2. STRAINS OF *XANTHOMONAS ORYZAE* IN ASIA AND THEIR VIRULENCE AGAINST RICE VARIETIES

Satoshi WAKIMOTO*

Introduction

The leaf blight disease of rice was first recognized as a bacterial disease in the late 19th century in Japan. Its distribution has gradually expanded in the 20th century. In recent years, an area amounting to 3 to 4 hundred thousand hectares, or ca. 10 per cent of the total paddy fields of Japan, has been affected annually. Thus the disease is now recognized as one of the most hazardous of the rice diseases, except the rice blast and sheath blight.

Many reports concerning the ecology and characteristics of the pathogenic bacterium, *Xanthomonas oryzae*, have appeared, especially in recent 15 years. Reports, however, are very rare from the countries belonging to South East Asia, despite the fact that the disease is widespread in these countries. It is feared that the disease in these countries will become more severe with the increasing adoption of intensive culturing methods of rice involving heavier fertilization and use of high yielding varieties.

It will be necessary, in the first step, to make clear the strains of the distributed bacterium and the grades of resistance of the common rice varieties of these countries.

Criteria and Practical Methods for the Classification of *X. oryzae* into Strains

There are two criteria for the classification of this bacterium into strains: One is due to phage sensitivity and the other to virulence against rice varieties.

1. Practical Method for Testing Phage Sensitivity.

Four kinds of bacteriophage, namely OP_1, OP_{1h}, OP_{1h2}, and OP_2, the host ranges of which are different for one another, are distributed in Japan. Of these phages, OP_1, OP_{1h}, and OP_{1h2} resemble each other in morphological and serological characteristics, so that they are treated as OP_1 group, while, OP_2 constitutes another group on account of its distinct characteristics.

The procedures for phage sensitivity test are as follows:

Table 1. Composition of PSA media.

Decoction of 300 gr of potato	
$Na_2HPO_4 \cdot 12H_2O$	2.0 gr
$Ca(NO_3)_2 \cdot 4H_2O$	0.5
Peptone	5.0
Sucrose	20.0
Agar	15.0
Water	1 ℓ
pH	6.8–7.0

* Division of Plant Pathology, Department of Plant Pathology and Entomology, National Institute of Agricultural Sciences, Tokyo, Japan.

(1) Plate the mixture of the following i) and ii) into a sterilized Petri dish.

(i) 2 ml of concentrated bacterial suspension prepared from PSA slant cultured for 3 days at 28°C. The composition of PSA media is as shown in Table 1.

(ii) 3 ml of PSA media, the temperature of which is regulated at 45 to 50°C.

(2) Streak the phage suspension by using a platinum loop, or drop it by a capillary pipette on the plate, sterilizing the loop, or cutting the tip of the pipette for every treatment.

(3) Incubate the plate at 25°C for 10 to 15 hrs. Plapues will appear if the bacterium is sensitive to the phage.

2. Practical Method for Testing Bacterial Virulence.

20 grains for each rice variety are seeded in 15×5×10 cm plastic pots in 2 lines. Inoculation is carried out during the stage of 4 to 5 leaves. Concentrated (ca. 10^9 per ml) bacterial suspension prepared from 3 days slant culture is inoculated by the side of the main vein at the middle part of each leaf blade by single needle prick method. The lesion will appear and extend up- and downwards from the pricked point. The length of the lesion is measured 10 to 15 days after inoculation, which gives a measure of the virulence of the inoculum. The length of the lesion on a given variety markedly differs according to bacterial isolates, as shown later.

For strain classification in our laboratory, 19 differential rice varieties as shown in Table 2 were used.

Table 2. Rice varieties used for strain classification of *X. oryzae* from Japan.

Susceptible ↑	Koganemaru
	Akashinriki
	Norin 27
	Asakaze
	Kōgyoku
	Shinzeki 1
	Nōrin 12
	Tōzan 38
	Oita-mii 120
	Nōrin 44
Resistant ↓	Sasashigure
	Nakasengoku
	Shinsen
	Nōrin 29
	Zensyo 17
	Taisyo-ako 66
	Jikkoku
	Aichi-asahi
	Kinmaze

The criteria shown in Table 3 were set up for the strain classification of Japanese isolates.

Table 3. Criteria for strain classification of *X. oryzae* according to virulence.

Strain		Reaction of the differential varieties
Group A	A I	Severely infecting all varieties.
	A II	Slightly infecting all varieties.
	A III	Slightly infecting most of the varieties except resistant ones.
Group B	B I	Severely infecting the varieties which are more susceptible than Nōrin 12.
	B II	Slightly infecting the varieties which are more suscepting than Nōrin 12.
	B III	Slightly infecting susceptible varieties only.

Strains of *X. oryzae* Classified by Means of Phage Sensitivity

Isolates of *X. oryzae* collected from various localities of Japan were classified by means of phage sensitivity, as shown in Table 4.

Table 4. Phage sensitivities of *X. oryzae* isolates from Japan.

Strain	Phages				Nos. of the isolates from Japan classified under the respective strain
	OP_1	OP_{1h}	OP_{1h2}	OP_2	
A	+	−	+	+	46
B	−	+	+	+	20
C	−	−	−	−	4
D	−	−	+	+	9
E	−	−	−	+	3
Total					82

From the result shown in Table 4, it is concluded that the strain A is the most commonly distributed in Japan, followed by B, D, C, and E in descending order.

Phage sensitivity of the isolates from Philippines are much different from those from Japan, as was shown by Goto (1965).

Table 5. Phage sensitivity of *X. oryzae* isolates from the Philippines (by Goto, 1965).

Strain	Frequency of isolation
A	0
B	3
C	24
D	36
E	3
Total	66

Most of the isolates from India, however, were found to belong to the strain E, as shown in Table 6.

This means that the 4 kinds of phages used in this experiment were not sufficient for the classification of Indian isolates. In order to set up an international standard for strain classification, it is necessary to carry out further detailed experiments using a larger number

Table 6. Phage sensitivity of *X. oryzae* from Inida.

Isolate	Phage strains				Strain
	OP_1	OP_{1h}	OP_{1h2}	OP_2	
N 6301	−	−	−	+	E
N 6302	−	−	−	+	E
N 6303	−	−	−	+	E
N 6304	−	−	−	+	E
N 6305	−	−	−	+	E
N 6306	−	−	−	+	E
N 6307	−	−	−	−	C
N 6308	−	−	−	+	E
N 6309	−	−	−	+	E

of isolates and other phage strains distributed in areas outside of Japan.

Knowledge about the host range of the phages and the sensitivity of the bacterium distributed in the paddy field is keenly needed when forcasting or ecological study of the disease is to be made by using bacteriophage techniques.

Strains of *X. oryzae* Classified according to Virulence

The isolates collected from Japan showed much difference in virulence against Japanese rice varieties, as shown in Table 7.

Table 7. Virulence of *X. oryzae* from Japan.

	A I	A II	A III	B I	B II	B III
Nos. of isolates classified under respective strain	37	21	20	18	13	9

On the other hand, the inoculation test against more than one hundred Indica varieties showed that most of the isolates from India are more virulent than those from Japan, while, the Philippine isolates show much variation in virulence, as shown in Table 8.

Table 8. Virulence of *X. oryzae* isolates from Asia against Indica rice varieties.

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

○ The isolates from the Philippines
△ The isolates from India
× The isolates from Japan

Although strain classification according to the virulence against differential Japanese rice varieties has not been examined yet, most of the isolates from India except N 6601 and N 6301 may be included into A–I group, and most of those from Philippines except B 69 into A–III or B group.

Resistance of Rice Varieties belonging to Indica Type

The resistance of 108 Indica varieties, which were furnished by the Division of Genetics of this Institute, against 21 *X. oryzae* isolates from the Philippines, India, and Japan was tested. In the case of Philippine varieties, inoculation was carried out using all the isolates shown in Table 8, however, in the case of Indian varieties, only 6 isolates, N 5861, H 6304, N 6302, H 5809, X. 0. 2, and B 69 were used. The figures shown in Table 9 are the average length of the lesions appeared ca. 10 days after inoculation.

Table 9. Markedly resistant and susceptible varieties of Indica rice.

		Resistant variety	Susceptibility	Susceptible variety	Susceptibility
Exp. 1	Philippine varieties	Binundock	0. 83	Mangarez	3. 08
		Raminad st-3	1. 03	Palawan	3. 06
		Binicol	1. 11	Dinalaga	2. 95
		BE-3	1. 16	Lubang Puti	2. 94
		Campena	1. 46	Nagdami	2. 93
		Kutapok	1. 51	Inabaca	2. 81
		Seraup Kechil 36	1. 53	Azucena	2. 74
				Curikit	2. 56
				Binaloyot	2. 49
Exp. 2	Indian varieties	Alursanna 199	0. 05	Rice 349 (Thona)	4. 18
		Gabra Paddy	0. 29	Dhali	3. 63
		Kolamba 540	0. 33	Dangaori	3. 29
		Chandramulshi	0. 49	Machang	3. 29
		TKM-6	0. 50	Gurguri	3. 24
				HR-22	3. 11
				TKM-1	2. 98
				Tauri	2. 98
				Sali	2. 89
				Badshabhog	2. 89
				Sorta 54-14	2. 89
				Ajan C	2. 83
				Ambemohar	2. 74
	Japanese varieties	Nakashin 120	0. 58	Kinmaze	2. 73

Susceptibility is shown by the average length of the lesions in cm which appeared ca. 10 days after inoculation.

The values of susceptibility in Exp. 1 and 2 can not be directly compared, as these were obtained under somewhat different experimental conditions.

It was concluded from the results shown partially in Table 9, that the variation in the resistance of Indica rice varieties is wider than that in Japonica varieties. Specific and complicated affinities between a host and a pathogen, such as are known in the case of rice

blast, were not observed.

Comment

While experiments on the strains of *X. oryzae* distributed in other countries of South East Asia are under way in our laboratory, it is concluded from the results obtained up to the present that the following measures are urgently required for the development of the research:

1. Collection of more phage and bacterial isolates.
2. Selection of phage strains useful for the classification of the bacterium.
3. Selection of rice varieties suited for rating the bacterial virulence.
4. The setting up of an international standard, based on phage sensitivity and virulence, for the classification of the bacterial isolates into strains.

Discussion

D. N. Srivstava, India: (1) To which isolate were the varieties from different countries tested for resistance (on Table 9.)? (2) Do you recommend free movement of seeds from one country to another? (3) Have you got any strain of the bacterium which is very virulent and yet non-sensitive to any phages?

Answer: (1) The susceptibility of rice varieties shown in Table 9 is the average length of the lesions appeared when inoculated with 21 isolates from Philippines, India and Japan. In the case of later experiments, however, 4 to 6 isolates having respective characteristics of virulent, moderately virulent and less virulent were inoculated. (2) We do not recommend, because overwintering of *X. oryzae* in rice seeds has already been confirmed. (3) We have not obtained any isolates which are very virulent and yet resistant against every phage strains. Most of the isolates belonging to strain C seem to be less virulent.

P. Kanjanasoon, Thailand: Did you collect any isolate from Thailand? If so, is it virulent or not?

Answer: I am sorry but we did not collect any isolate of *X. oryzae* from Thailand.

D. V. W. Abeygunawardena, Ceylon: Your work on phage sensitivity and strain virulence is based on *Xanthomonas oryzae*. Have you similar information in *X. oryzicola* or do you consider these two organisms to be the same.

Answer: We have 3 isolates of *X. translusence* f. sp. *oryzae* (*X. oryzicola* by Fang et al) from Philippines, and they are somewhat different in virulence among them. I think this bacterium will also be able to be classified like *X. oryzae*.

H. I. Oka, Japan: (1) When different rice varieties are inoculated with different strains of the bacteria, do we find some evidence for differential reaction? (2) If any differential reaction is found, is it related to the "phage sensitivity?"

Answer: (1) From the results obtained up to the present, we can say that the isolates of *X. oryzae* will be classified according to thier virulence against rice varieties. However, clearcut specific reactions between isolates and rice varieties which was reported in a case of rice blast have never been observed. (2) Any relationships between phage sensitivity and virulence were not observed.