Pathogenic Races of *Xanthomonas oryzae* pv. *oryzae* in South and East Asia

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Abstract

Variation in the pathogenicity of isolates of *Xanthomonas oyzae* pv. *oryzae* from 9 countries in South and East Asia was examined. The isolates were divided into 27 races on the basis of their pathogenicity to Japanese and Philippine differentials, Kinmaze, Kogyoku, Chugoku 45, IR 8, IR20, IR 1545-339, DV85, Java 14, Te-tep, Cas 209, PI 231129 and IR 24. Distribution of races was quite specific to the country. The isolates from Bangladesh showed high virulence, in contrast to those from Malaysia. Variety DV85 was resistant to most of the isolates except for those from Bangladesh, while, Cas 209 was susceptible to any isolates other than those from the Philippines. Two representative susceptible varieties Kinmaze and IR 24 showed complete resistance to isolates MY8417 and MY 8429 from Myanmar, indicating the presence of new resistance gene. All the 79 Japonica varieties were resistant to these two isolates. Twelve, 8, 7, 6, 5, 5, 5, 5 and 2 races were clarified from Bangladesh, Indonesia, Myanmar, China, India, Thailand, Nepal, Philippines and Malaysia, respectively.

Additional key words : *Xanthomonas oryzae* pv. *oryzae*, race, bacterial leaf blight, rice

Introduction

Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is a very serious disease in tropical and temperate rice growing countries. Although some chemicals have been developed in Japan to control this disease, none are fully effective under very severe conditions. Thus, the use of varietal resistance is the most effective and economical method for the control of this disease. Genetic analysis of resistance in rice and variation in the pathogenicity of the causal bacterium have been extensively conducted to assess the resistance of rice cultivars in Japan as well as at the International Rice Research Institute (IRRI) and in other Asian countries[11]. Following the initial etiological studies on the causal bacterium of rice bacterial leaf blight in Japan by Kuhara *et al.*[8], other reports have appeared on the variation in the pathogenicity of the bacterium and resistance genes of rice cultivars[1, 2, 3, 7, 8, 9, 11, 14]. The geographical distribution of pathogenic races of *X. oryzae* pv. *oryzae* should be carefully studied to develop methods for the control of this disease using resistant cultivars. Therefore, variation in the pathogenicity of the causal bacterium collected from 1982 to 1994 in several South and East Asian countries is discussed in the following.

Materials and Methods

Isolation of the causal bacterium : Rice leaves affected by bacterial leaf blight were collected from 1982 to 1994 in Bangladesh, India, Nepal, Myanmar, Thailand, Indonesia, Philippines, Malaysia and China. Diseased leaf samples were cut into small pieces, 1 cm in length including the marginal portion of fresh lesions. They were placed in 70 % ethyl alcohol for a few seconds, dipped in 1 % sodium hypochlorite solution for 1 minute and rinsed in sterile distilled water. Each sample was then homogenized with 10 ml sterile distilled water. The resulting suspension was diluted with sterile distilled water and appropriate dilutions were mixed with melting nutrient agar medium (Difco Laboratories, Detroit, Mich.) kept at 50°C in a waterbath. The mixture was poured into a plate, and the plates were incubated at 25°C for 4 days. Through this experiment, single-colony isolation was made. The viscous and yellow bacterial colonies that subsequently developed were subcultured on PSA medium and grown at 25°C for 2 days. For long-term preservation, the bacterial cells suspended in 10 % (w/v) skim-milk containing 0.05 % L-glutamic acid were lyophilized. For inoculation, the bacterium was grown on PSA[12] medium at 25°C for 2 days, and the culture was suspended in sterile distilled water to reach a concentration of 10^8 cfu/ml. The suspension was used as inoculum.

Pathogenicity test : Twelve differentials, Kinmaze, Kogyoku, Chugoku 45, IR 8, IR 20, IR 1545-339, DV 85, Java 14, Te-tep, Cas 209 and IR24, were used for the experiments. Gene symbols, as proposed by Ogawa[11], for resistance to the disease are shown in Table 1. The rice seedlings were grown in seedling box, and were transplanted individually to plastic pots (1/5000a) in an isolation greenhouse. Inoculation was performed by the clipping method[5] at the heading stage of the earliest cultivar. Tips of rice leaves were clipped off with scissors dipped in the bacterial suspension. Three weeks after inoculation, lesion length were measured. The degree of resistance was assessed based on the standard developed by Kauffman

Table 1. Resistance genes to bacterial leaf blight identified in differential cultivars

Differntials	Ecotype	Resistance genes
Kinmaze	Japonica	unknown
Kogyoku	Japonica	*Xa-1, Xa-12*
Chugoku 45	Japonica	*Xa-3*
IR 8	Indica	*Xa-11*
IR 20	Indica	*Xa-1, Xa-4*
IR 1545-339	Indica	*xa-5*
DV 85	Indica	*xa-5, Xa-7*
Java 14	Indica	*Xa-1, Xa-3, Xa-12*
Te-tep	Indica	*Xa-1, Xa-2, Xa-16*
Cas 209	Indica	*Xa-10*
PI 231129	Indica	*xa-8*
IR 24	Indica	*Xa-18*

et.al.[6] compared with that for standard isolates. The present study was conducted mostly in Japan and at IRRI, and several differential rice cultivars and bacterial races were used in each case[4, 10].

Results

Variation in the pathogenicity of bacterial isolates from several South and East Asian countries was examined. The population of the bacterial leaf blight pathogens of rice was very diverse, and the isolates were divided into 27 races according to pathogenicity to twelve rice varieties consisting of Japanese and IRRI's differentials (Table 2).

Bangladesh : Seventy-four isolates collected in Bangladesh were assessed for virulence and they were classified into 12 races based on the pathogenicity to the 12 differential rice varieties. All the 12 races were distributed throughout Bangladesh. Among the isolates, four of race 1 representing BD8422, were virulent to all the differential cultivars tested. Several isolates of race 2 and race 3 were avirulent to only one differential

Table 2. Interaction between differential rice cultivars and bacterial races of bacterial leaf blight and their distribution in Asian countries

Race[1]	Differential rice cultivars												Distribution[2]
	Kinmaze	Kogyoku	Chugoku 45	IR 8	IR 20	IR 1545-339	DV 85	Java 14	Te-tep	Cas 209	PI 231129	IR 24	
1	s	s	s	s	s	s	s	s	s	s	s	s[3]	BD
2	s	s	s	s	s	s	s	s	s	s	R	s	BD MY
3	s	s	s	s	R	s	s	s	s	s	s	s	BD CN
4	s	s	s	s	s	s	R	s	s	s	R	s	BD ID NP
5	s	s	s	s	s	R	R	s	s	s	s	s	IN
6	s	s	s	s	R	R	s	s	s	s	s	s	CN
7	s	s	R	s	s	s	s	R	s	s	s	s	BD ID
8	s	s	s	s	s	R	R	s	s	s	R	s	ID NP MY
9	s	s	R	s	s	s	s	R	s	s	R	s	BD
10	s	s	R	s	s	s	R	R	s	s	s	s	BD
11	s	s	R	R	s	s	s	R	s	s	s	s	BD TL
12	s	s	s	s	R	s	R	s	s	s	R	s	BD ID NP
13	s	s	s	s	R	R	R	s	s	s	s	s	IN CN
14	s	s	R	s	R	s	s	R	s	s	s	s	BD
15	s	s	R	s	s	R	R	R	s	s	s	s	IN
16	s	s	s	s	R	R	R	s	s	s	R	s	BD ID NP MY IN CN
17	s	s	s	R	R	R	R	s	s	s	s	s	IN
18	s	s	R	s	s	R	R	R	s	R	s	s	PP
19	s	s	R	s	s	R	R	R	s	s	R	s	IN PP
20	s	s	R	s	R	R	R	R	s	s	s	s	MY TL IN MS CN
21	s	s	R	s	s	R	R	R	s	R	R	s	PP
22	s	s	R	s	R	R	R	R	s	R	s	s	PP
23	s	s	R	s	R	R	R	R	s	s	R	s	BD NP MY TL PP MS CN
24	s	s	s	R	R	R	R	s	R	s	R	s	TL IN
25	s	s	R	s	R	R	R	R	R	s	R	s	TL
26	R	R	R	R	s	R	R	R	s	s	R	R	MY
27	R	R	R	R	R	s	R	R	s	s	R	R	MY

1) Tentative name.
2) BD : Bangladesh, ID : India, NP : Nepal, MY : Myanmar, TL : Thailand, IN : Indonesia, PP : Philippines, MS : Malaysia, CN : China.
3) R : Resistant reaction, s: Susceptible reaction.

Table 3. Percentage of isolates virulent to differential rice cultivars in each country

Country	Differential rice cultivars											
	Kinmaze	Kogyoku	Chugoku 45	IR 8	IR 20	IR 1545-339	DV 85	Java 14	Te-tep	Cas 209	PI 231129	IR 24
Bangladesh	100	100	27	97	78	90	78	27	100	100	60	100
India	100	100	68	100	57	71	23	68	100	100	24	100
Nepal	100	100	88	100	77	53	12	88	100	100	12	100
Myanmar	93	93	30	93	26	22	19	30	100	100	8	93
Thailand	100	100	11	91	3	3	3	11	94	100	63	100
Indonesia	100	100	12	98	88	0	0	12	99	100	48	100
Philippines	100	100	0	100	82	0	0	0	100	43	20	100
Malaysia	100	100	0	100	0	0	0	0	100	100	86	100
China	100	100	50	100	0	1	25	50	100	100	67	100

variety. All the isolates attacked 5 differentials, Kinmaze, Kogyoku, Te-tep, Cas 209 and IR 24, but only 27 % of them were virulent to Chugoku 45 and Java 14 (Table 3). Pathogenicity of the isolates from Bangladesh was very diverse.

India : Fifty-eight isolates collected from India in 1982 and 1983 were divided into 5 races. Among the isolates, isolates belonging to race 16 which predominated in India which were avirulent to IR 20, IR 1545-339, DV 85 and PI 231129. The percentages of isolates virulent to DV 85 and PI 231129 were 23 and 24 %, respectively.

Nepal : Seventeen isolates collected from Nepal in 1983 were divided into 5 races. All the isolates were virulent to Kinmaze, Kogyoku, IR8, Te-tep, Cas 209 and IR24. Of the isolates, however, only two were virulent to DV 85 and PI 231129.

Myanmar : Twenty-seven isolates collected in Myanmar in 1984 were divided into 7 races. Approximately, half of them belonged to race 23. Among the isolates, two isolates MY8417 and MY8429 were avirulent to Kinmaze and IR24. In this study for the first time, the two cultivars were found to harbour a resistance gene. Yamamoto et al.[13] identified *Xa-18* in IR24, Toyonishiki and Milyang 23 using these isolates.

Thailand : Thirty-five isolates collected from Thailand in 1983 were divided into 5 races. Twenty-one belonged to race 20, the most predominant race in Thailand. Only one isolate TL8203 of race 10, was virulent to IR 20, IR 1545-339 and DV 85.

Indonesia : Eighty-seven isolates from Indonesia in 1983 and 1984 were divided into 8 races. Of the 87 isolates tested, 37 belonged to race 19, and 29 belonged to race 15. The virulence of these two races was different only from that to PI231129. None of these isolates could attack IR 1545-339 and DV 85.

Philippines : Sixty-one isolates were collected from the Philippines in 1985 and 1986 and they were divided into 5 races. Twenty-two isolates belonged to race 21, 18 to race 19, 10 to race 18, 9 to race 23 and 2 to race 22, respectively. Cas 209 was resistant to races 18, 21 and 22 which were distributed only in the Philippines. All the Philippine isolates tested were avirulent to Chugoku 45, IR1545-339, DV 85 and Java 14.

Malaysia : Fourteen isolates from Malaysia in 1983 were divided into 2 races. Most of the isolates from Malaysia belonged to race 20, whereas two isolates were classified as race 23.

China : Twenty-four isolates were collected from the southern part of China in 1987 and 1994, and, they were divided into 6 races. Among the isoaltes, seven were classified as race 23, 5 as race 13, 5 as race 20, and 4 as race 6, respectively. Two isolates CN9408 and CN9409 of race 3 from Yuanjiang in Yunnan Province were virulent to all differential cultivars except IR 20.

Discussion

The pathogenic specialization of the causal bacterium of bacterial leaf blight was first reported in Japan[8], since then various papers have appeared on variation in the pathogenicity of the bacterium and resistance of rice cultivars[1, 2, 3, 12, 13].

Variation in the pathogenicity of the causal bacterium obtained from 1982 to 1994 in South East Asian countries was examined in this study. The isolates were divided into 27 races on the basis of their pathogenicity to twelve rice cultivars different in resistance to the disease. Race distribution was specific to each country. Isolates from Bangladesh showed considerable diversity in pathogenicity, while those from Malaysia showed simple pathogenicity. In Bangladesh, Indonesia, Myanmar, China, India, Thailand, Nepal, Philippines and Malaysia, 12, 8, 7, 6, 5, 5, 5, 5 and 2 races, respectively, were detected.

Such complexities have also been reported in Japan[9]. Cultivated cultivars in certain region may be considered a determining factor of race distribution. Japanese race II which is virulent to rice varieties with the resistant gene *Xa-1* was generally found in northern Japan where Kinmaze group cultivars, possessing no resistant genes, are cultivated extensively. Thus, other factors may possibly be associated with race distribution.

Resistance gene of DV 85 didn't operate to most isolates from Bangladesh but it was effective to most isolates from the other countries. Variety Cas 209 was not resistant to any isolate other than those in the Philippines. Kinmaze, IR 8 and IR 24, the most susceptible cultivars so far, showed high degree of resistance to MY8417 and MY8429 isolates from Myanmar, indicating the presence of new resistance gene[12]. All 79 Japonica cultivars were resistant to these two isolates.

A great diversity in pathogenicity of Bangladesh isolates appeared to be caused by the geographical factor that numerous rivers including those from India and Nepal join in this country. In addition, cultivated rice verieties may also affect the race distribution of *X. oryzae* pv. *oryzae*, since the diversity in pathogenicity is smaller in Malaysia and the Philippines than in Myanmar where many native rice varieties are still cultivated.

Bacterial races showed diverse distribution and thus, a genetic and pathological approach to use horizontal resistance should be considered to avoid damage due to the breakdown of race-specific resistance. The host-parasite relationship in bacterial leaf blight should be elucidated in greater detail. Examination should also be made of the physiological and ultrastructural aspects of this relationship.

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東南アジアにおけるイネ白葉枯病菌レースの分布

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摘　要

東南アジア各国からイネ白葉枯病罹病葉を採集し，分離した病原菌株の病原性検定を行ってレースの多様性と各国のレース分布を明らかにした。バングラデシュ，インド，ネパール，ミャンマー，タイ，マレイシア，インドネシア，中国，フィリピンの９カ国から収集した，399菌株について異なる抵抗性遺伝子を持つ日本およびＩＲＲＩの12種のイネ判別品種に接種し，それらの反応から病原性（レース）の判定を行った。

検定した399菌株は27のレースに類別され，本菌の病原性が極めて多様に分化していることが明かとなった。バングラデシュには全ての判別品種に病原性を示すレースを始め，相対的に病原力の強い菌株が多く分布しており，レースの種類も最も多様であった。ミャンマーには病原性範囲の広いレースから最も狭いレースまで多様な病原性を示すレースが分布していた。そのうち，BM8417とBM8429の２菌株はこれまで抵抗性遺伝子を持たないと考えられていた金南風，IR24の２品種に病原性を示さない点で特異であった。フィリピンには５種のレースが分布していたが，いずれも病原性範囲が比較的狭く，類似した反応を示した。マレーシアには２種のレースの分布が確認できただけであった。

バングラデシュは各地域からの河川が合流する地点であり，また，低地で洪水が多く恒常的多発地域である。ミャンマーでは在来品種が多く栽培されているのに対して，マレーシア，フィリピンではIRRI育成品種などの改良品種が多く栽培されている。各国のレース分布には，このような地理的条件や栽培品種が影響する可能性が示唆された。

キーワード：*Xanthomonas oryzae* pv. *oryzae*，レース，白葉枯病菌，イネ