Phenotypic Diversity of *Xanthomonas oryzae* pv. *oryzae* in Nepal

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ABSTRACT

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Fifty-three strains of *Xanthomonas oryzae* pv. *oryzae* collected from various rice-growing areas in Nepal were characterized for differences in biochemical and physiological characteristics, bacteriocin production, and virulence. Numerical analysis of phenotypic features revealed that all strains were associated at 82.4% similarity of coefficient (S_{sm}) in the dendrogram. No distinct phenons were observed, suggesting homogeneous populations of *X. o. oryzae*. Forty-four percent of the strains produced bacteriocin, and six bacteriocin groups were distinguished. Inoculation of *X. o. oryzae* strains on five IRRI differential rice cultivars (IR8, IR20, IR1545-339-2-2, Cas209, and DV85) and three Nepalese rice cultivars (Himali, Laxmi, and Sabitri) demonstrated significant cultivar–strain interaction. As a result, nine pathogenic races of *X. o. oryzae* were detected. Race 1 isolated from Nepal was similar to race 6 of the Philippines *X. o. oryzae*. No relationship was observed between virulence and physiological characteristics or bacteriocin production.

Additional keywords: bacterial blight of rice, differential interaction, epidemiology, *Oryza sativa*, pathogen variation

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (ex Ishiyama) Swings et al (35), is one of the most destructive diseases of rice in Nepal (1). The disease was reported as early as 1968 in Kathmandu Valley (14); however, it spread rapidly in subsequent years and is now prevalent throughout the Terai belts (plain) and hills. The occurrence of the disease in eastern Terai is usually early in the season (1). Major epidemics were reported in 1979 (12) and in 1987 (1). Yield losses, estimated up to 26%, have been recorded, with the greatest destruction being during the "kresek phase" of the disease, usually occurring 2–3 wk after transplanting (1,12).

Considerable variation has been observed among the strains of *X. o. oryzae* in other rice-growing countries in relation to biochemical and physiological characteristics (9,30,38,39), virulence (8,19,23), bacteriocin production (28), monoclonal antibodies (3,27), and restriction fragment length polymorphism (RFLP) (2,16,17). In addition, attempts to classify strains by biochemical and other vitro tests have not resulted in consistent groupings that relate to virulence. A set of differential rice cultivars with specific genes for resistance has been used to identify pathogenic races of *X. o. oryzae* (6,18). As a result, six pathogenic races of *X. o. oryzae* have been described in the Philippines (18,19,21). Twenty-one genes for resistance to *X. o. oryzae* have been identified worldwide and are being used in rice breeding programs.

In developing long-term strategies for BB disease management, it is important to assess variation in virulence of *X. o. oryzae* populations. Pathogenic variability of *X. o. oryzae* in Nepal has not been ascertained, and information on physiological and pathological characteristics are unknown. The objectives of this study were to characterize the strains of *X. o. oryzae* phenotypically and to demonstrate the occurrence of differential interactions between cultivars of rice and strains of *X. o. oryzae* indigenous in Nepal. The term "virulence" is used to differentiate pathogenicity, which is the ability of strains of *X. o. oryzae* to cause disease on a particular rice cultivar. "Phenotypic diversity" herein refers to relative differences in virulence or other attributes among strains of *X. o. oryzae*. A "race" is a group of strains that evokes a particular combination of susceptible (compatible) and resistant (incompatible) reactions when tested on a standard set of differential host cultivars (19).

MATERIALS AND METHODS

Bacterial strains. The strains of *X. o. oryzae* were collected from major rice-growing districts in Nepal between September 1987 and November 1989 (Table 1). Each strain was isolated from a portion of the leaf showing a typical BB lesion. A single colony of each strain was selected from the peptone sucrose agar (PSA) (24) plates and incubated at 28 C for 3 days. The cultures were maintained in 5% skim milk at 4 C and transferred regularly during the course of the experiments. For long-term storage, the strains were kept on 15% glycerol at −80 C or as lyophilized cultures and maintained at the departments of plant pathology at the University of Hawaii and Kansas State University.

Biochemical and physiological tests. The 53 strains used in this study were grown on PSA slants for 24 hr. Inoculum of each strain was prepared by suspending it in 10 ml of sterile distilled water and standardizing to 10^8 cfu/ml using a spectrophotometer. Uninoculated media were included in all tests to serve as controls. Unless otherwise specified, Dye's methods (4) were used for phenotypic characterization of *X. o. oryzae* strains. The Gram stain was performed by Huker's modified method (34). Growth of the strains at 5, 10, 20, and 40 C in PSA slant was examined after 7 days. Tolerance of the strains to three NaCl concentrations (1, 3, and 5%) and three glucose concentrations (10, 15, and 20%) was determined by the modified method of Dye on GYEA medium consisting of 1% (w/v) D-glucose, 0.5% (w/v) yeast extract, 3% (w/v) $CaCO_3$, and 2.5% (w/v) agar in distilled water (4). Fermentative and oxidative metabolism of glucose were tested by deep stab inoculations into duplicate tubes (11). Catalase activity, levan production, and nitrate reduction were detected by the methods of Hayward (10). Oxidase activity was tested according to Kovac's method (15). Egg yolk hydrolysis, acetoin production, litmus milk reactions, indole production, gelatinase activity, starch hydrolysis, and hydrogen sulfide production were determined by the methods of Dye (4).

Hydrolysis of Tween 20 [Polyoxyethylene (20) sorbitan monolaureate] and Tween 80 were observed after 6 days, following the method of Sierra (31). Growth of the strains in the presence of four tetrazolium salt concentrations (0.005, 0.01, 0.05, and 0.1%) and tests for 0.01% basic fuchsin and two concentrations of crystal violet (0.001 and 0.05%) were determined on GYEA medium after 7 days (4). Acid production from trehalose, glucose, sucrose, maltose, lactose, and starch were tested in the basal medium containing 0.15 g/L of bromocresol purple indicator (4). For this test, a 1% final solution of each carbohydrate was filter-sterilized, and 5-ml volumes were dispensed into test tubes. Strains were also checked in a

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carbohydrate-free control medium. Final observations were taken 21 days after incubation. Susceptibility of strains toward chloramphenicol (30 μg/ml), sisomicin (10 μg/ml), tetracycline (30 μg/ml), co-trimoxazole (25 μg/ml), gentamicin (10 μg/ml), penicillin (10 μg/ml), ampicillin (10 μg/ml), erythromycin (15 μg/ml), cephalexin (30 μg/ml), cloxacillin (5 μg/ml), and streptomycin (10 μg/ml) was tested in antibiotic disks (SPAN, Diagnostics, Surat, India) on PSA plates. A strain showing an inhibition zone ≥12 mm was recorded as sensitive to the tested antibiotic.

Analysis of phenotypic features. Results obtained from the biochemical and physiological tests were used for the computer-assisted numerical analysis. All tests were scored as positive (coded 2) or negative (coded 1). Missing and doubtful results were coded as 0 (no comparison). Both positive and negative matches were determined in the calculation by the unweighted average pair-group method (33). Similarity coefficients (S_{sm}) were calculated and data were transposed into a dendrogram using a Clustan program (42).

Bacteriocin production. Bacteriocin production was detected by the modified method of Echandi (5). Forty-five strains were randomly selected and used in this study (Table 1). The producer strains were transferred to PSA slants and incubated for 48 hr at 28 C. Strains were transferred from master plates with a multipoint replicator to fresh PSA plates. The bacterial colonies were killed by inverting the plates over 2 ml of chloroform for 60 min. The plates, with the lid off, were then placed in a transfer chamber with continuous air circulation for another 60 min. The indicator strain was prepared by adding 0.2 ml of a suspension (10^8 cfu/ml) to 4 ml of water agar at 40 C and pouring the mixture over the bottom layer of agar. A zone of growth inhibition was considered evidence of bacteriocin production.

Virulence analysis. Thirty-five strains were evaluated for their virulence on eight rice cultivars in the greenhouse of the Institute of Agriculture and Animal Science (IAAS), Rampur, Nepal. The rice cultivars employed were the following five IRRI differential rice cultivars: IR8 (possessing the *Xa-11* resistance gene) (36), IR20 (*Xa-4*) (25), IR1545-339-2-2 (*xa-5*) (henceforth referred to as IR1545) (25), Cas209 (*Xa-10*) (43), and DV85 (*xa-5, Xa-7*) (32). Three Nepalese rice cultivars, Himali, Laxmi, and Sabitri, were selected on the basis of preliminary studies which indicated differential interactions with the bacteria.

Seeds of each rice cultivar were planted in plastic buckets. After 20 days, three uniform seedlings of each rice cultivar were transplanted in 30-cm-diameter clay pots. The strains were subcultured on PSA slants and incubated for 48 hr at 28 C. The 2-day-old culture of each strain was used as inoculum. Inoculum was prepared by suspending the bacterial mass in 10 ml of sterile distilled water and adjusting to 10^8 cfu/ml by a spectrophotometer prior to inoculation. The experimental unit consisted of three plants per pot. Three fully expanded leaves of each plant per pot (total of nine leaves per experimental unit) were

Table 1. Strains of *Xanthomonas oryzae* pv. *oryzae* used in physiological and pathological studies in Nepal, 1989

Strain	Location	Variety source	Race[y]
NXO101	Jhapa	Masuli	ND[z]
NXO117	Dang	Simjira	1
NXO142	Bara	Dedawa	3
NXO146	Bara	Unknown	ND
NXO147	Parsa	Unknown	2
NXO149	Jhapa	B446	2
NXO151	Jhapa	Unknown	5
NXO152	Jhapa	Unknown	1
NXO153	Jhapa	Local	1
NXO156	Bara	Unknown	3
NXO157	Bara	Unknown	1
NXO159	Bara	Unknown	6
NXO160	Parsa	Unknown	ND
NXO161	Parsa	Unknown	ND
NXO163	Bara	Unknown	ND
NXO171	Parsa	Farm-45	1
NXO174	Parsa	Unknown	2
NXO175	Bara	Unknown	1
NXO176	Bara	Local	ND
NXO180	Rautahat	Unknown	2
NXO181	Rautahat	Unknown	7
NXO188	Gorkha	Himali	1
NXO190	Jhapa	Local	2
NXO191	Jhapa	Unknown	1
NXO194	Jhapa	Unknown	ND
NXO195	Dang	Bindeshwori	6
NXO196	Dang	Bindeshwori	ND
NXO197	Dang	Unknown	ND
NXO198	Dang	Unknown	4
NXO199	Dang	Unknown	8
NXO200	Rupendehi	Unknown	1
NXO201	Rupendehi	Local	1
NXO202	Dang	Bindeshwori	1
NXO205	Dang	Local	9
NXO207	Butwal	Unknown	1
NXO208	Dang	Bindeshwori	1
NXO209	Rupendehi	Kalomasino	3
NXO210	Rupendehi	Local	4
NXO211	Rupendehi	Sarju-49	1
NXO212	Rupendehi	Unknown	1
NXO213	Rupendehi	Local	1
NXO214	Rupendehi	Unknown	1
NXO215	Butwal	IR20	1
NXO216	Rupendehi	Kabara	1
NXO228	Syanja	Jarneli	ND
NXO235	Kaski	Local	ND
NXO236	Palpa	Unknown	ND
NXO237	Syanja	Battisara	ND
NXO239	Syanja	Unknown	ND
NXO240	Kaski	Gurdi	ND
NXO244	Palpa	Masuli	ND
NXO245	Palpa	Ampjhutte	ND
NXO253	Tanahua	Kalnakhari	ND
NXO256	Lamjung	Sali	ND
NXO257	Tanahua	Masuli	ND
NXO259	Tanahua	Battisara	ND
NXO261	Jhapa	Masuli	ND
NXO262	Jhapa	Laxmi	ND
NXO263	Jhapa	Janaki	ND
NXO264	Jhapa	Unknown	ND
NXO270	Banke	Salidhan	ND
NXO274	Kailali	Alka	ND
NXO275	Bardia	IR64	ND
NXO282	Banke	Unknown	ND
NXO296	Banke	Tilka	ND

[y] Determined by inoculation to the rice cultivars IR8 (*Xa-11*), IR20 (*Xa-4*), IR1545 (*xa-5*), Cas209 (*Xa-10*), DV85 (*xa-5, Xa-7*), Himali, Laxmi, and Sabitri. Differential reactions induced by races of *X. o. oryzae* on eight rice cultivars are given in Table 3.
[z] ND = not determined.

inoculated with each strain 35 days after sowing (13). The resistance or susceptibility was rated from nine inoculated leaves of each rice cultivar per treatment. Lesion length from the leaf tip was measured (in centimeters) 21 days after inoculation (20). Disease reactions were categorized according to lesion length, where 0–3 cm was classified as resistant (R) and more than 3 cm as susceptible (S). The experiment was arranged in a split plot with the rice cultivar as main plot and the bacterial strain as subplot (7). The entire experiment was performed twice in 1989, and the means of each treatment from the two experiments were averaged to obtain the overall mean. Data on the lesion length of each cultivar–strain combination were analyzed using the SAS analysis of variance (ANOVA) procedure (29). Comparisons of main and interaction effects ($P < 0.01$) between the rice cultivar and the bacterial strain were calculated using Duncan's multiple range test.

RESULTS

Clustering of strains. The phenotypic similarities among the 53 strains of *X. o. oryzae* are illustrated in the dendrogram (Fig. 1). All strains were associated at 82.4% S_{sm}. Thirteen features were positive to all 53 strains investigated; that is, all strains were aerobic; catalase positive; grew at 10, 20, and 30 C; hydrolyzed starch, egg yolk, and Tween 80; produced levan; liquefied gelatin; reduced and peptonized litmus milk; and produced acid from glucose. The following tests were negative to the 53 strains: acetoin production; growth at 5 and 40 C; anaerobic growth; nitrate reduction; oxidase activity; growth in the presence of 0.1% (w/v) tetrazolium chloride salt; hydrolysis of Tween 20; oxidase activity; indole production; alkalinization of litmus milk; acid production from maltose and lactose; growth in the presence of tetracycline (30 μg/ml), penicillin (10 μg/ml), cloxacillin (5 μg/ml); and 0.05% (w/v) crystal violet. Several tests, such as growth on glucose concentrations; basic fuchsin; 0.01% and 0.005% (w/v) tetrazolium salt; acid production from carbohydrate; and susceptibility to sisomicin (10 μg/ml), chloramphenicol (30 μg/ml), co-trimoxazole (25 μg/ml), ampicillin (10 μg/ml), gentamicin (10μg/ml), cephalexin (30μg/ml), streptomycin (10μg/ml), and erythromycin (15 μg/ml) varied with the strains.

Bacteriocin production. Strains differed in bacteriocin production and sensitivity. Based on bacteriocin production and sensitivity patterns of all strains tested, four strains were selected as indicator strains. The indicator strains were used to categorize the 45 strains into six bacteriocin groups (Table 2). Eight strains were in group A, 2 in B, 3 in C, 4 in D, 3 in E, and 25 in E.

Virulence analysis. Differences in virulence as measured by the lesion length on eight rice cultivars were apparent among strains of *X. o. oryzae* (Table 3). No associations between virulence and origin of strain or rice cultivar were observed. Rice cultivars differed in their degree of resistance to *X. o. oryzae*. The three Nepalese rice cultivars, Himali, Laxmi, and Sabitri, showed differential reactions to 18 strains. DV85 was resistant to six strains, Cas209 to one strain, and IR8 to one strain. IR20 and IR1545 were susceptible to all strains.

Using the five IRRI differentials and three Nepalese rice cultivars, the 35 strains were classified into nine pathogenic races (Table 3). Race 1 consisted of 19 strains that were highly virulent on all rice cultivars. Race 2 was composed of five strains that had a virulent reaction on all rice cultivars except Sabitri. Race 3 contained three strains that were avirulent on Laxmi and Sabitri. Race 4 consisted of two strains that were avirulent only on DV85. Race 5 contained only one strain, which was avirulent on Himali but virulent on other rice cultivars. Race 6 was avirulent on DV85 and Laxmi. Race 7 was avirulent on DV85, Laxmi, and Sabitri but virulent on other rice cultivars. Race 8 caused shorter lesions on Cas209, Himali, and Sabitri. Race 9 was avirulent on IR8, DV85, Himali, Laxmi, and Sabitri but virulent on other rice cultivars. Significant ($P < 0.01$) cultivar–strain interaction on lesion length was demonstrated (Table 3).

Table 2. Strains of *Xanthomonas oryzae* pv. *oryzae* used for bacteriocin production in Nepal, 1989

Strain	Indicator strain				Group
	NXO181	NXO211	NXO240	NXO275	
NXO142	±[x]	–[y]	–	–	A
NXO147	±	–	–	–	A
NXO149	±	–	–	–	A
NXO171	+[z]	–	–	–	A
NXO176	+	–	–	–	A
NXO146	±	–	–	–	A
NXO195	±	–	–	–	A
NXO296	±	–	–	–	A
NXO264	±	–	–	±	B
NXO236	±	–	–	±	B
NXO159	+	–	±	–	C
NXO201	±	–	±	–	C
NXO194	+	–	±	–	C
NXO156	+	–	+	±	D
NXO157	±	–	+	±	D
NXO160	+	–	+	–	D
NXO202	±	–	±	±	D
NXO101	+	+	–	+	E
NXO117	±	+	–	+	E
NXO161	+	+	–	±	E
NXO207	–	–	–	–	F
NXO212	–	–	–	–	F
NXO174	–	–	–	–	F
NXO163	–	–	–	–	F
NXO209	–	–	–	–	F
NXO239	–	–	–	–	F
NXO244	–	–	–	–	F
NXO240	–	–	–	–	F
NXO215	–	–	–	–	F
NXO253	–	–	–	–	F
NXO256	–	–	–	–	F
NXO259	–	–	–	–	F
NXO275	–	–	–	–	F
NXO282	–	–	–	–	F
NXO216	–	–	–	–	F
NXO261	–	–	–	–	F
NXO151	–	–	–	–	F
NXO228	–	–	–	–	F
NXO180	–	–	–	–	F
NXO191	–	–	–	–	F
NXO211	–	–	–	–	F
NXO237	–	–	–	–	F
NXO197	–	–	–	–	F
NXO181	–	–	–	–	F
NXO274	–	–	–	–	F

[x] ± = Partial inhibition zone (diameter 0.5–2 mm).
[y] – = No inhibition.
[z] + = Complete inhibition zone (diameter > 2 mm).

DISCUSSION

The phenotypic dendrogram of the 53 strains of *X. o. oryzae* collected in Nepal was structured on biochemical and

physiological characteristics. No gross phenotypic differences among the strains tested were observed, and the results were similar to those obtained in previous studies (26,37,39). Some variations in acid production from trehalose and sucrose, and susceptibility toward antibiotics, were observed; however, the variations did not correlate with location, original host, or virulence. Our results showed that 44% of *X. o. oryzae* strains produced bacteriocin. Differences in bacteriocin production and sensitivity to indicator strains were distinct. No correlation was observed between bacteriocin groups and virulence on rice cultivars or biochemical and physiological characteristics. Similar findings were reported for other plant pathogenic bacteria (5,40,41).

Our results demonstrated highly significant cultivar–strain interaction ($P < 0.01$), confirming pathogenic specialization of *X. o. oryzae* in Nepal. This supports the hypothesis of differences in the populations present in Nepal. The pathogenic specialization of *X. o. oryzae* has also been reported from other rice-growing countries on the basis of specificity on differential rice cultivars (8,9,18, 19,21). Based on reactions on five IRRI differentials and three Nepalese rice cultivars, the 35 Nepalese strains of *X. o. oryzae* were classified into nine races. The strains differed widely in their virulence and should be considered to represent races. Race 1 isolated in this study was similar to race 6 of the Philippines *X. o. oryzae* (18). This study

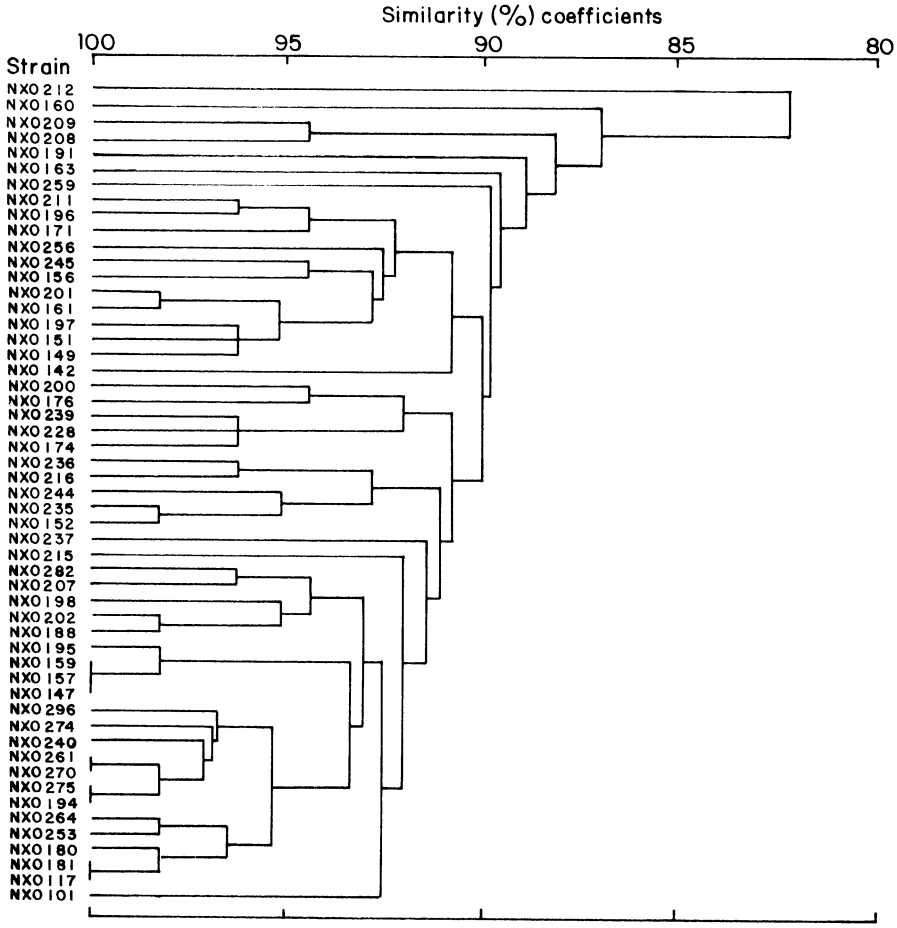

Fig. 1. Dendrogram showing phenotypic similarities among 53 strains of *Xanthomonas oryzae* pv. *oryzae* collected in Nepal. Similarity coefficients were clustered by unweighted average pair-group method.

Table 3. Virulence of representative strains of *Xanthomonas oryzae* pv. *oryzae* on eight rice cultivars[w] planted in the greenhouse, Rampur, Nepal, 1989

Representative strain	Race	Lesion length (cm) on rice cultivar[x]								Total strains	
		IR8 (*Xa-11*)	IR20 (*Xa-4*)	Cas209 (*Xa-10*)	IR1545 (*xa-5*)	DV85 (*xa-5, Xa-7*)	Himali	Laxmi	Sabitri	No.	%
NXO207	1	9.3 ab[y] (S[z])	18.3 ab (S)	20.5 a (S)	16.0 ab (S)	16.0 a (S)	20.2 a (S)	6.0 ab (S)	13.8 a (S)	19	54
NXO149	2	9.9 a (S)	10.0 b (S)	12.8 c (S)	11.3 b (S)	9.2 b (S)	8.9 bc (S)	6.8 ab (S)	0.3 d (R)	5	14
NXO156	3	11.1 a (S)	12.8 b (S)	10.7 c (S)	11.8 b (S)	10.5 ab (S)	12.5 b (S)	0.4 c (R)	1.6 c (R)	3	9
NXO198	4	12.4 a (S)	12.6 b (S)	14.5 bc (S)	10.4 b (S)	0.7 c (R)	10.6 b (S)	5.1 ab (S)	7.1 b (S)	2	6
NXO151	5	8.3 ab (S)	19.2 a (S)	13.0 bc (S)	15.0 ab (S)	14.5 ab (S)	2.6 de (R)	10.0 a (S)	4.4 bc (S)	1	3
NXO159	6	10.7 a (S)	10.3 b (S)	10.1 bc (S)	3.4 c (S)	1.3 c (R)	10.1 b (S)	2.2 b (R)	3.6 bc (S)	2	6
NXO181	7	11.4 a (S)	10.6 b (S)	14.1 bc (S)	12.9 b (S)	1.5 c (R)	11.8 b (S)	0.1 c (R)	0.8 d (R)	1	3
NXO199	8	3.4 c (S)	11.2 b (S)	0.1 e (R)	3.7 c (S)	14.4 ab (S)	0.1 e (R)	3.5 b (S)	1.6 c (R)	1	3
NXO205	9	2.1 c (R)	14.6 ab (S)	13.8 bc (S)	7.0 bc (S)	0.4 c (R)	0.6 e (R)	0.3 c (R)	2.4 bc (R)	1	3

[w] Experimental unit consisted of three plants per pot. Three fully expanded leaves of each plant per pot (total nine leaves/experimental unit) were clip inoculated with each strain of *X. o. oryzae* 35 days after sowing. Each treatment was replicated twice. Data are overall means of two independent experiments.

[x] Lesion length (<3 cm = resistance [R], and >3 cm = susceptible [S]) was measured 21 days after inoculation.

[y] Strain–cultivar interaction = 3.0** (highly significant at $P < 0.01$).

[z] R = resistant, and S = susceptible. Race was determined by inoculation to the five IRRI differentials, IR8 (*Xa-11*), IR20 (*Xa-4*), IR1545 (*xa-5*), Cas209 (*Xa-10*), and DV85 (*xa-5, Xa-7*), and three Nepalese rice cultivars, Himali, Laxmi, and Sabitri, as described by Mew (18).

indicated that the strains of *X. o. oryzae* collected from Terai (plain) regions of Nepal were more virulent on rice cultivars than were the strains of *X. o. oryzae* isolated from hills and valleys. The disease survey results also suggested that many rice cultivars, such as Masuli, Bindeshwori, CH-45, and Sarju-49, were highly susceptible to *X. o. oryzae* in Terai regions of Nepal (1). The occurrence of the disease in Terai could coincide with the introduction of the rice cultivars susceptible to *X. o. oryzae* from 1970 to 1980 and with the increase in double cropping. The combined effects of host, environment, and cultural practices possibly exerted a high selection pressure on the pathogen. Consequently, the disease became severe due to rapid adaptation of the pathogen to a particular host cultivar (22).

The results suggested that the strains of *X. o. oryzae* from Nepal are highly virulent on most of the resistance genes that are presently used in rice breeding at IRRI. High susceptibility of IR20 (*Xa-4*), IR1545 (*xa-5*), and DV85 (*xa-5, Xa-7*) to *X. o. oryzae* has been reported from India (8). Recently, RFLP analysis and monoclonal antibodies have been used to compare strains of *X. o. oryzae* from rice-growing countries in Asia. A high degree of DNA polymorphism was detected on strains of *X. o. oryzae* from Nepal and India compared to strains from China, Indonesia, Malaysia, Korea, and the Philippines (2). In addition, serologically distinct groups of strains were found in Nepal and India when *X. o. oryzae* was tested with three monoclonal antibodies (27). The apparent pathogenic specialization of *X. o. oryzae* in Nepal complicates the rice-improvement program. Greenhouse inoculation would be useful for selecting and using the most virulent strains in a rice-breeding program for resistance to BB in Nepal.

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