Genetic Diversity of *Xanthomonas oryzae* pv. *oryzae* Strains from Sri Lanka

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ABSTRACT

Ochiai, H., Horino, O., Miyajima, K., and Kaku, H. 2000. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* strains from Sri Lanka. *Phytopathology* 90:415-421.

Sixty strains of *Xanthomonas oryzae* pv. *oryzae*, collected from 29 locations in Sri Lanka in 1995, were analyzed by restriction fragment length polymorphism using either polymerase chain reaction-amplified 16S and 23S rDNA or the repetitive DNA element *IS1112* from *X. oryzae* pv. *oryzae* as hybridization probes. Two different ribogroups were observed in the Sri Lankan strains using rDNA probes, whereas five clusters were identified by the *IS1112* probe. Bootstrap analysis revealed that the five clusters defined by *IS1112* were relatively robust. Our results suggest that the Sri Lankan strains are phylogenetically composed of five different groups.

Each cluster was partially associated with climatic conditions (intermediate zone and wet zone) and was related to groups based on ribotyping. Based on virulence analysis using 12 rice cultivars, each containing a single resistance gene, 14 pathotypes were identified among the Sri Lankan strains. All strains were virulent to resistance genes *Xa1*, *Xa2*, *Xa4*, *Xa10*, *Xa11*, and *Xa14*. Only one strain (pathotype 1) was virulent to all major resistance genes including *Xa21*, while strains of the other pathotypes were all avirulent to *Xa21*. A partial relationship was found between the determined phylogenetic groups using the *IS1112* probe and pathotypes for all but two clusters. The results of this study will facilitate the further understanding of the population structure of *X. oryzae* pv. *oryzae* in Sri Lanka.

Additional keywords: bacterial blight of rice, pathogen populations, phylogeny.

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (33) is one of the most destructive diseases of rice in Asia (25). Resistant rice varieties and pathogenic variation in the bacterium have been studied extensively, because the use of resistant cultivars is the most promising control method against the disease. However, most resistance genes provide effective, stable protection only against subpopulations of the pathogen (23). Changes in race (pathotype) structure within the population may result from several factors such as genetic change (mutation or recombination in response to agricultural or environmental constraints) or migration from other geographic areas. Therefore, to guide the selection and deployment of resistance genes, the population structure of the pathogen needs to be understood. Population structure refers to the amount of genetic diversity within a population, the phylogenetic relationships within and among subpopulations, and the partitioning of variation in time and space (23). The research reported here was undertaken to provide information on the current population structure of this pathogen in Sri Lanka.

In Asia, previous studies concerning the pathotypic and genetic diversity of *X. oryzae* pv. *oryzae* were based on differential interactions with resistance genes (1,2,9-11,13,15,26,28,29), restriction fragment length polymorphism (RFLP) using repetitive DNA elements (insertion sequences) as probes (3,4,17,20,27,42), and polymerase chain reaction (PCR)-based DNA fingerprinting (1,12,36). Furthermore, a survey of race distribution showed temporal and geographic differentiation of pathogen populations in the Philippines (4,26,27). Molecular techniques such as RFLP analyses have also been successfully used to detect genetic variability within populations of *X. oryzae* pv. *oryzae* in the Philippines and other Asian countries (1,3,4,20,27,42). In the Philippines, Leach et al. (20) and Nelson et al. (27) found that groupings based on a repetitive DNA

element (*IS1112*) were the most robust and that some phylogenetic lineages were related to pathotypes (races). Within Asia, Adhikari et al. (3) found that the pathogen population exhibited regional differentiation and suggested that, based on the clustering of strains from different countries, the pathogen migrated between countries.

In Sri Lanka, bacterial blight was first reported in 1958. Since kresak, the wilt phase of the disease, first occurred in the Batalagoda area in 1967, the disease has caused serious damage to rice production. However, Sri Lanka is one of the countries where race distribution and genetic diversity of the pathogen has not previously been studied, although resistance in rice cultivars has been analyzed genetically (39,40). Geographically, Sri Lanka is of interest, since rice on this island grows in diverse climatic conditions (dry, intermediate, and wet zones) at altitudes ranging from sea level to approximately 2,000 meters. Therefore, genetic diversity of the pathogen is expected.

The objectives of this study were to assess the genetic structure of this pathogen in Sri Lanka using two probes (rDNAs and the repetitive DNA element *IS1112*) as an initial approach, to determine the relationship between pathogenic races and phylogeny of the pathogen, and to relate information on the population structure of this pathogen in Sri Lanka to environmental factors.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. Rice leaves naturally infected with *X. oryzae* pv. *oryzae* were collected in farmers' fields from 29 locations in 1995. When more than one strain is listed for the same location, each strain was isolated from a different field. All isolations were performed in Japan under controlled conditions. From each infected leaf sample, three single colonies were isolated and one strain/field was selected as a representative strain for this study. The purified strains were maintained in 10% skim milk at -40°C and as lyophilized cultures. The strains were revived on Wakimoto's agar (32) for DNA isolation and pathogenicity tests. Information on bacterial

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strains, geographic locations, climatic conditions, and host cultivars is shown in Table 1.

Preparation of DNA. Total bacterial DNA was prepared using a modified cetyltrimethylammonium bromide (CTAB) extraction method (5). Briefly, cultures were incubated for 16 h at 28°C in 5 ml of Luria-Bertani medium (per liter: 10 g of Bacto-tryptone, 5 g of Bacto-yeast extract, and 5 g of NaCl [Difco Laboratories, Detroit]). Cells were lysed with sodium dodecyl sulfate (Wako Pure Chemicals Industries, Osaka, Japan), and the resulting lysate was treated with proteinase K (Wako Pure Chemicals Industries). DNA was

TABLE 1. *Xanthomonas oryzae* pv. *oryzae* strains collected from different locations in Sri Lanka

Strain	Location	Climate ^a	Rice cultivar ^b
SL9501	Peradeniya, Kandy District	I	BG34-6
SL9504	Nanu-Oya, Kandy District	I	
SL9508	Nanu-Oya, Kandy District	I	
SL9510	Nanu-Oya, Kandy District	I	
SL9511	Nanu-Oya, Kandy District	I	
SL9514	Kadugannawa, Kandy District	I	
SL9517	Hingula, Kandy District	I	
SL9521	Hingula, Kandy District	I	
SL9523	Hingula, Kandy District	I	
SL9524	Mawanella, Kegalla District	I	
SL9526	Mawanella, Kegalla District	I	
SL9527	Penideniya, Kandy District	I	
SL9528	Penideniya, Kandy District	I	
SL9530	Meewathura, Kandy District	I	BG11
SL9532	Meewathura, Kandy District	I	BG11
SL9533	Koshinna, Kandy District	I	
SL9536	Udukumbura, Kegalla District	I	
SL9539	Udukumbura, Kegalla District	I	
SL9542	Warakapola, Kegalla District	I	
SL9545	Danovita, Kurunegala District	I	BG11
SL9548	Weuda, Kurunegala District	I	
SL9549	Weuda, Kurunegala District	I	
SL9551	Polonnaruwa, Polonnaruwa District	D	
SL9552	Polonnaruwa, Polonnaruwa District	D	
SL9554	Ranweli, Matale District	I	
SL9557	Ranweli, Matale District	I	
SL9561	Palapathwela, Matale District	I	
SL9564	Totagamuwa, Matale District	I	
SL9568	Alawathugoda, Kandy District	D	
SL9571	Alawathugoda, Kandy District	I	
SL9574	Alawathugoda, Kandy District	I	
SL9575	Alawathugoda, Kandy District	I	
SL9577	Akurana, Kandy District	I	
SL9582	Akurana, Kandy District	I	
SL9585	Halbodagama, Nuwara Eliya District	W	H4
SL9587	Halbodagama, Nuwara Eliya District	W	H4
SL9589	Kalupahana, Badulla District	I	BG400-1
SL9592	Kalupahana, Badulla District	I	BG400-1
SL9595	Halpe, Ratnapura District	W	BG94-1
SL9599	Halpe, Ratnapura District	W	BG94-1
SL95100	Halpe, Ratnapura District	W	BG94-1
SL95101	Halpe, Ratnapura District	W	BG94-1
SL95103	Belihul-oya, Ratnapura District	W	BG104
SL95107	Rathpalawinna, Ratnapura District	W	BG94-1
SL95111	Palakand, Ratnapura District	W	BG300
SL95113	Palakand, Ratnapura District	W	BG300
SL95114	Palakand, Ratnapura District	W	BG300
SL95115	Opanyake, Ratnapura District	W	
SL95119	Meegahagoda, Ratnapura District	W	
SL95120	Meegahagoda, Ratnapura District	W	
SL95122	Meegahagoda, Ratnapura District	W	
SL95123	Kuruwita, Ratnapura District	W	
SL95127	Eheliyagoda, Ratnapura District	W	
SL95130	Eheliyagoda, Ratnapura District	W	
SL95133	Kaluaggale, Colombo District	W	
SL95136	Kaluaggale, Colombo District	W	
SL95138	Kaluaggale, Colombo District	W	
SL95139	Polonnaruwa, Polonnaruwa District	D	Mahasana
SL95143	Halpe, Ratnapura District	W	BG94-1
SL95144	Halpe, Ratnapura District	W	BG94-1

^a D = dry zone, I = intermediate zone, and W = wet zone.

^b Blank = cultivar unknown.

purified with a solution of CTAB/NaCl (10% CTAB [Sigma-Aldrich Japan, Tokyo] in 0.7 M NaCl) followed by chloroform/isoamyl alcohol and phenol/chloroform/isoamyl alcohol (Wako Pure Chemicals Industries) extractions. DNA was recovered with isopropanol precipitation and washed in 70% ethanol. The pellet was resuspended in 100 µl of Tris-EDTA buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA, pH 8.0) with 1 µl of ribonuclease A (10 mg/ml; Sigma-Aldrich Japan) and stored at 4°C.

Hybridization probes. PCR-amplified 16S and 23S rDNA and plasmid pJEL101 containing the repetitive DNA element *IS1112* (21) were used as hybridization probes. The pJEL101 probe was used previously to assess genetic diversity within this pathogen in the Philippines and other Asian countries (3,20,42). PCR amplification of rDNA probes was performed with a GeneAmp PCR System 2400 (Perkin-Elmer Applied Biosystems, Tokyo) using the Expand High Fidelity PCR System (Boehringer Mannheim, Tokyo). The PCR reactions were carried out in a 100-µl reaction volume by mixing template DNA with reaction buffer containing 200 µM each of deoxynucleotide triphosphates (Perkin-Elmer Applied Biosystems), 1 µM of each primer, 2 mM MgCl₂, and 2.6 U of polymerase. Amplification followed standard protocol: an initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min; and a final extension at 72°C for 7 min. The PCR primers were designed from the published 16S and 23S rRNA sequences from *Escherichia coli* and *Pseudomonas* sp. (8,14,35). Primers 1F (5'-AGTTTGATCCTGGCTC-3') and 3R (5'-AAGGAGGTGATCCAGCC-3') amplified 16S rRNA genes, and primer 4F (5'-ACTAAGCGTACAGGTGGAT-3') and 6R (5'-TCACGGTTCATTAGTACCGG-3') amplified 23S rRNA genes. PCR-amplified probes were labeled with the ECL direct labeling system (Amersham, Tokyo) according to the manufacturer's instructions and pJEL101 was labeled with photobiotin (Gibco BRL, Tokyo) as described by McInnes et al. (24).

Southern hybridization and detection. Approximately 1 µg of total DNA from each strain was completely digested with each restriction enzyme (Toyobo, Tokyo) at 37°C. For ribotyping, restriction enzymes *EcoRI* and *HindIII* were used. For hybridizing with pJEL101 as a probe, restriction enzymes *BamHI* and *EcoRI* were used. Digested DNA was separated by electrophoresis in 0.7% agarose (Agarose S; Nippon Gene, Tokyo) gels at 2 V/cm for 15 h in 0.5× Tris-borate-EDTA buffer (0.045 M Tris-borate and 0.001 M EDTA). Separated DNA fragments were transferred to a nylon membrane (Hybond-N or Hybond-N+, Amersham) using a

Fig. 1. Restriction fragment length polymorphism (RFLP) analysis of Sri Lankan strains of *Xanthomonas oryzae* pv. *oryzae*. Southern blots were probed with polymerase chain reaction-amplified 16S or 23S rDNA. Sizes (in kilobases) are indicated on the left. Lane numbers refer to the RFLP patterns given in Table 2. **A**, *HindIII*-digested DNA was probed with 16S rDNA. **B**, *EcoRI*-digested DNA was probed with 23S rDNA.

vacuum blotting system (VacuGene XL; Pharmacia Biotech, Tokyo) according to the manufacturer's protocol. For probes labeled with the ECL system, hybridization and detection procedures followed the manufacturer's instructions (Amersham). The blots were exposed to XAR5 X-ray films (Eastman Kodak, Rochester, NY) at

room temperature. When probes were labeled with photobiotin, the BluGENE system (Gibco BRL) was used for hybridization and detection according to the manufacturer's instruction.

RFLP data analysis. The banding pattern (haplotype) of each strain was coded in binary form by scoring the presence or ab-

TABLE 2. Restriction fragment length polymorphism (RFLP) and pathogenicity analysis of *Xanthomonas oryzae* pv. *oryzae* strains from Sri Lanka

Strain	RFLP type no.								Pathotype ^c
	rDNA ^a				IS1112 ^b				
	16S	23S	Ribotype	Ribogroup	B	E	Haplotype	Cluster	
SL9501	1	1	r1	R1	1	1	1	A	6
SL9504	2	2	r4	R2	2	2	6	C	5
SL9508	1	1	r1	R1	1	4	2	A	6
SL9510	2	1	r3	R2	3	3	11	D	2
SL9511	2	1	r3	R2	3	5	12	D	3
SL9514	2	1	r3	R2	3	5	12	D	3
SL9517	2	1	r3	R2	3	5	12	D	3
SL9521	2	1	r3	R2	3	5	12	D	3
SL9523	2	1	r3	R2	3	5	12	D	3
SL9524	2	1	r3	R2	4	5	13	D	3
SL9526	1	1	r1	R1	1	1	1	A	4
SL9527	2	1	r3	R2	5	6	14	D	3
SL9528	2	2	r4	R2	2	7	7	C	5
SL9530	2	2	r4	R2	2	8	8	C	5
SL9532	2	2	r4	R2	2	22	10	C	5
SL9533	1	1	r1	R1	1	4	2	A	6
SL9536	1	3	r2	R1	6	10	16	B	7
SL9539	1	3	r2	R1	6	10	16	B	8
SL9542	1	1	r1	R1	1	1	1	A	4
SL9545	1	1	r1	R1	1	11	3	A	4
SL9548	1	3	r2	R1	7	12	18	B	8
SL9549	1	3	r2	R1	7	12	18	B	8
SL9551	1	3	r2	R1	6	12	17	B	7
SL9552	1	3	r2	R1	8	12	19	B	8
SL9554	1	3	r2	R1	6	12	17	B	8
SL9557	1	3	r2	R1	6	12	17	B	8
SL9561	1	1	r1	R1	9	4	20	A	4
SL9564	1	3	r2	R1	6	10	16	B	8
SL9568	2	2	r4	R2	10	13	21	C	5
SL9571	2	1	r3	R2	17	14	30	D	3
SL9574	2	2	r4	R2	2	13	9	C	9
SL9575	2	1	r3	R2	17	14	30	D	3
SL9577	1	3	r2	R1	11	15	22	B	7
SL9582	1	3	r2	R1	11	15	22	B	8
SL9585	2	2	r4	R2	10	13	21	C	5
SL9587	1	3	r2	R1	6	10	16	B	8
SL9589	2	1	r3	R2	12	16	23	E	1
SL9592	2	1	r3	R2	13	17	24	E	5
SL9595	2	1	r3	R2	13	18	25	E	10
SL9599	2	2	r4	R2	14	19	27	A	5
SL95100	2	2	r4	R2	14	19	27	A	7
SL95101	2	1	r3	R2	17	14	30	D	3
SL95103	2	2	r4	R2	15	13	28	C	9
SL95107	1	1	r1	R1	9	4	20	A	12
SL95111	1	1	r1	R1	9	4	20	A	14
SL95113	1	3	r2	R1	6	9	15	B	8
SL95114	1	1	r1	R1	9	4	20	A	14
SL95115	2	1	r3	R2	18	5	31	D	3
SL95119	1	1	r1	R1	1	19	4	A	12
SL95120	2	1	r3	R2	13	18	25	E	11
SL95122	1	1	r1	R1	1	19	4	A	12
SL95123	1	1	r1	R1	1	23	5	A	6
SL95127	1	1	r1	R1	9	4	20	A	12
SL95130	1	1	r1	R1	1	19	4	A	4
SL95133	2	1	r3	R2	13	20	26	E	11
SL95136	1	1	r1	R1	1	19	4	A	6
SL95138	2	1	r3	R2	13	20	26	E	9
SL95139	1	3	r2	R1	16	21	29	B	8
SL95143	1	1	r1	R1	1	4	2	A	13
SL95144	2	2	r4	R2	2	2	6	C	9

^a The 16S and 23S columns indicate the RFLP patterns given in Figure 1A and B. Ribotype was determined by the combination of RFLP patterns with 16S and 23S rDNA as probes. Ribogroup corresponds to RFLP cluster in Figure 2.

^b The B and E columns indicate the RFLP patterns obtained with *Bam*HI and *Eco*RI, respectively, presented in Figure 3A and B. The IS1112 type (haplotype) was determined by the combination of RFLP patterns obtained from the two enzymes. Cluster types correspond to RFLP cluster in Figure 4.

^c Pathotype was defined by Table 3.

sence of each band. To confirm band identities and differences in unique banding patterns, samples to be compared were electrophoresed in the same gel. In hybridizations with pJEL101, low intensity bands and fragments ≥ 4 kb in size were not considered, because they were ambiguous and difficult to compare between strains. Therefore, data obtained with two restriction enzymes for bands ≤ 4 kb in size were combined to discriminate haplotypes. This was easier and more reliable to use for scoring each strain. When using two different probes, rDNA and pJEL101, the analysis was performed separately for each probe. Pairwise distances for each RFLP data set were computed with the Dice similarity coefficient using the WinDist program (41). Dendrograms were constructed from the distance matrix data by UPGMA using the NEIGHBOR and DRAWGRAM program in the PHYLIP package (version 3.5c; J. Felsenstein, Department of Genetics, University of Washington, Seattle). The confidence limits of dendrograms were determined by bootstrap analysis with 1,000 replications using the WinBoot program (41).

Pathogenicity tests. To compare quantitative differences in virulence of the strains within a given cluster or among the five putative clusters defined by the *IS1112* probe, all strains were evaluated for virulence on the following 11 near-isogenic lines and one cultivar, each carrying a specific resistance gene: near-isogenic lines IRBB1 (*Xa1*), IRBB2 (*Xa2*), IRBB3 (*Xa3*), IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB7 (*Xa7*), IRBB8 (*xa8*), IRBB10 (*Xa10*), IRBB11 (*Xa11*), IRBB13 (*xa13*) (30,31), IRBB21 (*Xa21*) (16,19); and one cultivar, Taichung Native 1 (*Xa14*) (34). IR24 was used as a susceptible check. Seeds of rice cultivars were sown in plastic boxes, and 3 weeks later, seedlings were transplanted to 30-cm-diameter plastic pots. Rice plants were grown under greenhouse conditions for 3 months. Bacterial suspensions for inoculations were prepared in 10 ml of sterile distilled water at 10^8 CFU/ml. To test the virulence of the strains, plants with fully expanded leaves were inoculated by the leaf-clipping method (18). Twenty leaves per cultivar were inoculated with each strain. Lesion lengths on inoculated leaves were measured at 21 days after inoculation. Disease reactions were categorized according to lesion lengths as described by Adhikari et al. (1). The pathogenicity tests were replicated three times.

RESULTS

Ribotyping with PCR-amplified 16S and 23S rDNA probes.

In the analysis employing the 16S rDNA probe, restriction enzyme *Hind*III was used to digest total DNA, and two different patterns were obtained among the strains tested. In the analysis with the

Fig. 2. Dendrogram constructed with UPGMA on the basis of the combined restriction fragment length polymorphism data sets using 16S and 23S rDNA probes. Numbers refer to the ribotype numbers given in Table 1. The numbers on the branches of the dendrogram indicate the percent bootstrap values for 1,000 replicates.

23S rDNA probe, restriction enzyme *Eco*RI was used to digest total DNA, and three different patterns were observed. Each pattern is shown in Figure 1A and B, and the pattern number corresponding to each strain is given in Table 2. From the combined results of analyses with the 16S and 23S rDNA probes, the Sri Lankan strains can be divided into four ribotypes named r1, r2, r3, and r4 (Table 2). The number of strains in each ribotype was about equal. Cluster analysis based on the combined RFLP data set resulted in two major ribogroups designated R1 and R2 (Fig. 2). Ribogroup R1 was composed of ribotypes r1 and r2, and ribogroup R2 consisted of ribotypes r3 and r4. Bootstrap analysis was used to provide an indication of the degree of support for these ribogroups (Fig. 2). The bootstrap values for each cluster were 84.5 and 85.5%, respectively, which indicates that, although the clusters were not strong, each ribogroup was clustering at the 0.1 distance level ($>90\%$ similarity level).

RFLP analysis with the *IS1112* repetitive DNA element. All the strains were examined for DNA polymorphisms using plasmid pJEL101, which contains the *IS1112* repetitive DNA element, as a probe. Hybridization profiles with each of the two enzymes are shown in Figure 3A and B. Each haplotype was defined using the combined data of the hybridizing bands ≤ 4 kb in size (Table 2). After *Bam*HI digestion, the probe hybridized with 6 to 11 fragments per strain, a total of 20 bands was scored, and 18 different patterns were observed (Fig. 3A). After *Eco*RI digestion, the probe hybridized with 6 to 16 fragments per strain, a total of 35 bands was scored, and 23 different patterns were observed (Fig. 3B). When data from each enzyme were combined, a total of 31 RFLP types were detected (Table 2). Based on the combined data for both restriction enzymes, a dendrogram was constructed using

Fig. 3. Southern hybridization profiles of A, *Bam*HI- and B, *Eco*RI-digested DNA of Sri Lankan strains of *Xanthomonas oryzae* pv. *oryzae* using the photo-biotin-labeled pJEL101 probe. Lane numbers refer to the restriction fragment length polymorphism types given in Table 2. Sizes (in kilobases) are indicated on the left.

UPGMA. Bootstrap analysis provided a measure of the clusters' relative robustness (Fig. 4). The Sri Lankan strains were divided into five clusters named A, B, C, D, and E (Fig. 4), and the percentage of strains belonging to these clusters were 31.7% ($n = 19$), 23.3% ($n = 14$), 15.0% ($n = 9$), 20.0% ($n = 12$), and 10.0% ($n = 6$), respectively. The bootstrap values for the internal branches of clusters C, D, and E were 100, 97.8, and 99.6%, respectively, indicating that these are strong clusters. However, the internal branches of clusters A and B were 54.5 and 82.9%, indicating that the interrelationship among strains in these clusters was not clearly established, although the bootstrap value for the branch with these clusters (91.0%) showed that these clusters were robust. Clusters A and B corresponded to ribogroup R1, whereas clusters C, D, and E corresponded to ribogroup R2. Two strains, SL9599 and SL95100, belonging to cluster A were exceptions; they belonged to ribogroup R2. Our results suggest that the Sri Lankan strains are phylogenetically composed of five different groups.

Pathogenicity analysis. Sixty strains from various locations in Sri Lanka were evaluated for virulence on 11 near-isogenic lines and one cultivar, Taichung Native 1, each carrying a specific resistance gene: *Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa21*, and *Xa14*, respectively. All strains were virulent to IRBB1, IRBB2, IRBB4, IRBB10, IRBB11, and cv. Taichung Native 1 with resistance genes *Xa1*, *Xa2*, *Xa4*, *Xa10*, *Xa11*, and *Xa14*. Only one strain, SL9589, was virulent to IRBB21 with the *Xa21* gene. Based on the virulence analysis, 14 pathotypes were detected among the Sri Lankan strains tested (Table 3). The strain of pathotype 1 was virulent to all major resistance genes tested, including *Xa21*, while strains of the other pathotypes were all avirulent to *Xa21*. Strains of pathotype 3, one of the major groups, were virulent to all major resistance genes except *Xa21*. All but two strains belonging to pathotypes 2 and 10 were virulent to IRBB13 with the *xa13* gene. Strains in other pathotypes were classified by virulence to IRBB3, IRBB5, IRBB7, and IRBB8 with resistance genes *Xa3*, *xa5*, *Xa7*, and *xa8*.

Association between phylogenetic groups (clusters) and pathotypes. A partial relationship was found between the phylogenetic groups determined using the *IS1112* probe and the pathotypes based on virulence against the 12 resistance genes for all but two clusters. Three clusters consisted of strains of only two pathotypes: cluster B contained only pathotypes 7 and 8, cluster C contained only pathotypes 5 and 9, and cluster D contained only pathotypes 2 and 3. The two pathotypes in each cluster differed in their reaction to one resistance gene: *xa5* for cluster B, *xa8* for cluster C, and *xa13* for cluster D. The other two clusters, A and E, were heterogeneous groups and contained strains of more than five pathotypes.

When the strains were grouped according to climatic conditions (dry, intermediate, and wet zones) in Sri Lanka, a partial correlation was found. Strains belonging to clusters B, C, and D were mainly found in the intermediate zone, while those of cluster E were found in the wet zone. However, strains of cluster A were found in both zones, and the proportion of strains in each zone was about equal.

DISCUSSION

Ribotyping with the 16S + 23S rRNA probes from *E. coli* has been used to assess genetic diversity and geographic distribution within and among *X. campestris* pathovar(s) (6,7,37). Verdier et al. (37) reported that ribotypes delineated geographic groups within *X. campestris* pv. *manihotis* collected from Africa and South America. Based on our ribotyping among Sri Lankan strains, two different ribogroups (R1 and R2) were observed and the number of strains of each ribogroup was almost equal. Bootstrap analysis indicated that they were not strong groups, but the level of similarity within each group was >90%. Thus, we conclude that Sri Lankan strains are composed of two major groups. The two groups did not

have a distinct geographic location in Sri Lanka, which may reflect that the strains were collected at a single time, and only 60 strains were examined. When we examined strains from India, they were mainly ribogroup R1 (H. Ochiai, unpublished data). Adhikari et al. (3) grouped strains from different Asian countries using probes *IS1112* and *avrXa10* and suggested that the pathogen migrated within Asia. George et al. (12) also showed that some strains from Indonesia and the Philippines were very similar using PCR- and RFLP-based fingerprinting of the insertion sequence. The same haplotypes were widespread in these two countries. Our ribotyping results suggest that strains of ribogroup R2 are indigenous to Sri Lanka and that strains of ribogroup R1 might have migrated from India. More information is needed to clarify this possibility.

RFLP analysis with the repetitive DNA element revealed a high level of polymorphism in the Sri Lankan strains. These results are similar to those of other studies (3,20,27,42). As the *IS1112* probe

Fig. 4. Dendrogram constructed with UPGMA on the basis of the combined rDNA and *IS1112* restriction fragment length polymorphism data sets. The *IS1112* type (haplotype), pathotype, and ribogroup numbers correspond to those in Table 2. The numbers on the main branches of the dendrogram indicate the percent bootstrap values for 1,000 replicates.

TABLE 3. Pathogenicity analysis of the 60 strains of *Xanthomonas oryzae* pv. *oryzae* on the 12 rice cultivars containing a single gene for resistance^a

Pathotype	No. of strains	Rice cultivars (known resistance genes)											
		IRBB1 (<i>Xa1</i>)	IRBB2 (<i>Xa2</i>)	IRBB3 (<i>Xa3</i>)	IRBB4 (<i>Xa4</i>)	IRBB5 (<i>xa5</i>)	IRBB7 (<i>Xa7</i>)	IRBB8 (<i>xa8</i>)	IRBB10 (<i>Xa10</i>)	IRBB11 (<i>Xa11</i>)	TN 1 ^b (<i>Xa14</i>)	IRBB21 (<i>Xa21</i>)	IRBB13 (<i>xa13</i>)
1	1	S	S	S	S	S	S	S	S	S	S	S	S
2	1	S	S	S	S	S	S	S	S	S	S	R	R
3	11	S	S	S	S	S	S	S	S	S	S	R	S
4	5	S	S	S	S	R	S	S	S	S	S	R	S
5	8	S	S	R	S	S	S	S	S	S	S	R	S
6	5	S	S	R	S	R	S	S	S	S	S	R	S
7	4	S	S	S	S	S	S	R	S	S	S	R	S
8	11	S	S	S	S	R	S	R	S	S	S	R	S
9	4	S	S	R	S	S	S	R	S	S	S	R	S
10	1	S	S	R	S	R	S	R	S	S	S	R	R
11	2	S	S	R	S	R	S	R	S	S	S	R	S
12	4	S	S	S	S	R	R	S	S	S	S	R	S
13	1	S	S	R	S	R	R	S	S	S	S	R	S
14	2	S	S	S	S	R	R	R	S	S	S	R	S

^a Pathogenicity tests were performed and scored as described in text. R = resistant and S = susceptible.

^b TN 1 = cv. Taichung Native 1.

gives a large number of bands per strain, it provides useful information to assess genetic relationships. Genetic analysis showed that the Sri Lankan strains consist of five groups that proved to be relatively robust by bootstrap analysis. Based on the two genetic analyses, the results suggest that Sri Lankan strains of *X. oryzae* pv. *oryzae* are broadly composed of two different subpopulations.

To investigate pathotype diversity of the Sri Lankan strains, we analyzed virulence using the 12 resistance genes. Recently, Nepalese strains of *X. oryzae* pv. *oryzae* were evaluated for virulence on a series of 11 near-isogenic lines, each carrying a single resistance gene, and 26 pathotypes were identified by Adhikari et al. (1). Our results showed that 14 pathotypes were detected, and Sri Lankan strains were also diverse. Comparing pathotypes between Nepal and Sri Lanka indicated that only five pathotypes were similar to those of Nepalese strains, although we used rice cv. Taichung Native 1 with the resistance gene *Xa14*. Sri Lankan pathotypes 1, 4, 7, 12, and 14 corresponded to Nepalese pathotypes 1, 20, 26, 10, and 11, respectively, suggesting that the pathogen population differs. In this study, we conducted pathogenicity tests including the *Xa21* gene that conferred resistance to strains of *X. oryzae* pv. *oryzae* from many countries (38). Recent studies showed that strains virulent to this gene were widely distributed in Korea (22) and Nepal (1). However, only one strain virulent to *Xa21* has been detected in our limited samples from Sri Lanka.

On the basis of cluster analysis derived from hybridization to *IS1112*, a partial association was found between phylogenetic groups and pathotypes. Adhikari et al. (1) observed a partial relationship between the groups determined by PCR-based fingerprinting and virulence, but the molecular polymorphism was largely independent of virulence. The transposable element used in these studies was assumed to be phenotypically neutral and suitable for phylogenetic analysis (27). To understand pathogen evolution and differentiation, further research is necessary.

We have evaluated genetic diversity in the *X. oryzae* pv. *oryzae* population in Sri Lanka as an initial step to understanding the pathogen population structure. Although our sample size was limited, the results indicated diversity in the population structure for much of Sri Lanka. Additional samples are needed to fully understand the population structure. Combined with information already published for other parts of Asia on the population structure of *X. oryzae* pv. *oryzae*, a more comprehensive understanding of this pathogen is emerging.

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