, 2nd and 3rd 10 days of the month respectively.

Some of the bacteria which have multiplied in the leaf tissues are secreted as bacterial ooze on the margin or on the surface of the leaf. The ooze which is dried and turned to yellow is often observed on an affected leaf (Photo 23). The bacteria thus exuded outside are washed by rain and morning dews, into paddy field water and transferred from one field to another with irrigation water.

Pathogenic bacteria are also carried by storm. Strong wind can blow them off over a distance of a few scores of meters. Therefore, a typhoon accompanied by strong wind and heavy rain hurts leaves of rice plants, opens an entrance to their invasion and contributes very much to the occurrence and expansion of bacterial leaf blight in the latter part of rice growing stage.

5. Ecology of Bacterial Leaf Blight Bacteria in Tropical Areas

In rice producing areas in tropical region in Asia, winter is much different from that in Japan. On the contrary, however, they are afflicted by the dry season when rice culture cannot be performed. It is very important to know where and how the pathogenic bacteria keep their life during such dry season lasting several months.

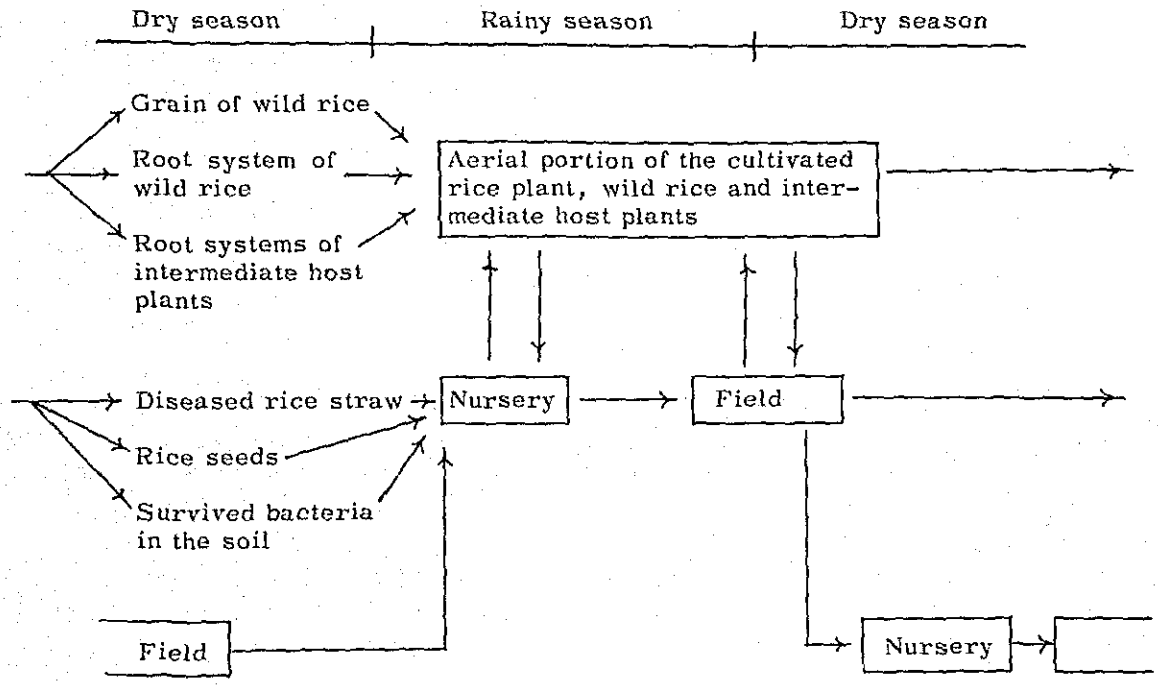
In the Philippines, however, the life of pathogenic bacteria in affected paddy grains lasts only for a month or less. Consequently it seems to be considered that they are not important as the primary source of infection. But it is reported from India that in the tropics the bacteria survive through the dry season in affected seed grains as the very important primary source of infection[30]. Whether they survive in affected straws from a season to the next or not is still unknown the same as the case of those in affected paddy grains. This problem must be solved urgently in the tropical region. In the provinces of Bihar and Gujarat in India, it was evidenced that wild rice plants grown in irrigation canal or ditches were commonly affected by bacterial leaf blight (Photo 11) and considered to be an important source of infection[42]. The under ground part of these plants seem to remain alive all the year round to help surviving of leaf blight bacteria. It is also urgent to study how long the pathogenic bacteria survive in dry soil under the tropical sun.

In the tropical region, although rice culture is carried on mainly in the wet season, it is practicable even in the dry season in some areas where irrigation system is provided. In such areas, rice plants on various stages of growth are grown at the same time and nursery beds are often prepared adjoining a paddy field where matured rice plants already seriously affected by the disease stand. In such a case, the pathogenic bacterialare carried by irrigation water to infect rice plants on different stages of growth one by one and the problem of the bacterial survival through the dry season is out of question.

Fig. 7 shows an assumption regarding the general course of life cycle of the bacteria in the tropical region. The true course must be traced through studies actually made in tropical areas.

No much difference seems to exist in the process of primary infection, occurrence and spread of the disease between Japan and the tropical region. But in the latter, as the temperatute is higher even at the nursery stage of rice plants, nurseries are more frequently flooded and the density of pathogenic bacteria is also higher, the occurrence of disease is often observed on rice plants immediately after transplanting, being accompanied by the kresek symptoms. Besides, in the tropical areas, there supposed to be no stagnancy in disease development in summer such as seen in Japan. Thus the pathogenic bacteria multiply straightly along with the growth of rice plants and symptoms develop gradually from lower leaves to the upper.

Fig. 7 Assumed Life Cycle of Leaf Bright Bacteria in the Tropics (Wakimoto)



Note: ☐ represents disease occurrence.

→ shows direction of bacterial translocation.

VIII. BACTERIOPHAGE OF LEAF BLIGHT BACTERIA

A bacteriophage (or a phage) is a virus that propagates by living bacteria, and also called a bacterial virus. The phage of leaf blight bacteria was isolated for the first time in 1953 in Japan[48] and since then has been utilized in studying the ecology of leaf blight bacteria[37, 41]. Thereafter, some kinds of phages different in characteristics were isolated not only from Japan but also from Southeast Asia.

1. Kinds and Characteristics of Phages

The bacteriophage of leaf blight bacteria isolated at first in Japan was named OP_1, and after then different strains such as OP_1h, OP_1h_2, OP_1t, OP_2, and OP_2m were isolated in Japan[39, 49]. OP_1h, and OP_1h_2 are the same as Op_1 in terms of their morphological and serological characteristics, but are different from OP_1 with regard to host range. OP_1t differs from OP_1 in the optimum temperature for their propagation. All of them belong to the group of OP_1 (Photo 24). But OP_2 (Photo 25) and OP_2m are different from OP_1 not only in their shapes, but also in the serological characteristics and host range, belonging to OP_2 group different from the group of OP_1. OP_2 and OP_2m are not the same in respect of the form of their plaques (Photo 27).

Many isolations of bacteriophages of *X. oryzae* have been collected from diseased leaves from the Philippines[6], Formosa[13], Malaysia[28], Thailand, Cambodia, India, and Ceylon[27]. There are widely distributed phages belonging to the groups of OP_1 and OP_2 throughout the Philippine Islands, and their host range is much different from that of the phages isolated in Japan (Table 6). In Formosa there have been isolated the phages belonging to the group of OP_1, and also a filamentous phage which has not been found in Japan. In Malaysia, Cambodia, Thailand and India, various kinds of phages belonging to the group of OP_1 or OP_2 have been isolated from diseased leaves (Table 7). In Ceylon, some kinds of phages with the different host range have also been isolated, but their nature and the group to which they belong have not yet been identified.

Table 7. Phage Isolation of Leaf Blight Bacteria Collected from Southeast Asia (Wakimoto et al.)

Provisional nomenclature of phage isolation	Place of collection	Group	Provisional nomenclature of phage isolation	Place of collection	Group
M 1	Malaysia	OP_1	BP 1	Philippines	OP_2
M 2	do	do	BP 3	do	?
M 3	do	do	BP 4	do	OP_1
M 4	do	do	BP 5	do	do
M 5	do	do	BP 6	do	do
M 6	do	do	BP 7	do	do
C 1	Cambodia	OP_2	OP_1h_3	India	OP_1
C 2	do	do	I 1	do	do
C 3	do	OP_1	I 2	do	do
Th 1	Thailand	?	I 3	do	do
Th 2	do	?	I 4	do	do
Th 3	do	?	I 5	do	do
			I 6	do	OP_2
			I 7	do	OP_1

2. Method for Isolation and Quantitative Assay of Phages

A bacteriophage of leaf blight bacteria is widely distributed among the endemic areas and can be easily isolated from the soil or the paddy water of affected paddy fields, river water irrigated the area, and diseased leaves. The ordinary procedure of isolating phages from diseased leaves is as follows: firstly, some isolates of the pathogenic bacteria shall be collected, and are cultivated at 28° C for a few days. These bacteria shall be suspended in sterilized distilled water to make suspension of high concentration. Each two ml of this suspension are distributed to sterilized test tubes to be used as indicators.

Secondly, small pieces of a diseased leaf shall be ground in mortal with a small quantity of sterilized water, and then centrifuge it at 10,000 rpm for 10 minutes. After then 0.1~1.0 ml of the supernatant shall be added to the suspension of indicator bacteria. Thirdly, about 3 ml of PSA medium which was dissolved and has been kept under 45°~ 50° C shall be added to the suspension, and then promptly plated into a sterilized dish. After incubation of the plate at 25° C for ca. 15 hours, there will appear distinct plaques (Photo 27). These plaques illustrate the presence of phages in the sample, and by counting these plaques the phages can be quantitatively measured. This method is called the plaque count method.

If the phage is to be isolated from soil, small amount of soil must be suspended in sterilized water and if from the paddy water or river water, some of

the water shall be centrifuged, and then the same process above mentioned shall be operated on the supernatant fluid as a sample. In this way, the phage easily can be isolated.

3. Use of Phages

The phage propagates only by living host bacteria. Thus, *X. oryzae* phaged can be used to detect and quantatively assay leaf blight bacteria, as has been mentioned. In Japan, forecasting the outbreak of leaf blight is carried out by periodical assay of the population of phages in nature. In the near future, these phage methods will be applied in many parts of Southeast Asia, resulting in a big progress in study on leaf blight.

As the bacteriophage attacks leaf blight bacteria and kill it, the former is likely to be used as a natural enemy to the latter, but it has not yet been successfully taken into practical use, perhaps because (1) the phage is adsorbed by leaf blight bacteria only by chance and not actively, (2) the particle of the phage it too large to penetrate into and translocate in the rice tissues, (3) host range of the phage is rather limited on one hand and leaf blight bacteria with resistance to the phage may easily appear on the other hand.

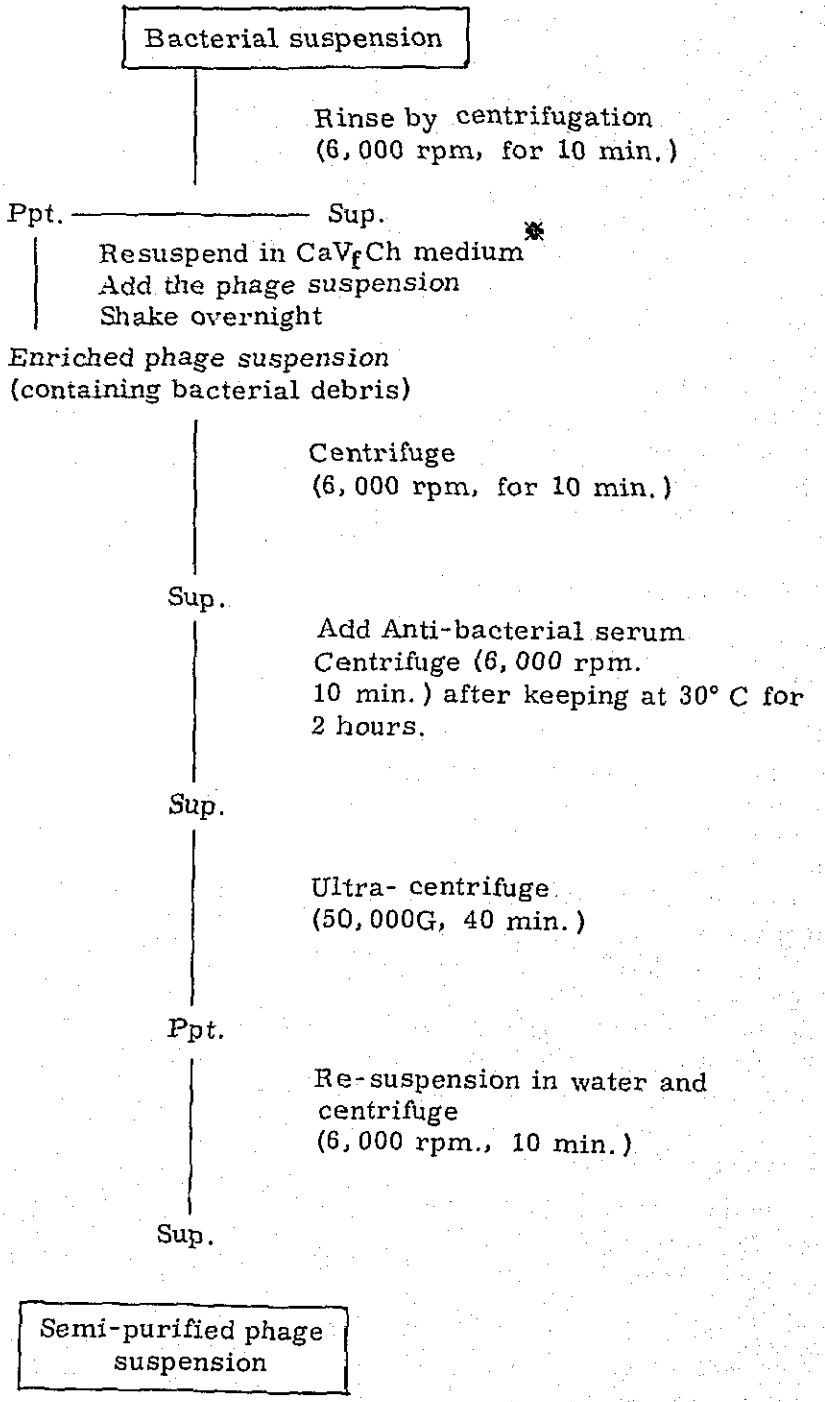
4. Enrichment and Purification of Phages

The phage, being added to leaf blight bacteria cultured in a culture solution and incubated under suitable temperature, will rapidly propagate. But the upper limit of propagation is set within $10^9 \sim 10^{10}$/ml. On the other hand, if the concentrated bacterial suspension is added onto the plaques in a dish and kept still under appropriate temperature over night, the phage can propagate to the concentration of $10^{10} \sim 10^{11}$/ml. By using these enriched suspension the phage can be purified under the method as shown in Figure 8. This purification is required for making anti-phage serum, observing the form of the phage through an electronmicroscope or studying on the physico-chemical attributes of the phage. The anti-bacterial serum used in this process can be made under the following method. The leaf blight bacteria cultured for $1 \sim 2$ days shall be suspended in a physiological saline solution, and each $0.5 \sim 2.0$ ml of this suspension shall be injected ca. 5 times repeatedly into the vein of a rabbit at the intervals of $5 \sim 7$ days. Thus, the serum will be able to be prepared from the blood drawn from the rabbit not before a week after final injection.

5. Preservation of Phages

If the centrifugalized supernatants of enriched phage suspension is stored in a refrigerator at $0 \sim 5°$ C, the activity of phage can be kept during some months, although it may be reduced to some extent. It can also be stored more stably, if kept in a cold storage under the condition of entirely free from contaminated microorganisms by passing through millipore filter (pore size 0.45 micron). If, too, a dish with plaques on it is tightly sealed and stored cool in order to avoid drying, the phage can survive for about two months. The safest method of storing the phage is freezing-drying. If the centrifuged supernatants of enriched phage suspension is frozen and dried in an ampoule, the phage can keep up its activity semi-permanently without fear of deterioration. 0.1% gelatine solution or PS medium protects the activity of purified phages when it was used as suspending medium before freezed-drying. If many discs of filter paper impregnated with the phage suspension are freeze-dried and stored in refrigerator, they can be used conveniently by piece to the extent necessary and be easily transmitted to any person or place.

Fig. 8. Purification method for phages (Wakimoto, 1960)



※ $CaCl_2$ added Vitamin free casein hydrolysate medium

IX. *BDELLOVIBRIO BACTERIOVORUS*, A PARASITIC BACTERIUM TO LEAF BLIGHT BACTERIA

Engaging in isolating the phage from soils, Stolp and Petzold, both plant-pathologist in Germany, found out in 1962 that there appeared other plaques later than those of the phage. They determined and pronounced that they are caused by a kind of bacteria. Moreover, the same kinds of bacteria have been isolated from in soils and sewage water in California. Stolp and Starr named it *Bdellovibrio bacteriovorus*. The word "Bdello" originates from "bdella" in Greek (a leach in English) meaning the vibrio type of bacteria that attacks other bacteria like a leech. Afterwards Bdellovibrio also has been isolated in Israel, Canada and so on, and is under study by bacteriologists and biochemists there.

In Japan, this Bdellovibrio at the first time was isolated from soils and irrigation water in the paddy fields affected by leaf blight in Ibaragi and Shizuoka prefectures, using a leaf blight bacteria as the indicator.(Uematsu *et al* 1969) This Bdellovibrio is thought to be widely distributed among rice-producing areas in Southeast Asia as well as in Japan.

1. Method for Isolation

Bdellovibrio is a kind of bacteria which adsorb to other bacteria, penetrates into them, propagates in their body, and bursts out after a certain period killing the hosts entirely. Therefore, if Bdellovibrio is mixed with the suspension of susceptible bacteria (an indicator), and poured into a dish along with the agar culture medium, and kept under the temperature optimum for propagation for two or three days, there will appear plaques (the part where the bacteria cannot propagate) (Photo 28). By making use of this character Bdellovibrio can be isolated from a sample in nature.

The process of isolating Bdellovibrio from soil can be summarized as follows: firstly, add an appropriate volume (about two times that of the soil) of sterilized water, and centrifugalize at 2,000 ~ 3,000 rpm for about 5 minutes. Secondly, the supernatant fluid shall be gradually passed through a Millipore filters of pore-size 3 to 0.45μ in order to expel other bacteria. Thirdly, a part (0.1 ~ 1.0 ml) of the final filtrate shall be added to the concentrated suspension (about 10^{10}/ml) of leaf blight bacteria. To this mixture about 3 ml of YPDA medium (the main components -- peptone 0.006%, dextrose 0.3%, agar 0.6%) is added and poured into a dish and kept still at 30° C. Plaques of Bdellovibrio will appear only after more than 24 hours, contrasting to 10 ~ 15 hours for phage plaques. Bdellovibrio can be separated from phages by operating repeatedly, therefore, the above process of plating method followed by selection of plaque two or three times.

2. Morphological and Physiological Characteristics

The sizes of Bdellovibrio is in the range of one-thirds to one-sixth of common bacteria, and the form varies from a comma type to a spherical type or a spiral type. Each type has a polar flagellum stretching out of the cell, thicker than those of common bacteria. The cell of the typical comma type is 0.8 ~ 1.0 in length, 0.3 in width and has a flagellum of about 50 mμ thick, as can be seen in Photo 29.

Bdellovibrio attacks not only *X. oryzae*, but also a number of Gram-negative bacteria, pathogenic to plants and animals and soil bacteria. The host range of Bdellovibrio differs by isolates. Some isolates of Bdellovibrio collected from a paddy field can be parastic to wider range of bacteria, while others from the same paddy field have narrow host range (Table 8).

Table 8. Host Range of *B. bacteriovorus* (Uematsu & Wakimoto, 1970)

Bacteria	BdN 6801	BdN 6802	BdN 6804	BdN 6805	BdN 6806
Xanthomonas					
oryzae	+	+	+	+	+
citri	+	+	+	+	+
hyacinthi	+	+	+	+	-
phaseoli	+	+	+	+	-
translucens	+	-	+	+	+
pruni	+	-	+	+	-
Pseudomonas					
mori	+	-	+	+	+
rugosa P1-15-1	+	+	+	+	+
solanacearum	+	-	+	+	-
marginalis	+	-	+	+	-
eriobotryae	+	-	+	+	-
apatata P1-3-1	+	-	+	+	-
oryzicola	-	-	-	-	+
Erwinia					
aroideae	+	-	+	+	-
herbicola	+	-	+	+	-
Escherichia					
coli	+	-	-	-	-
Aerobacter					
aerogenes	+	-	+	+	-
cloacae	+	-	+	+	-

Five isolates of Bdellovibrio collected in Japan, as illustrated in Table 9, can penetrate in all strains of leaf blight bacteria collected from many parts of Southeast Asia as well as from Japan.

Table 9. Host Range of *B. bacteriovorus* to Leaf Blight Bacteria (Uematsu & Wakimoto, 1970)

Isolates of Leaf Blight Bacteria (Country of origin)	BdN 6801	BdN 6802	BdN 6804	BdN 6805	BdN 6806
X. oryzae					
H5809 (A) (Japan)	+	+	+	+	+
N5807 (A) (")	+	+	+	+	+
N5802 (B) (")	+	+	+	+	+
N5805 (B) (")	+	+	+	+	+
N5874 (C) (")	+	+	+	+	+
N5843 (D) (")	+	+	+	+	+
N5868 (D) (")	+	+	+	+	+
N5842 (E) (")	+	+	+	+	+
N5801 (E) (")	+	+	+	+	+
N6602 (E) (India)	+	+	+	+	+
N6702 (E) (Ceylon)	+	+	+	+	+
No. 1 (C) (Philippines)	+	+	+	+	+
N6802 (E) (Cambodia)	+	+	+	+	+
N6807 (C) (Malaysia)	+	+	+	+	+
N6813 (C) (Thailand)	+	+	+	+	+

Note: A - E in parentheses show strains classified by phages.

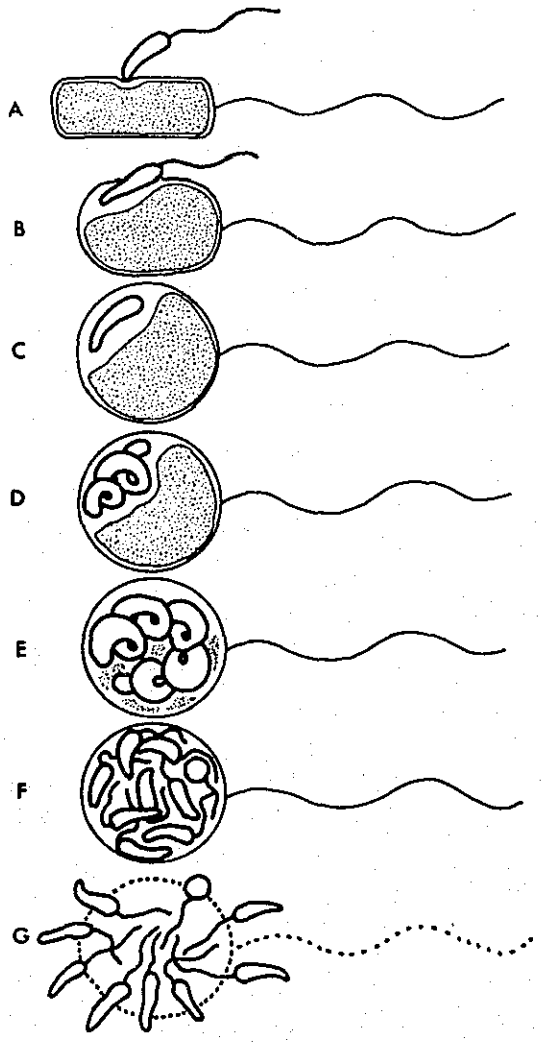
Bdellovibrio can be assayed quantitatively by almost the same method of plaque counting as in the case of phages. However, since the efficiency of plaque formation greatly depends upon kind and concentration of the indicator, incubation temperature and agar concentration of the media, the conditions required for quantitative assay of Bdellovibrio are to be more exact than those for phage of leaf blight bacteria. The concentration of the indicator is required to be $10^9 \sim 10^{10}$/ml. The lower it becomes, the efficiency of plaque formation decreases. Optimum temperature is 30°～32° C, and as it deviates out of this range, the efficiency goes down.

Bdellovibrio tends to die rather rapidly even at the normal temperature in distilled water and be killed entirely by putting it at 45°～ 50° C for 10 minutes.

3. Propagation

Once Bdellovibrio encounters a host, the former adsorb to the latter violently (Photo 9-A), breaks the cell wall of it and penetrates into the cell possibly by the function of enzyme excreted from Bdellovibrio (Fig. 9-B). Then, cells of the host become swell and spherical so that they finally cease from moving. The cytoplasm of the host cell separates from the cell wall and there appears a cavity in which

Fig. 9. Diagrammatic Expression of Propagation of Bdellovibrio (Uematsu & Wakimoto, 1970)



Bdellovibrio establishes itself (Photo 9-C). Bdellovibrio gradually decompose the cytoplasm of the host by enzyme and digest it as nutrient, and grows further (Phto 9-D). About at the time when cytoplasm of the host is completely digested, Bdellovibrio grown into a spiral type begins to break up into normal size ones and to burst out from the host by dissolving the cell wall (Photo 9-E). This process has been illustrated by Starr and others through electron microscopic observation of the ultra thin sections.

It is possible through one-step growth experiment to examine the latent period and the average burst size (average numbers of Bdellovibrio liberated from single host cell when Bdellovibrio attacks to leaf blight bacteria. It has been made clear that 8 9 cells of Bdellovibrio are newly liberated from a single leaf blight bacterium in the course of three and half hours after the start of penetration.

4. Artificial Culture

At the time when Bdellovibrio was first isolated, it was thought that Bdellovibrio was a kind of parasitic bacteria which could not propagate unless adsorption and penetration into living host bacteria. After then, there have been isolated several variants of Bdellovibrio which can be cultured on an artificial culture medium. Some mutants of Bdellovibrio which have been isolated are following: (1) a mutant which can be cultured on a synthetic medium, but lose parasitic ability after cultivation, (2) one which can be cultured on a medium composed of heat killed bacteria and does not lose its parasitic ability, (3) one which can be cultured on both of the above culture media and does not lose its parasitic ability.

Bd-N6801, one isolate of Bdellovibrio that has been isolated from soil of a paddy field in Japan, can attack various kinds of bacteria, and moreover, can propagate on the culture medium of these bacteria killed at 120° C, resulting in the formation of light yellowish white colonies. Bdellovibrio obtained from these colonies is the same as that propagated on living bacteria in terms of morphology and parasitic ability. In other words, it can be said that Bd-N6801 has both parasitic and saprophytic ability. Besides Bd-N6801, other isolates collected in Japan, though to a lesser degree, contains some population of cells having both abilities. This explains that the parasitic ability of Bdellovibrio is subject to change, and the change of "parasitic→ parasitic + saprophytic → saprophytic" is likely to occur frequently.

5. Bacterial Leaf Blight and Bdellovibrio

Bdellovibrio seems, from its characteristics, to be widely distributed among paddy fields in Japan and Southeast Asia where leaf blight frequently outbreaks, and to play the same role in controlling the population of leaf blight bacteria in nature as the phage above mentioned. Bdellovibrio, however, so far has been isolated from paddy fields only in Japan and not in any other country. Bdellovibrio which has been isolated from soil or sewage in some countries except Japan, too, not yet has been determined to be able to attack leaf blight bacteria.

The distribution of Bdellovibrio in paddy fields, seasonal change of its population, the relationship between Bdellovibrio and bacterial diseases in paddy fields such as leaf blight and bacterial stripe, the usage of Bdellovibrio and so on, will be made clear by further studies.

X. RESISTANCE OF RICE VARIETIES TO BACTERIAL LEAF BLIGHT

In Japan it has long been known that the resistance of rice to leaf blight greatly varies among varieties[15]. The resistance of various rice varieties which are grown at present has been made clear, and the breeding has brought out a number of resistant new varieties.

Leaf blight is widely distributed in many parts of Southeast Asia. But the areas where local rice varieties are grown without commercial fertilizer, in general, have experienced only a little outbreak of leaf blight. Serious damage of leaf blight has occurred only after the introduction of a highly susceptible short stem variety which requires much amount of fertilizers. This illustrates the strong relationship between the damage by leaf blight and resistance of rice varieties to leaf blight.

1. Methods for Assessment of Resistance

To test the resistance to leaf blight of rice varieties, two methods can be thought as appropriate. The first is to grow various varieties at an endemic area, and to detect the difference of natural occurrence of leaf blight among varieties. The second is to inoculate the pathogen to young seedlings or adult plants of various varieties, and to compare the length of the lesions which appeared on such variety. Formerly the first method was used predominantly, but for the reason that the incidence of leaf blight greatly changes by year and at the same time often become uneven within the same plot of paddy fields, experiments through many years were required to determine accurately the resistance of a variety. In Japan as late as in 1951, the needle prick inoculation was devised, and thereafter, the spray inoculation, the inoculation by dipping and so on were developed for testing resistance.

(a) Test of Resistance by Needle Prick Inoculation

For the needle used for inoculation, inoculators with one to twenty needles, depending upon the growing stages of rice plants, have been prepared. To young seedlings the bacteria should be inoculated into the middle parts of upper three leaves aside of the main veins by an inoculator with one or two needles. In the case of adult plants, the bacteria should be inoculated into the middle parts of the upper first flag leaf to second leaves by an inoculator with four to twenty needles. The inoculated plants shall be kept at the appropriate temperature of 25°~30° C. By measuring the length or size of lesions which appear after 7~10 days of inoculation in the case of young seedlings and after 20~25 days in the case of adult plants, the resistance of rice varieties to leaf blight can be determined.

Three kinds of bacterial strains, virulent, moderate and less virulent ones shall be cultured on the slant of PSA medium for two days, and then be suspended in sterilized distilled water to make the concentration of 10^{8}~^{9}/ml. The suspension is to be used for inoculation.

The research results so far obtained show that there is a close correlation between the resistance during the seedling stage and that during the

adult stage both varieties of Japonica and Indica, and that the approved results for the resistance of young seedlings and adult plants by needle prick inoculation method closely coincide with those by the method of detecting natural outbreak of the disease.

(b) Test of Resistance by Spray Inoculation

This method is to confirm the resistance by measuring the incidence of diseases after the bacteria have been inoculated to young seedlings or adult plants by spray inoculation. Pathogenic bacteria for inoculation should be newly isolated ones from diseased leaves, and cultures for 2 days have to be suspended at the concentration of about 10^9/ml and sprayed. Spray inoculation in the field needs to be carried out in the evening and not during daytime in the field. When plants are inoculated in a glass-house kept under high humidity will raise the rate of infection.

(c) Test of Resistance by Dip Inoculation

The point of this method is to dip the roots of young rice seedlings in bacterial suspension (bacterial concentration: $10^{6\sim7}$/ml) immediately before transplanting for several hours and thereby to make the disease occurrence uniform in the field in order to obtain accurate results.

(d) Other Testing Methods

A variants of the spray inoculation method, called vat method and bed method, is also used. The vat method refers to the one by which rice seedlings shall be planted in hydroponic solusion (Kasugai's formula) added with 1% of agar in a vat of suitable size (ca. 5 cm in depth) and suspension of bacteria is sprayed to seedlings at the beginning of sprouting of the second leaf, and then by measuring the rate of diseased seedlings after the inoculated seedlings have been kept at 25°~27° C for 15~17 days. Bed method refers to vat method enlarged in scale using a nursery bed in the field or in a glass-house rather instead of a vat.

These testing methods are highly usable for the selection of the resistant local varieties as well as for examining leaf blight resistance of bred varieties.

2. Resistance of Japonica Rice

Since the beginning of 1900's many research and examination results have been reported about the leaf blight resistance of various varieties of Japonica Rice.

Selected results of them are shown in Table 10-A~10-C.

The typical highly resistant varieties of Japonica are as shown in Fig. 10-A 10-C, varieties developed from Shobei, Shiga-Sekitori 11 or Kono 35[3].

Table 10. Resistance of Japonica Rice Varieties to Bacterial Leaf Blight

A. (Kuwazuka, 1933)

Resistant	Shigasekitori 11, Butsuriki, Owaribozu 14, Dotoku, Hosui
Fairly resistant	Shobei, Suga-ippon, Okushirozasa, Nishinomiya, Fuji, Kyushuban 8, Mikawaho, Kawachiho, Banshuho, Akashinriki, Akaho, Taishoakaho, Konishiki, Soginishiki, Akebono, Sotoku, Musashi, Kameji 1, Kameji 2, Tangochusei, Ginbozu, Aikokuzatsu-ban 28, Aichu 9, Miishinriki, Hayaozeki 3, Saigoku, Satsumanishiki, Nakatetakamiya, Kichishinshu, Mokkomochi
Rather susceptible	Mikawanishiki, Aichiasahi, Gen-ippon 1, Miyashinriki, Furuseshinriki, Shin-akashiho, Yotsugi, Shirosembon, Aichu 8, Kinai-goriki, Sengoku 3, Sengoku 4, Toramaru 7, Sagawashinriki, Saga 172, Yokichisen, Asahi, Yaemugura, Kumasen, Otadane, Kobozu, Hinode 19, Senshutsu, Fukujin, Fukuso 2, Asahimochi, Aichimochi
Susceptibel	Shinriki, Shinriki 3, Sakaeshinriki, Senbon-asahi, Honen-asahi, Shinriki 404, Nakayoshi, Kiryoyoshi, Waseasahi, Kyoto-asahi, Fukuban 38, Omachi, Dochu 1, Doban 2, Iyobenkei 1, 2, Nakaben 122, Shiban 37, Shiban 102, Yamatonishiki, Konihon, Tangoshinriki, Harimanishiki, Heiwamochi, Shinrikimochi

B. (Kiryu et al., 1951 - 1954)

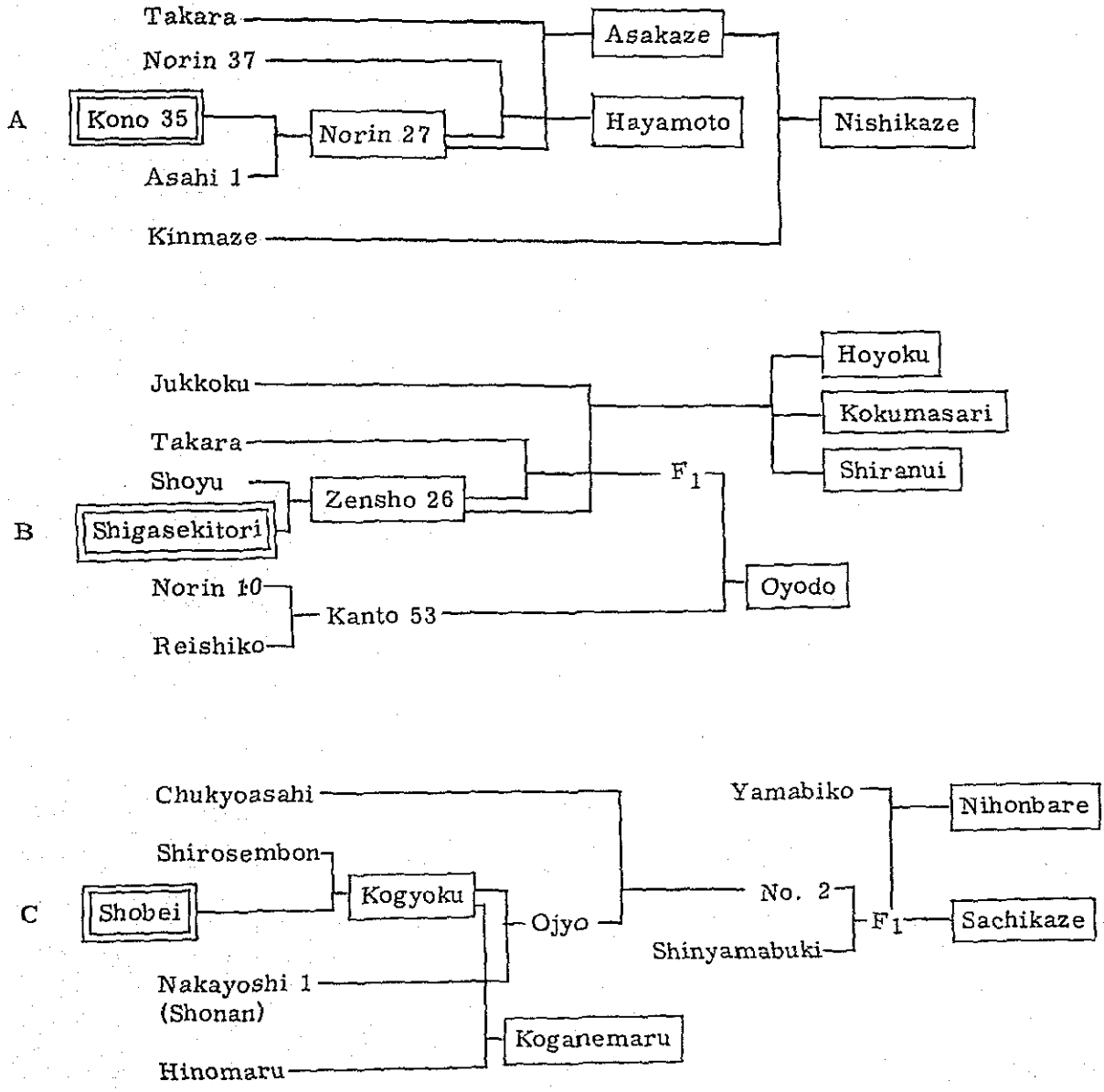
Highly resistant varieties	Zensho 26, Kogyoku, Akashinriki, Shinseki 1, Norin 27, Fukujin
Resistant varieties	Saikai 46, Koganemaru, Norin 12, 29
Moderately resistant varieties	Norin 18, 37, Oitamii 120, Saikai 41, 45, Shin-ai, Benisengoku, Hyuganishiki, Shinrikimochi, Norinmochi 5, Takasago, Koken, Kakei 92, 93, 94
Susceptible varieties	Higomochi, Tsurugiba, Shinseki (Saga), Norin 22
Very susceptible varieties	Asahi, Takara, Kamiyama, Zuiho, Norin 39, 40, Hozakae, Saikai 44, Jukkoku

C. (Yoshida et al., 1961)

Resistant varieties	Norin 27, 35, Kogyoku, Koganemaru, Zensho 17
Intermediate varieties	Moderately resistant varieties Norin 13, 23, 31, 38, 41, Kameji, Shinsen, Oitamii 102, Aikokusai 1, Muboaikoku
	Escaping varieties Norin 1, 7, 14, 16, 17, 21, 24, 30, 41, 43, 45, 46, 47, 48, 50, 55, 56
Susceptible varieties	Norin 3, 6, 8, 10, 12, 18, 22, 25, 26, 29, 32, 36, 37, 39, 40, 51, 52, 53, 54, 57, Asahi*, Sembon-asahi, Acihiasahi, Asahi*, Waseasahi, Chukyo-asahi, Omachi, Takara, Shinriki, Shinriki 798, Shinrikimochi, Nurebaine, Murasakinurebaine

* Different from each other

Fig. 10 Pedigrees of Rice Varieties Resistant to Bacterial Leaf Blight (Fujii, et at, 1967)



Legend: ▭ : Parent varieties for the breeding of resistant varieties.

▭ : Bred resistant varieties.

3. Resistance of Indica Rice

Since it turned out as late as in 1960's that a leaf blight is widely distributed throughout Southeast Asia, much attention has been concentrated on the leaf blight resistance of Indica varieties which is extensively grown in that region.

The resistance to leaf blight varies greatly among varieties both of Indica and Japonica. But the difference of resistance among varieties of Indica is much wider than Japonica. There are several researches aiming to examine the resistance of various varieties of Indica and classify them into some groups according to resistance. Goto[7] reported that the leaf blight bacteria isolated in the Philippines and Japan were inoculated into 85 varieties of Indica and 17 varieties of Japonica, and consequently the greater parts of them were susceptible to the disease. Washio and others [43] inoculated 3 strains of bacteria of different virulence, A, B, C, into total 44 varieties of Japanese and foreign rice, and classified them into four groups, I (resistant to A, B, C), II (resistant to A, B, but susceptible to C), III (resistant to A, but susceptible to B, C), IV (susceptible to A, B, C), according to resistance. Sakaguchi and others[24] inoculated 3 bacterial strains of different virulence, isolated in Japan, into 159 varieties of Japanese and 704 varieties of foreign rice to test their resistance. The results show that there are considerably many varieties both of Japonica and foreign rice with resistance to the first group of less virulent, that there is no Japonica variety with resistance to the second group of moderately virulent while the resistant varieties are found among those from tropical Asian countries, and that there is only a few varieties such as Lead Rice, Nigeria 5, TKM with resistance to the third group of virulent. Thereafter, there has been isolated other strains of bacteria which can affect even the resistant varieties. Thus, it has so far been thought that there is no variety with immunity to leaf blight. Table 11 shows the results of the test on the resistance of varieties conducted at the International Rice Research Institute in the Phillippines (Ann. Rept., IRRI., 1964~67). Four isolates of different virulence, collected from Japan and many parts of Southeast Asia, were inoculated by single-needle prick inoculation into young seedlings of 169 rice varieties from Southeast Asia and 6 typical resistant and susceptible varieties from Japan. The results are shown in Table 12. This table reveals that among Indica varieties there are some with higher resistance, while more than a few are more susceptible to leaf blight than any Japanica variety. Most rice varieties in the world are susceptible to leaf blight, and the proportion of varieties with resistance is only several per cent (Table 13).

The results of single-needle prick inoculation to young rice seedlings are shown in Table 14. This shows resistance during the seedling stage and a few varieties change in their resistance as they grow. Therefore, it is necessary to test the resistance of rice varieties taking account of their growing stages, too.

Table 11. Rice Varieties Found Resistant to Bacterial Leaf Blight at IRRI.

IRRI Ann. Report 1965	1966	1967	1968
Athikraya Ptb 9	Tainan 1	Zenith	H59. PI 245704-2
Adt 25	Zenith	TKM 6	Taihoku-iku 73
Ca 902/b/2/1	PI 209938	Wase Aikoku 3	Tainan No. 7
D 44-1	Tainan 8	M. Sungsong	Tainan-iku 504
E. L. Gopher	Tainan 9	Keng Chi-ju	Takao No. 20
Hsinchu 56	Tainan-iku 446	Mortgage Lifter	Takao No. 25
PI 209938	Takao 21	Early Prolific	Takao-iku No. 12
Zenith	Early prolific	Early Prolific	Takao-iku No. 39
Khao Tah Oo	Yen Tu	Early Prolific	Takao-iku No. 49
Kwangfu 1	PI 162319	Early Prolific	Takao-iku No. 55
Lantjang	Baifufugova	Lacrosse x Zenith	CI 8920-4
Mean-don-tsi	BJI	Sel from 9210	Boa Vista
M 302	TKM-6	Lacrosse Zenith-Nira	Secano do Brazil
Remadja	268b/Pr/22/1/2	Bluebonnet x Rexark	H59-50
Secano do Brazil	DF-1	PI 162319	H30-9
221b/57/1/4	DNJ-152	PI 209938	Fukei 71
Sigadis	DD-100	Tainan-iku 512	Vary Lava 4
Sonacalif	UCP-28	Giza 38	Vary Vato 277
Tainan 1	DM-36	Balilla	Royofotsy 743
Tainan 9	DV-29	BJ 1	Vary Lava 701
Tainan-iku 512	M. Sungsong	Sigadis	
Tainan-iku 516	Nagkayat	Semora Mangga	
Takao No. 21	Bolillo	Tainan 9	
	Lang Chung Yi Lung Ju	221C/BC111/Bn/62/	
	Taino 38	DZ-78	
	Sollana	DZ-60	
		DD-96	
		DV-29	
		DV-52	

Table 12. Resistance of Southeast Asian Rice Varieties to Bacterial Leaf Blight (Wakimoto et al., 1968)

Varieties \ Average length of lesions (cm)	0 ~ 1	1 ~ 2	2 ~ 3	3 ~ 4	4 ~ 5	5 ~	Number of varieties tested
Vietamese	8	9	5				22
Thai	4	12	9	3			28
Philippine		2	2	3			7
Indo-Chinese		6	5	1			12
Burmese			1		3	1	5
Indian and Pakistani	11	7	13	10	2	1	44
Ceylonese	10	19	10	4	1		44
Sumatran	1	4	2				7
Japanese		3	3				6*

* Kogyoku, Norin 27, Asakaze, Akashinriki, Jukkoku and Kinmaze

Table 13. Resistance of Rice Varieties to Bacterial Leaf Blight in the World (IRRI, Ann. Rept., 1966)

Disease scale		Number of varieties	%
0)		67	1.82
1)		63	1.71
2)	R	33	0.90
3)		100	2.72
4)		155	4.22
5)	I	230	6.26
6)		788	21.43
7)		1029	28.00
8)	S	1137	30.93
9)		74	2.01
Total		3676	100

Note: R : Resistant I : Intermediate S : Susceptible

Table 14-A. Resistance of Indica Rice Varieties to Bacterial Leaf Blight (Wakimoto et al.)

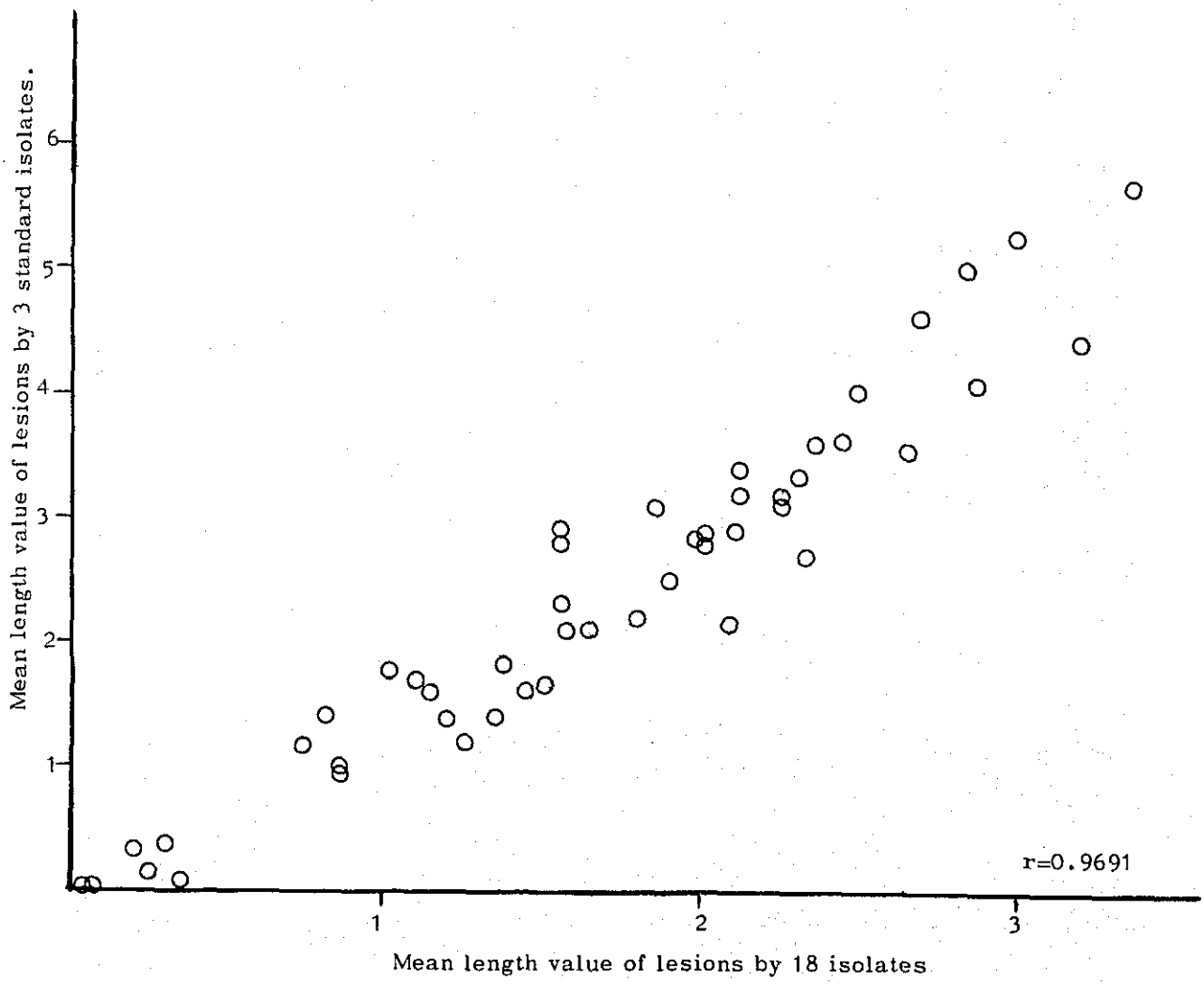
Philippine varieties of rice	Mean length value of lesions by 21 bacterial strains from Japan, Philippines and India
Kutapok	1.51
Dinalaga	2.95
Fortuna	2.67
Magsanaya	2.16
Binicol	1.11
Azusena	2.74
Nagdami	2.93
Seraup Kechil 36	1.53
Intan	1.67
B-E-3	1.16
Raminad St.-3	1.03
Palawau	3.06
Kinanclang Puti	2.41
Inabaca	2.81
Binundok	0.83
Bulastog	1.77
Consejala	1.72
Binacroy	1.80
Ketang	1.59
Macanining	1.91
Ginata	2.12
Curikit	2.56
Lingleng	2.41
Mangarez	3.08
Binaloyot	2.49
Tadukan	1.69
Alasan	2.12
Milagrosa	1.99
Lubang Puti	2.94
Campena	1.46

Table 14-B. Resistance of Indica Rice Varieties to Bacterial Leaf Blight (Wakimoto et al.)

Indian and other varieties of rice	Mean length value of lesions by 6 bacterial strains from Japan, Philippines and India	Varieties	Mean length value of lesions by 6 strains
Ambemohar 157	2.78	Ramtulasi	2.45
Antersal 67	1.65	Pedong 32	1.21
Antersal 90	0.62	Machang	3.29
Bhadas 1303	2.48	Sali	2.89
Bhurarata 4-10	2.18	Tauli	2.98
Kamod 253	1.30	Kumari	1.59
Kolamba 42	1.56	Dangaori	3.29
Kolamba 540	0.33	Harinmuda	1.84
Mugad 161	2.11	Keshipanja	2.21
Panwel 61	0.94	Peswari	2.25
SR 47-22	1.75	Ajan C	2.83
Waner 1	1.31	Chandra mulshi A	0.49
Zinia 14	2.10	Chandra Rana	1.61
Zinia 31	0.99	Chandra Main	1.31
Zinia 63	1.17	Dhali	3.63
Mugad 249	1.79	Gurguri	3.24
Kagisal 44-1	1.90	Kanaibashi	1.87
Antersal 200	0.98	Roal	1.61
Yellikerisal 4	0.99	Mahisdal	1.91
AKP-9	0.81	Bhutumuri	1.56
Honnekattu 15-2	0.89	Gabra Paddy	0.29
Valya 1-4	1.71	Modan	2.53
Honsu 28-2	1.65	Rice 370 (Basmathi)	2.57
Valitaya 4-5	0.78	Sindur Kata	2.31
Walya 26	1.86	T636	2.42
Alursanna 199	0.05	S-1092	1.69
Mysore Kaddi 139	1.67	Patnai 23	1.26
Kembhuti (S-67)	0.85	Co-4	0.95
Halubbalu 317	1.14	RDR 7	1.49
Puttabhatta 1370	1.19	Patnai 6	1.68
Coimbattore Sanna 701	0.81	Early Kolipi 70	2.01

Coimbattore Sanna 661	0.79	Badshabhog	2.89
Ratnachudi 749	1.58	Co 25	1.08
Co-13	1.98	Mugad 81	1.75
HR-19	0.83	Nagupur Sanna (S246)	1.83
HR-21	1.13	Bangarkaddi 9 (S705)	2.61
HR-31	1.68	Henzado 1 (Lead Rice)	0.89
HR-67	0.83	SR-26-B	1.22
Addy	1.39	Niyershail	1.71
Ratnachudi 718	0.62	Marichsail	1.11
HR-22	3.11	T 608	1.83
TKM-6	0.50	Sorta 54-14	2.89
TKM-1	2.98	Sorta 17-1	1.87
T-141	1.31	Co 21	1.77
Latisail	1.23	Bluestick	0.40
APT-10	0.81	Daudkhani	0.67
Surjankhi	2.68	Daudin	1.39
Dharial	0.97	Hatishil	1.79
Altai	2.13	Bhasamanik	2.37
T-136	2.98	Lead Rice	0.71
Rice 349 (Thona)	4.18	Pokkali	1.56
Dodiga 6-2-2	2.09	Nakashin 120	0.58
PTB-16	1.01	Waseaikoku 3	0.88
Dular	1.48	Kinmaze	2.73

Fig. 11 Correlation between Mean Length Value of Lesions by 18 Bacterial Isolates and that by 3 Standard Bacterial Isolates (Wakimoto et al.)



There is no marked specific affinity between variety of rice and kind of bacterial isolates, and as shown in Fig. 11, there is a close correlation between the mean length value of the lesions obtained by inoculation of many isolates and that by one or a few isolates. It turns out therefore that resistant varieties to some isolate have a relatively resistant to every bacterial isolate. In testing leaf blight resistance of a rice variety, it is enough to inoculate 3 standard strains of virulent, moderately virulent, and less virulent instead of many isolates. N6805 (Cambodian isolate virulent), N6810 (Malaysian isolate, moderately virulent), N5861 (Japanese isolate, less virulent), have been established as these standard isolates. An example of classification by this method is shown in Table 15.

Table 15. Classification of Tested Varieties of Rice According to Their Resistance (Wakimoto et al.)

R	M R	MS	S
Chinsura Boro 2	Pi 1	Rantaj-emas 2	Rice 349 (Thona)
Mueynong 62	Latisail	Sangkendan	DCS
Pusur	Kutapok	Malinja (DC 4)	Karalath
Alursanna 199	Gangsale Bhatta	Nep Vai	Dianalaga
Khao Kod 31-24-14	Gia Gao 89-B	BM 5	Tadukan
Lead Rice	Asd 8	Leuang Hawn	Rimbang
	(Akashinriki)	Pinulpot 1	Pin Gaew Bow
		Beoloe Pote	Katoentjar 1
		Sirenteng	Maeha
		Basilanon	Bangender
		Nggeroekmacring	Ketan-eson
		Var Padi Oerang Oerangan	Jaguary
			Bandgender
			Bomba
		Texas Fortuna	Mee Sabe
		(Tsutsui-ine)	Rantaj-emas 3
		(Murasaki-Muyozetsu)	Singadogabo
		(Kinmaze)	Kazza 77
		(Asakaze)	Davao 1
		(Jukkoku)	Nihh
		(Kogyoku)	(Kitareba-Chogoei)

Note: Varieties in parentheses are Japanese or Chinese ones.

4. Heredity of Resistance

Washio and others[43] classified the leaf blight resistance of rice varieties into the resistance to affection and resistance to lesion extension, and reported that for the former, the resistance of the rice variety Kogyoku of Group III to the bacterial strain belonging to Group A (less virulent) is controlled by 2 complimentary dominant genes (X_1, X_2), and the resistance of the variety Shimotusku of Group IV to the same strain by 2 complimentary dominant genes (X_1, X_3), while for the latter, the determinant is polygenes in some case and several active genes in another. Later, Sakaguchi[24] classified rice varieties into 3 groups, that is Rantaj-emas Group (resistant), Kogyoku Group (intermediate), Kinmaze (susceptible), and clearly showed that Rantaj-emas Group has 2 dominant allelomorphic genes (X_{a1}, X_{a2}), and Kogyoku Group one dominant allelomorphic gene (X_{a2}). X_{a1} is the gene which control the resistance to Group I bacterial strain (less virulent), X_{a2} the gene that control the resistance to Group II bacterial strain (moderately virulent). They reported that these genes lie closely linked on the eleventh chromosome.

The study on genes controlling resistance to leaf blight was only started, and with respect to resistance the relationship between genes above-mentioned and gene which control the virulence of bacteria, remains to be further studied. Also, the analysis of the group of genes which control resistance to extension of lesions will be an especially important matter.

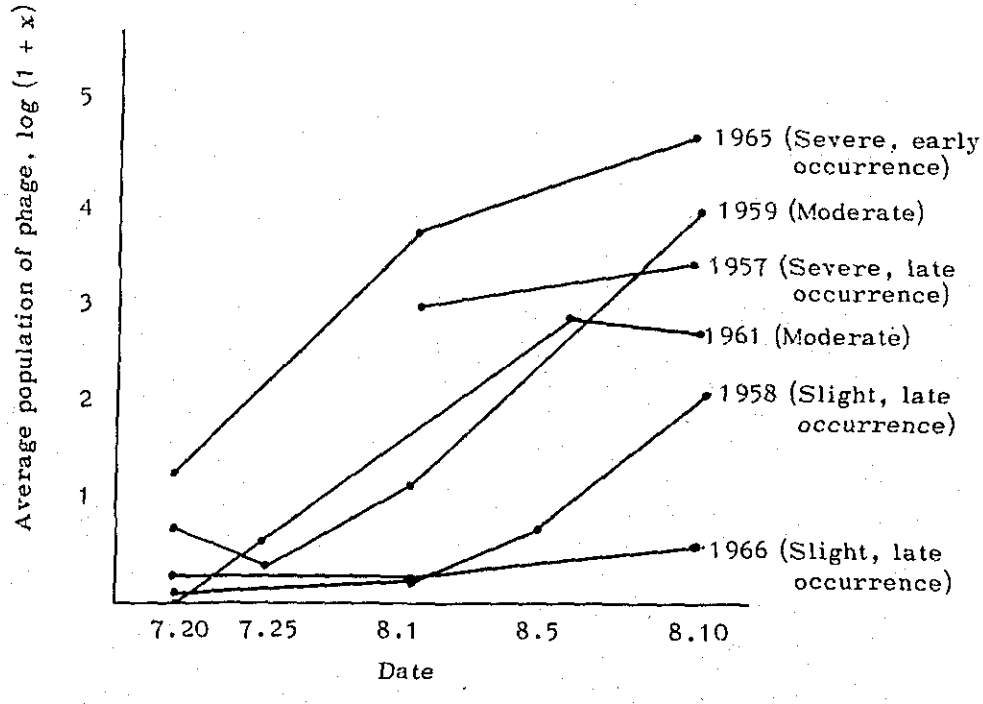
XI. FORECAST OF DISEASE OCCURRENCE

The occurrence of Bacterial leaf blight of rice plant is greatly influenced by climatic conditions during the crop season; such as rain, sunshine, temperature and humidity, strong winds (typhoons), etc. In forecasting the outbreak of the blight, therefore, weather conditions have been closely observed and long term forecasts based on climatic investigation have shown results to some extent so far. More recently, with the advance in studies concerning the behavior and ecology of the pathogenic bacteria in natural fields, a method of forecasting the occurrence of the blight by periodically assaying the population of pathogenic bacteria or bacteriophages closely corresponded to it, has been developed. Particularly, the method of quantitatively assaying bacteriophages is much easier compared with direct assay of the pathogenic bacteria, and this method have been practised actually[34].

1. Forecast by Phage

Bacteriophages in the paddy field water can be detected before the on set of the blight in rice. Researches conducted at the Kyushu Agricultural Experiment Station between 1957 and 1966 showed that changes in the population of phage corresponds to early or late of blight occurrence as well as to the degree of the disease (Fig. 12).

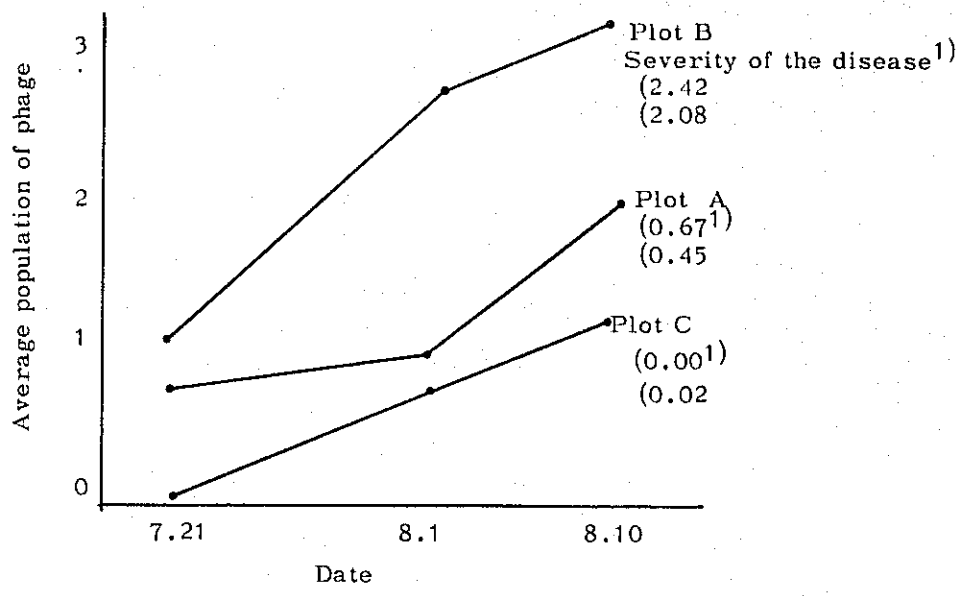
Fig. 12 Relationship between Periodical Change of Phage Population in Paddy Field Water and Disease Occurrence (Tagami, 1968)



Note: Phage population is expressed by logarithmic value of the number of phages per ml. of water

The outbreak of bacterial leaf blight in each paddy field can thus be predicted by periodically assaying the population of bacteriophages in the paddy water. It has also been made clear that the amount of bacteriophages in irrigation channels feeding a large area, has a close relation to the occurrence of blight in the area (Fig. 13).

Fig. 13 Relationship between Periodical Change of Phage Population in Canal Water and Disease Occurrence (Tagami, 1968)



Note: 1) Upper figures show the severity in August and lower figures that in October.

A number of items should be borne in mind in collecting samples for the quantitative assay of bacteriophages for the purpose of forecasting the occurrence of bacterial leaf blight of rice: (1) Survey beforehand the distribution of strains of bacteriophages and use the bacteria sensitive to the phage as indicator. (2) For the sampling of water, choose a paddy field of susceptible variety of rice, if there are many fields of different varieties in the area, while little care is needed for an area of a single variety. (3) Sample water should be taken close to the drainage outlet in each paddy field. (4) Water from the irrigation channel should be collected at a spot where drainage waters from as many fields possible flow together. (5) The time for collecting water would differ with early or late varieties and with local conditions. It should be decided through experience. (6) As bacteriophages in paddy and channel waters rapidly increase after rain, the weather conditions before and after the picking of sample water should be recorded to help correct the data. (7) Sample water picking in early morning is recommended as the population of bacteriophages decreases with sunshine.

2. Correction by Climatic Conditions

The occurrence of bacterial leaf blight of rice is influenced by temperature, rain, typhoons, etc., during the crop season. Therefore, these factors should be added to the data obtained in bacteriophage assay for the adjustment of later forecasting. Heavy rains that inundate rice in seedling stage or soon after trans-

planting promote infection of leaf blight bacteria, while extremely high temperature and sunshine during the summer time retard the expansion of the blight disease. Typhoons, coming from summer to autumn, help spreading of leaf blight bacteria by wind, and, damaging the plant body cause bacteria to penetrate into the plant easier, leading to the resulting rampance of the disease.

3. Forecast in Tropical Rice Growing Areas

The ecology of leaf blight bacteria and the phage in the tropics has not yet been made clear. Forecast of the disease occurrence is accordingly a matter of future researches. The following steps are required for conducting the research. (1) Accumulation of data for finding out the relation between climate and blight occurrence is necessary. (2) Strains of bacteriophages in the area and their distribution should be made clear. (3) In each area, in the light of the cultivation pattern of rice, annual surveys are to be carried out on the seasonal change in the population of bacteriophages in the water of paddy fields and rivers. (4) Find out the relation of the change in population of bacteriophage and weather conditions during crop season to the tendency of the occurrence of leaf blight.

With the advance in these researches, it is expected that the bacteriophage method can be applied as an effective measure of forecast of the outbreak of bacterial leaf blight in tropical regions.

The bacteriophage method is assumed to be particularly effective in finding the degree of contamination of rice seedlings in the nursery, and also in forecasting the occurrence of Kresek symptom on rice during the early stage after transplanting through the tillering stage. The success of Shigemura and his colleagues in Ceylon illustrates this[27].

XII. CONTROL OF THE DISEASE

1. Control by Cultural Practices

Experiments on the protection of rice against bacterial leaf blight have been conducted throughout Japan from the 1960's. During the early stages of study, emphasis was put on cultivation side: such as the type of nursery, control of irrigation water, fertilizer application and variety selection. Through these studies it was found out that, plants grown in upland nurseries had a lower rate of blight occurrence after being transplanted to the fields than those from low land nurseries, that early drainage promotes outbreak of the disease and that excessive nitrogenous fertilizers help accelerate the blight. Marked difference was observed in the resistance of each variety to the leaf blight, and the adoption of resistant varieties was seemed to be essential for the protection. In the 1950's later, since many facts concerning the hibernation of the blight bacteria and the important role of intermediate hosts, mud grass and its variety (*Leersia oryzoides* (L.) Schwarz and *L. oryzoides* var. Sayanuka Ohwi), were made clear, several important methods were added to then recommended cultivation practices for the protection. Problems connected with the cultivation method have already been explained and are not further discussed here.

2. Chemical Control

Chemical protection has been in use on the other hand since the discovery of the bacterial leaf blight. At first, experimental use of chemicals, then generally applied, such as bordeaux mixture and other copper compounds, sublimed sulfur and lime sulfur, showed some effects against the disease. No remarkable progress was made in protective chemicals for many years thereafter. With the coming into use of mercury compounds and other new agricultural chemicals in rice culture around 1950, strong interest in the development of new protective chemicals against bacterial leaf blight was stimulated. The chemicals tested in the 1950's included copper compounds (or copper-mercury compounds), mercury compounds and antibiotics.

Copper compounds generally show some protective effects against bacterial leaf blight, but sometimes cause brown spot on leaves and hulls, and particularly raise problem of bad effect upon yields. Although injuries by chemicals were more or less dicreased by the use of bordeaux mixture of higher content of lime, and copper-mercury compounds instead of single copper compounds, yields did not increase significantly.

But, for the reason that there were no other effective chemicals to replace them, these chemicals were used partly. Although mercury compounds show marked effect on the bacteria in vitro test, they show little protective effects when sprayed on infected rice plants.

Streptomycin, chloramphenicol, aureomycin, fradiomycin, actidion and many other medical antibiotics were tested. Of these, streptomycin and chloramphenicol showed protective effects in some extent. However, streptomycin of high concentration caused spots on leaves, and decreased crop yields if sprayed during the later growth stage of rice, and was not extensively used.

Chloramphenicol cause less injury and is widely used in Japan. In the

1960's, the screening of chemicals was further accelerated and many effective chemicals were developed.

a. Screening of Chemicals

The screening for effective chemicals against bacterial leaf blight had been carried out by the following three steps. First, the sterilizing effect in vitro is examined by cup method, filter paper disc method or agar dilution method. Biological test using rice seedlings follows for the selected chemicals to know the real protective effect and about the occurrence of spray injury. Lastly, the effect under natural conditions on yield is examined in field tests.

But recently a number of chemicals were discovered, which, in vitro showing no sterilizing effect, prove highly protective when sprayed to the inoculated plants. This fact showed that sterilizing effect in vitro does not necessarily correlates with the results of in vivo tests, and the methods of primary screening of chemicals can be classified into needle inoculation method, spray method, dip method, etc., according to the way of inoculation; and pot method, vat method, and bed method, etc., according to the instruments employed for growing seedlings (Kyushu Agr. Expt. Sta.). Various inoculation methods have been discussed earlier in the foregoing section and are not repeated here. All of these methods testify the protective effects by spraying chemical before or after inouclation and comparing the development of blight lesions after the spray. The pot method use young seedlings or grown rice plants cultivated in pots. In vat method rice plants are cultivated in vats with agar added Kasuagi's culture solution and inoculated by spray at 1 - 2 leaf stage. Protective effect of chemical spraying is tested by examining the percentage of diseased seedlings. Bed method is rather large in scale, and uses nursery bed plotted in the field.

Field tests are conducted to determine the effects under natural conditions of the chemicals that showed effects through the forementioned test methods. A suitable field is chosen in the area frequently and evenly affected by the disease. The field is divided into plots of proper size. Spraying the chemical 1 - 3 times at due stages (Usually between young ear formation and heading stages). Effects are compared by disease occurrence and at the same time spray injuries are also examined. Final conclusions are to be reached after assessment of the yields. On the other hand, separate tests are required for chemicals having effects on bacterial leaf blight to clarify their poisonous effect to fish, including acute and chronic toxicity and the amount of residue in the harvested crop.

b. Kinds and Effects of Chemicals

The chemicals now in use in Japan for the protection against bacterial leaf blight are as follows; Cellocidin, Delan (dithianone), Shirahagen (L-chloramphenicol), Sankel (dimethyl-dithiocarbamate-Ni), Phenazin (Phenazin-5-oxide) and Celdion (fentiazon). The sterilizing power of Cellocidin is competitively suppressed by the presence of cystine or glutathion and is thought to inhibit SH base. It is also assumed to accure to its selective impedance to various NAD requiring dehydrogenases, especially α-ketoglutarate $\rightarrow$ succinate[21]. When sprayed on rice, brown spots by the chemical may appear sometimes.

Delan is insoluble in water, and its function on blight's bacteria is quite unknown, but shows protective effects to some extent when sprayed in the field. Although its control effect is unstable, often lead to increase in yield.

Shirahagen's effective ingredient is chloramphenicol which is widely used medically. It shows the sterilizing effect by inhibiting protein synthesis, but its function on leaf blight bacteria has not been studied. It has a comparatively stable effect when sprayed on rice, but sometimes may cause slight spray injury.

Functioning mechanism of Sankel is unknown too, but it is widely applied as it has a comparatively stable effect. Sterilizing effect is probably due to the decomposed products, dithiocarbamate or nickel. Under some condition spray injury (brown spots) may appear.

Phenazine is absorbed by blight bacteria and produces the system of phenazin $\rightleftarrows$ oxiphenazin which competitively inhibit the electron transfer system of the bacteria[25]. Its protective effects are comparatively stable causing no spray injury practically and is rather generally used.

Celdion is almost insoluble in water and its function mechanism is unknown. When sprayed on rice, it shows stable and considerably high effects without any spray injury.

These chemicals were all developed in the 1960's but none of them has yet shown satisfactory results. All of them need to be sprayed 2 - 3 times.

c. Effects of 2-amino-1, 3, 4 - thiadiazole (TF-128) and TF-130 in Disease Control

In 1970, it was reported that 2-amino-1, 3, 4-thiadiazole has a highly protective effect against bacterial leaf blight[45]. This chemical can inhibit the growth of *X. oryzae* even at the concentration of less than 1 ppm on synthetic culture medium. The solution of 100 ppm or above, demonstrates strong protective effect when sprayed on rice, causing no spray injury. It was also made clear that the chemical shows remarkable effects when applied to paddy field soil so that the concentration in the soil reaches 1 ppm or above.

This chemical always showed highly protective effects in experiments using a number of Indica-type rice varieties and bacterial strains collected from Southeast Asian countries (Tables 16, 17). TF-130 is more effective than TF-128 at the same concentration, but the composition of this chemical is not reported yet.

Table 16. Effect of 2-amino-1, 3, 4-thiadiazole (TF-128) on Bacterial Leaf Blight of Indica Type Rice Varieties

(Wakimoto et al.)

Variety		Number of leaves inoculated	Percentage of affected leaves	Mean length value of lesion
Bulastong	TF-128	27	14.9	0.2
	Check	32	100	4.8
Consejala	TF-128	35	45.7	0.9
	Check	30	96.7	6.0
Pi No. 1	TF-128	37	8.1	0.1
	Check	46	90.0	3.6
Nep Vai	TF-128	33	24.2	0.3
	Check	41	90.3	5.0
Pinulupot 1	TF-128	24	33.3	0.9
	Check	26	96.2	6.3
Bangender	TF-128	16	93.8	2.8
	Check	20	100	7.1
Asd 8	TF-128	27	55.6	2.0
	Check	17	94.1	5.4
Kogyoku	TF-128	54	63.0	1.1
	Check	44	97.7	4.3
Kazza 77	TF-128	26	50.0	0.5
	Check	26	84.6	4.4
Katoentijar 1	TF-128	23	73.9	1.1
	Check	28	89.3	4.0
Sirenteng	TF-128	19	21.1	0.3
	Check	24	100	6.5
Pusur	TF-128	33	39.4	0.4
	Check	27	77.8	1.9
Pin Gaew Bow	TF-128	25	28.0	0.4
	Check	22	77.3	4.8

Tadkan	TF-128	30	26.7	0.5
	Check	39	92.3	5.9
B-E-B	TF-128	23	13.0	0.2
	Check	24	66.1	1.8
Lead Rice	TF-128	34	41.2	0.4
	Check	42	88.1	2.7
Boelone pote	TF-128	26	15.4	0.2
	Check	27	92.6	3.3
Bomba	TF-128	26	50.6	0.8
	Check	26	92.3	5.0
Basilanon	TF-128	23	47.8	0.5
	Check	34	97.1	4.9
Rantaj-emas 3	TF-128	26	84.6	1.5
	Check	32	100	5.6
Giao-Cao	TF-128	53	17.0	0.2
	Check	32	96.9	4.7
Latisail	TF-128	26	11.5	0.1
	Check	16	81.3	2.1
Kutopok	TF-128	16	12.5	0.1
	Check	13	92.3	2.5
Kinmaze	TF-128	50	26.0	0.3
	Check	39	97.4	3.1

Note: Inoculated isolate: N6303-1 (Indian isolate)

The chemical is applied so that its concentration in the soil of the pots comes to 1 ppm or higher.

Table 17. Effect of 2-amino-1, 3, 4-thiadiazole (TF-128) on Southeast Asian Isolates of Leaf Blight Bacteria

		Number of leaves inoculated	Percentage of affected leaves	Mean length value of lesion
N6803-1	TF-128 500 ppm	113	23.0	0.3
(Cambodia)	Check	117	84.6	3.6
N6805-1	TF-128 500 ppm	120	36.7	0.9
(Cambodia)	Check	119	95.0	5.2
N6815	TF-128 500 ppm	111	32.4	0.6
(Thailand)	Check	118	90.7	5.7
N6822	TF-128 500 ppm	110	22.7	0.3
(Philippines)	Check	117	92.3	4.5
N6302-1	TF-128 500 ppm	120	18.3	0.3
(India)	Check	120	82.5	2.7
N6303-1	TF-128 500 ppm	120	44.2	0.8
(India)	Check	120	90.8	4.5

		Number of leaves inoculated	Percentage of affected leaves	Mean length value of lesion
N6807-1	TF-128 500 ppm	87	6.9	0.1
(Malaysia)	Check	89	78.7	1.7
N6809-1	TF-128 500 ppm	68	39.7	0.9
(Malaysia)	Check	75	77.3	2.6
N6305	TF-128 500 ppm	68	33.8	0.9
(India)	Check	78	48.7	1.7
N6701	TF-128 500 ppm	89	14.6	0.2
(Ceylon)	Check	86	76.7	2.1
N6710	TF-128 500 ppm	80	21.3	0.2
(Ceylon)	Check	113	87.6	4.2
X. oryzae 1	TF-128 500 ppm	83	41.0	0.7
(Philippines)	Check	77	74.0	2.3
X. oryzae 3	TF-128 500 ppm	78	32.1	0.5
(Philippines)	Check	87	67.8	2.0

d. Integrated Control

As a measure against bacterial leaf blight, emphasis tends to be laid on the cultivation of resistant varieties and the development of better protective chemicals. At present, however, integrated protection method combining cultivation practices and chemical application is urgently needed.

(1) Adoption of resistant varieties: Out of suitable varieties of each area more resistant varieties should be chosen for cultivation.

(2) Selection of the location of rice nursery: Select a place with no fear of the danger of overhead flooding.

(3) Selection of the type of nursery: Preferably the type of nursery that may not induce deep water condition is recommended, and if possible, upland nursery is to be prepared.

(4) Elimination of infection sources: Disposal of diseased straws of the previous year, cutting and burning during winter time of the weeds (especially the mud grass) in irrigation canals and paddy fields, autumn plowing of paddy fields (to cause death to rice stabbles, and if possible, readjustment of land help removal of infection sources.

(5) Disinfection of seeds: Disinfect seeds using mercury compounds or antibiotics.

(6) Improvement of fertilizer application: Special care should be taken not to apply excessive nitrogenous fertilizers. Top dressings are to be conducted in small quantities several times instead of applying large quantity at a time.

(7) Spray of chemicals: During the later period of nursery water is to be drained to the level of ground surface and sprayed chemical once or twice. If these overhead flooding is occurred, spray chemicals right after the water recedes. From the maximum tillering to heading time in the field, spray 2 - 3 times. Spray of chemicals is also needed immediately after a typhoon.

If TF-128 or TF-130 becomes available in practical use, a drastic change in control measure will be occur.

e. Control in Tropical Areas

There are no essential difference between the methods in tropics and other regions for the protection of plants against bacterial leaf blight. There comes in tropics, however, the dry season instead of the winter in Japan, and the mechanism of surviving of blight bacteria through the dry season is unknown. At the same time the degree of resistance of many rice varieties to bacterial leaf blight is not yet thoroughly clarified. These facts make it impossible to enlist practical methods of selecting resistant varieties of rice nor of eliminating sources of infection. At present, the most important matter is to endow the high yielding short stem varieties with a nature of resistance to the disease and to avoid those varieties of high susceptibility. Many research institutions are now interested in the selection and breeding of resistant varieties to bacterial leaf blight. It can be hoped that in the near future improved rice varieties having high yielding ability together with the short stem nature of high resistance to bacterial leaf blight will be developed. There is none the less a growing interest in chemical protection and we expect that chemicals with excellent effects will soon appear and come into extensive use.

XIII. CONCLUSION

Bacterial leaf blight of rice is one of the most important plant diseases in tropical rice growing areas. Establishing a protection method against the disease is of interest not only for those engaging in researches on plant diseases but also for researchers in various fields of study concerning rice plant. In many of the tropical Asian countries the number of specialized researchers on bacterial leaf blight is steadily increasing and the desire to promote studies with the cooperation of Japanese and American experts is also growing among them. It is our sincere hope that Japan, with the longest history of research in this fields can answer to the demands of tropical Asian countries and contribute to an early establishment of the protection method, fully utilizing the accumulated study results and technology. The most urgent task is the breeding of short stem high yielding varieties resistant to bacterial leaf blight and the developing of effective chemicals. Present status may be expressed as evolving a good hope in both of these fields under the cooperation on international level. With a step further, a successful protection method will be established before long.

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