Genetic Diversity within the Population of *Xanthomonas oryzae* pv. *oryzae* in India

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ABSTRACT

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Xanthomonas oryzae pathovar *oryzae* causes a serious disease of rice in India and is endemic in all of the major rice-growing areas of the country. Sixty-seven *X. oryzae* pv. *oryzae* strains, collected mostly in 1994 and 1995, from 18 locations in India were analyzed by DNA fingerprinting methods using two separate repeat element probes from the *X.*

oryzae pv. *oryzae* genome. These results show that strains belonging to a single pathogen lineage can be isolated from 16 of the 18 locations sampled; many of these locations are separated from each other by hundreds of kilometers and represent ecologically diverse rice-growing areas. Pathotyping analysis indicated that the strains in this lineage belong to pathotype 1b of *X. oryzae* pv. *oryzae*.

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

Xanthomonas oryzae pathovar *oryzae* (25) causes bacterial leaf blight, a serious disease of rice. Since the first reports of the pathogen in India (2,23), it has been found in all of the major rice-growing areas (24). Variation in the spectrum of virulence among different strains has been reported (3,5,11,20). Pathotype studies conducted under the All India Coordinated Rice Improvement Project during the last 20 years indicate that pathotypes 1a and 1b are widely distributed in India (18,19). These two pathotypes exhibit a similar virulence spectrum and are unable to cause disease on rice cultivars BJ1 and DV85. They are distinguished from each other on the basis of their reaction pattern on the IR20 rice cultivar, which carries the *Xa-4* disease-resistance gene. Pathotype 1a strains are incompatible on IR20, whereas pathotype 1b strains are compatible. However, race studies based on differential cultivars may not indicate the true level of genetic diversity within a pathogen population. Molecular techniques such as restriction fragment length polymorphism (RFLP) have provided new tools to more accurately assess the genetic diversity of pathogen populations and have been used extensively for this purpose.

RFLP analyses have been successfully used to detect genetic variability within populations of *X. oryzae* pv. *oryzae* in the Philippines (14,16) and in a broad survey of strains from different countries in Asia (1). We have initiated RFLP studies, with probes that were used in these earlier studies, to assess the population structure of this pathogen in India. The results reported here indicate that a single lineage consisting of closely related *X. oryzae* pv. *oryzae* strains is present in widely separated locations within India. The reactions of these strains on rice differential cultivars are similar to those of pathotype 1b.

MATERIALS AND METHODS

Strain collection. Strains of *X. oryzae* pv. *oryzae* were isolated from naturally infected rice plants collected from 18 widely dis-

persed geographical locations within India (Fig. 1). Information on bacterial strains, geographical locations, host cultivars, and year of collection are listed in Table 1. The strains were collected by A. P. K. Reddy in collaboration with other scientists in the All India Coordinated Rice Improvement Project. The locations that are listed in Table 1 are the base sites from which farmers' fields in the neighboring villages were surveyed. A few infected leaf samples were collected at random from these fields. The names of these villages and their geographical locations are available upon request. From each village, isolations were made and one strain/village was maintained as a representative strain. When multiple strains were referred from one location, each strain was obtained from a different village in the surveyed area. Bacterial strains collected from the Taichung Native-1 (TN-1) rice cultivar were not obtained from farmers' fields, but from the campuses of agricultural research stations. BXO4, BXO5, and BXO6 were collected from farmers' fields and provided by C. L. Raina, Agricultural Research Station, Kapurthala. BXO2 and BXO3 were provided by V. S. Trimurthy, Indira Gandhi Agricultural University, Raipur.

Growth of bacteria. All the strains of *X. oryzae* pv. *oryzae* were isolated and routinely grown on peptone sucrose agar (8,26) at 28°C. For long-term storage, strains were grown in peptone sucrose and frozen at -70°C in 20% glycerol. All the strains collected were test-inoculated onto the susceptible TN-1 rice cultivar to confirm their pathogenicity.

Hybridization probes. Two different repeat elements from the *X. oryzae* pv. *oryzae* genome were used as hybridization probes—the avirulence gene probe called *avrXa10* (9) and the insertion element probe IS1112 (14). These probes have been previously used to detect genetic variability within the population of this pathogen in the Philippines and other locations in Asia (1,13,16).

DNA isolation and Southern hybridization. Bacteria were grown for 18 h in peptone sucrose medium on a rotary shaker at 28°C, and genomic DNA was isolated according to the protocol of Leach et al. (14) and stored at 4°C. Genomic DNA was digested with restriction endonucleases *Bam*HI or *Eco*RI (New England Biolabs Inc., Beverly, MA) for 3 h at 37°C. Three micrograms of completely digested DNA from each strain were separated electrophoretically on 0.7% agarose gels, denatured, neutralized, and

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vacuum-transferred to Hybond N (Amersham Life Science, Buckinghamshire, England) membranes according to the procedure given by Sambrook et al. (21). DNA was cross-linked to membranes using an UV Stratalinker (Stratagene Inc., La Jolla, CA). Blots were prehybridized in a solution of 0.5 M sodium phosphate (pH 7.2), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, and 1 mM EDTA for 3 h at 65°C. Probes were labeled with $\alpha^{32}\text{P}$ -dATP and hybridized for 18 h at 65°C with constant shaking. Blots were washed at 65°C with $2\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), 0.1% SDS, and 5 mM sodium phosphate (pH 7.0) three times for 20 min each and with $0.5\times$ SSC, 0.1% SDS, and 3 mM sodium phosphate buffer (pH 7.0) three times for 20 min each. Autoradiography was done with X-ray film at -70°C . A kilobase DNA ladder (Stratagene Inc.) was added to all the gels as a marker. After transfer, the membrane was cut, and the lane carrying the marker was hybridized separately.

RFLP analysis. The haplotype (banding pattern) of each strain obtained by Southern hybridization was compared with all the other strains, and presence or absence of a particular band was recorded as 1 and 0, respectively (4). The data was analyzed by the similarity coefficient of Nei and Li (15): $(2N_{xy})/(N_x + N_y)$. N_{xy} is the number of bands common to strains x and y ; N_x and N_y are the number of bands in strains x and y , respectively. Dendrograms were prepared by the UPGMA (unweighted pair group method of averages) option of the NEIGHBOR program of PHYLIP (Phylogeny inference package) (7). For each set of data, the program was run 10 to 15 times by randomizing the sequence of input data and using different seed numbers as prescribed (7). Identical clusters of strains were obtained in different runs. The confidence limits of dendrograms were determined by bootstrap analysis with 2,000 replications using the WinBoot program (27). The bootstrap values are expressed as a percentage of these 2,000 replications. The higher the bootstrap value for a particular group, the greater is the confidence that this group is real and not pro-

Fig. 1. Collection sites of *Xanthomonas oryzae* pv. *oryzae* strains from India. Details of the collection sites are provided in Table 1. Filled circles indicate collection sites of strains belonging to a widely distributed lineage (described in Results). Open squares indicate strains that are not part of this lineage.

TABLE 1. *Xanthomonas oryzae* pv. *oryzae* strains collected from rice cultivars grown at different locations in India

Strain	Location	Rice cultivar	Year
BXO1	Chinsuria, West Bengal	NA ^a	1992
BXO2	Raipur, Madhya Pradesh	NA	1991
BXO3	Raipur, Madhya Pradesh	NA	1991
BXO4	Kapurthala, Punjab	PR106	1992
BXO5	Ferozpur, Punjab	PR106	1992
BXO6	Patiala, Punjab	PR106	1992
BXO7	Titabar, Assam	NA	1992
BXO8	Nellore, Andhra Pradesh	NLR9672	1993
BXO9	Jammu & Kashmir	Jaya	1993
BXO10	Cuttack, Orissa	NA	1993
BXO11	Cuttack, Orissa	NA	1993
BXO12	Maruteru, Andhra Pradesh	Krishnaveni	1994
BXO13	Maruteru, Andhra Pradesh	Krishnaveni	1994
BXO14	Maruteru, Andhra Pradesh	Krishnaveni	1994
BXO15	Maruteru, Andhra Pradesh	Krishnaveni	1994
BXO16	Maruteru, Andhra Pradesh	Krishnaveni	1994
BXO17	Pantnagar, Uttar Pradesh	NA	1994
BXO18	Pantnagar, Uttar Pradesh	NA	1994
BXO19	Pantnagar, Uttar Pradesh	NA	1994
BXO20	Pantnagar, Uttar Pradesh	NA	1994
BXO25	Raigad, Maharashtra	Jaya	1994
BXO26	Raigad, Maharashtra	Jaya	1994
BXO27	Raigad, Maharashtra	Jaya	1994
BXO28	Kapurthala, Punjab	PR106	1994
BXO29	Kapurthala, Punjab	PR106	1994
BXO30	Kapurthala, Punjab	PR106	1994
BXO31	Kapurthala, Punjab	PR106	1994
BXO32	Kapurthala, Punjab	PR106	1994
BXO33	Kurukshetra, Haryana	PR106	1994
BXO34	Karnal, Haryana	Basmati	1994
BXO35	Karnal, Haryana	Basmati	1994
BXO36	Karnal, Haryana	Basmati	1994
BXO37	Karnal, Haryana	Basmati	1994
BXO38	Hyderabad, Andhra Pradesh	TN-1 ^b	1994
BXO40	Pusa, Bihar	TN-1	1994
BXO401	Maruteru, Andhra Pradesh	Chaitanya	1995
BXO403	Maruteru, Andhra Pradesh	Krishnaveni	1995
BXO405	Maruteru, Andhra Pradesh	Krishnaveni	1995
BXO407	Karjat, Maharashtra	KJT184	1995
BXO409	Karjat, Maharashtra	Jaya	1995
BXO411	Karjat, Maharashtra	Jaya	1995
BXO413	Kaul, Haryana	Basmati	1995
BXO414	Kaul, Haryana	Basmati	1995
BXO415	Kaul, Haryana	Basmati	1995
BXO416	Kaul, Haryana	PR106	1995
BXO417	Kaul, Haryana	PR106	1995
BXO418	Kaul, Haryana	PR106	1995
BXO419	Nellore, Andhra Pradesh	NA	1995
BXO421	Nellore, Andhra Pradesh	NA	1995
BXO423	Nellore, Andhra Pradesh	NA	1995
BXO432	Raigad, Maharashtra	Ratna	1995
BXO433	Raigad, Maharashtra	Ratna	1995
BXO434	Raigad, Maharashtra	Ratna	1995
BXO435	Raigad, Maharashtra	Ratna	1995
BXO436	Raigad, Maharashtra	Ratna	1995
BXO437	Raigad, Maharashtra	Ratna	1995
BXO438	Raigad, Maharashtra	Ratna	1995
BXO439	Kaul, Haryana	PR106	1995
BXO440	Kaul, Haryana	Jaya	1995
BXO441	Kaul, Haryana	Jaya	1995
BXO442	Kaul, Haryana	Jaya	1995
BXO443	Pantnagar, Uttar Pradesh	NA	1995
BXO444	Pantnagar, Uttar Pradesh	NA	1995
BXO445	Pantnagar, Uttar Pradesh	NA	1995
BXO446	Pantnagar, Uttar Pradesh	NA	1995
BXO447	Pantnagar, Uttar Pradesh	NA	1995
BXO448	Kaul, Haryana	Jaya	1995

^a NA indicates that information is not available.

^b TN-1 = Taichung Native-1 rice cultivar.

duced as an artifact of the clustering process (27). Felsenstein (6) proposed a very stringent criterion and suggested that only groups having a bootstrap value >95% should be considered to be robust.

Pathotype analysis. The seeds of the rice differential cultivars were obtained from the Directorate of Rice Research. The plants were grown in plastic pots (17-cm diameter $\times$ 15-cm height) under flooded conditions in the greenhouse on soil obtained from the rice farm of the Directorate of Rice Research. The pots were filled with a mixture of soil and farmyard manure at a ratio of 3:1. N-P-K were supplied to the plants at the rate of 100-75-75 kg/ha as the

basal dose in the form of urea, superphosphate, and muriate of potash. Fifteen-day-old rice seedlings were transplanted to these pots. Five seedlings were planted in each pot, taking care to give equal spacing between the seedlings. The pots were irrigated every day. The plants were top-dressed with urea at the rate of 50 kg/ha at the tillering stage. Adequate plant protection measures were taken to ensure healthy and vigorous growth of the plants. Forty-day-old plants were clip-inoculated (12) with bacterial suspensions of 10^9 cells/ml. An average of 20 leaves per pot were inoculated for each strain-cultivar combination.

Disease scores were evaluated 15 days after inoculation based on a 0 to 9 scale (10,12): 0 = no visible lesion; 1 = lesions restricted to 0.5 to 1.0 mm; 3 = lesions elongated, but less than one-fourth of the leaf blade; 5 = lesions extended to half of the leaf blade; 7 = lesions extended from three-fourths to the base of the leaf blade; and 9 = lesions completely destroyed the leaf blade and sheath. A disease score of 4 and below was rated as a resistant reaction, 4 to 5 as a moderately susceptible reaction, and 5 and above as a susceptible reaction (10,12).

RESULTS

RFLP analysis with the *avrXa10* probe. Sixty-seven strains of *X. oryzae* pv. *oryzae* were analyzed for RFLPs using the avirulence gene probe *avrXa10*. There were nine distinct haplotypes (Fig. 2A), based on the 19 distinct bands observed. DNA fragments that hybridized ranged from 12 to 2 kb. The number of bands in each haplotype varied from a minimum of seven bands in BXO38 to as many as 11 bands in BXO8 and BXO17. The most common haplotype among the strains analyzed was the BXO1 type of banding pattern. Figure 2B shows the BXO1 haplotype exhibited by strains collected from different geographical locations in India. Among the 67 strains analyzed, 55 strains from 12 different locations had this haplotype.

Similarity coefficients for pairwise combinations of haplotypes were obtained, and a dendrogram was prepared (Fig. 3A) with the UPGMA option of PHYLIP. A single dominant lineage (clustering at the 88% similarity level) was formed by 59 strains from 15 locations; this lineage included the haplotype represented by BXO1. The strains BXO5 and BXO17 formed a second group (85% similarity level). BXO6 and BXO8 did not cluster strongly with any of the other lineages; BXO38 clustered with the dominant lineage at the 80% similarity level. Low bootstrap values were obtained for all of these clusters. These values improved to 97.7% for the dominant lineage and 95.2% for the group of BXO5 and BXO17 when BXO6, BXO8, and BXO38 were left out of the bootstrapping analysis. Cluster analysis of the data using the NEIGHBOR JOINING option of the PHYLIP program gave the same clusters as with UPGMA (J. Yashitola, unpublished data).

Haplotype variability of multiple strains from a single location. It was observed that, in many cases, multiple strains from the same locality (Table 1) had an identical haplotype. All of the strains from Maruteru (BXOs 12, 13, 14, 15, 16, 401, 403, and 405) had an identical haplotype, and only the haplotype of BXO12 is shown (Fig. 2B). Similarly, all of the strains collected from Pantnagar in 1994 (BXOs 17, 18, 19, and 20) had a haplotype identical to that of BXO17 (Fig. 2A). Identical haplotypes were observed for multiple strains collected from each of several other locations including Cuttack, Kapurthala, Karjat, Karnal, Maruteru, and Raigad. The Kapurthala strains (BXOs 4, 28, 29, 30, 31, and 32) had the same haplotype, even though BXO4 was collected in 1992, while the rest were collected in 1994. In contrast, the Nellore strain BXO8, which was collected in 1993, had a haplotype distinct from BXO419, BXO421, and BXO423, which were collected in 1995 from the same location. All of the 1995 strains exhibited one haplotype. Similarly, Pantnagar strains BXO17, BXO18, BXO19, and BXO20 obtained in 1994 had a different haplotype when compared with the strains collected

Fig. 2. Haplotype diversity within Indian strains of *Xanthomonas oryzae* pv. *oryzae* revealed by the probe *avrXa10*. Genomic DNA was isolated from bacterial strains collected from different locations in India. *Bam*HI-digested genomic DNA was probed with radio-labeled *avrXa10*. The strain number is indicated on each lane. A kilobase DNA ladder was added as a size marker. Each strain was analyzed at least three times, and the same haplotype was obtained every time. **A**, Haplotype diversity. **B**, Haplotypes of a group of closely related strains.

in 1995 (BXOs 443, 444, 445, 446, and 447), which had the BXO1 haplotype.

The two strains from Raipur (BXO2 and BXO3) had distinct haplotypes (Fig. 2A). These haplotypes were, nevertheless, closely related to each other and to the BXO1 haplotype (Fig. 3A). The total number of bands was the same in each of these three strains. The haplotypes of BXO2 and BXO3 differed from that of BXO1 by the absence of one band (albeit a different band in each strain) and the presence of another. As the *avrXa10* gene probe detects differences in the number of copies of a 102-bp repeat element within individual members of the avirulence gene family of *X. oryzae* pv. *oryzae* (9), the haplotypes of BXO2 and BXO3 could have arisen by deletion of several copies of this repeat element from a member of the avirulence gene family in BXO1. The possible effect of this change on the virulence of these strains is being investigated. Strains BXO1, BXO2, and BXO3 were found to have identical haplotypes when analyzed with another repeat element probe (discussed below), confirming that they are indeed very closely related.

RFLP analysis with the IS1112 probe. The second probe used was IS1112, a repeat element cloned from the *X. oryzae* pv. *oryzae* genome (14). A sample of the different haplotypes obtained using this probe is shown in Figure 4A. Eleven distinct haplotypes (seven of them are shown in Fig. 4A) were observed among the strains examined. Band resolution was observed from 8 to 1 kb, therefore, comparisons of bands were made using the 30 bands within this size range. A number of strains from different locations that had identical haplotypes when probed with *avrXa10* were also found to have very related haplotypes when probed with IS1112 (Fig. 4B).

Similarity coefficients were obtained and a dendrogram was prepared (Fig. 3B). One lineage contained 60 strains from 16 different locations and included all the strains that formed a dominant lineage when *avrXa10* was used as the probe. Strains within this group clustered together at the 93% similarity level, and the bootstrap value was 99.9%, indicating that it is a very strong cluster. The BXO38 strain was part of this cluster (haplotype identical to that of BXO33). The bootstrap values for the internal branches within the dominant lineage were <53%, which suggests that, although the dominant lineage is a very strong cluster, the interrelationships among the strains within this lineage are unclear. Another cluster was formed by BXO5, BXO17, BXO6, and BXO8, with a bootstrap value of 81%; within this cluster, BXO5 and BXO17 (similarity level of 84%) were more closely related to each other.

Construction of dendrogram by combining RFLP data obtained with the two probes. The RFLP data obtained by using the probes *avrXa10* and IS1112 were combined and a dendrogram was prepared (Fig. 3C). A dominant lineage comprising 60 strains (including BXO38) from 16 locations was formed at the 93% similarity level. The bootstrap values were 100%, indicating that this is a very strong cluster. The bootstrap values for the internal branches within the dominant lineage were <52%, indicating again that, although the dominant lineage is a very strong cluster, the interrelationships between the strains within this lineage are not clear. BXO5, BXO17, BXO6, and BXO8 were related to each other at the 68% similarity level, but the 64.3% bootstrap value indicates that this is not a strong cluster. Within this group, strains BXO5 and BXO17 were more closely related to each other (clustering at the 87% similarity level), with a bootstrap value of 93%.

Virulence of pathogen strains on differential rice cultivars. The results in Table 2 indicate that the majority (10/14) of the strains tested exhibited a similar virulence spectrum, and these strains belonged to the BXO1 lineage. BXO5, BXO6, BXO8, and BXO17 were compatible on rice cultivars BJ1 and DV85. The haplotypes of these strains were distinct (Figs. 2 and 4) and quite unrelated (Fig. 3) to those strains that were incompatible with rice

Fig. 3. Dendrograms of Indian strains of *Xanthomonas oryzae* pv. *oryzae* derived from restriction fragment length polymorphism (RFLP) studies. The dendrograms were constructed using the UPGMA option of PHYLIP on the basis of RFLP data obtained with **A**, *avrXa10*; **B**, IS1112; and **C**, the two probes combined. The number of strains that have identical haplotypes are given in parentheses. Numerals or symbols at the internal branches indicate bootstrap values for clusters to the right of the numeral or symbols. Values for the symbols are given in the inset.

cultivars BJ1 and DV85. The strain BXO8 was interesting, as it was compatible with the highly resistant rice cultivar IET8585.

DISCUSSION

The RFLP studies reported here were initiated to gain a better understanding of the genetic diversity of *X. oryzae* pv. *oryzae* in India. The results indicate that strains belonging to a single lineage (henceforward referred to as the BXO1 lineage) constitute the majority of this collection of the pathogen. Results obtained with the two probes (combined) indicate that the BXO1 lineage is a very strong cluster (present in 100% of the clusters obtained by bootstrapping analysis). Although there is diversity within this lineage (the probes used can detect a total of 11 different haplotypes), these strains appear to be closely related to each other, as they cluster together at the 93% similarity level in the dendrogram. The non-BXO1 lineage strains form a group of loosely related strains, but, among these, BXO5 and BXO17 are most closely related to each other.

The collection sites of the BXO1 lineage strains are, in many cases, separated by several hundred to several thousand kilometers and represent diverse ecological rice-growing areas of the country (Fig. 1). Since multilocus probes were used in this study, it is unlikely that the related haplotypes arose independently at various locations. This suggests that gene flow due to pathogen migration, followed by subsequent divergence in some cases, has occurred. One way that the pathogen could migrate across such large distances is by inadvertent dispersal through seed. We are, therefore, examining the possibility that *X. oryzae* pv. *oryzae* might be seed-borne in India.

The results indicate that all 10 BXO1 lineage strains that were tested are incompatible on rice cultivars BJ1 and DV85. The strains (BXOs 5, 6, 8, and 17) that are compatible on BJ1 and DV85 have haplotypes that are easily distinguishable from the BXO1 lineage. This suggests that, at least at this level, the molecular haplotype might be useful in predicting the pathological properties of Indian strains of *X. oryzae* pv. *oryzae*. The strains in the BXO1 lineage are compatible on rice cultivar IR20. This suggests that these strains are similar to the previously described pathotype 1b of *X. oryzae* pv. *oryzae*. Pathotype studies conducted during the last 20 years have indicated that pathotype 1b is widely distributed in India (18, 19). The results presented here suggest that this continues to be the case.

The rice cultivars BJ1 and DV85 and their derivatives have been consistently resistant to bacterial leaf blight in the International Rice Bacterial Leaf Blight Nursery, as observed over a number of years (1981 to 1987) and a number of locations in India (18,

Fig. 4. Haplotype diversity within Indian strains of *Xanthomonas oryzae* pv. *oryzae* revealed by the probe IS1112. Genomic DNA was isolated from bacterial strains collected from different locations in India. *Eco*RI-digested genomic DNA was probed with radio-labeled IS1112. The strain number is indicated on each lane. A kilobase DNA ladder was added as a size marker. Each strain was analyzed at least three times, and the same haplotype was obtained every time. **A**, Haplotype diversity. **B**, Haplotypes of a group of closely related strains.

TABLE 2. Virulence of *Xanthomonas oryzae* pv. *oryzae* strains on differential rice cultivars^{a,b}

Bacterial strain	Rice cultivar					
	BJ1	DV85	Cempo Selak	IR20	IET8585	TN-1 ^c
BXO1 ^{d,e}	R (3.0)	R (2.5)	S (6.0)	S (6.0)	R (1.0)	S (7.0)
BXO6 ^{d,f}	S (5.6)	S (6.0)	S (7.0)	S (6.0)	R (1.0)	S (7.0)
BXO8 ^d	S (7.0)	S (5.0)	S (6.0)	S (6.0)	S (5.0)	S (7.0)

^a The virulence assays were performed and scored as described in Materials and Methods. R = resistant and S = susceptibility. Disease scores are in parentheses.

^b The resistance genes that have been reported (17) to be in these rice cultivars are BJ1 (*xa-5* and *xa-13*), DV85 (*xa-5* and *xa-7*), Cempo Selak (*xa-3*), and IR20 (*xa-4*). The resistance genes in IET8585 have not been identified. IET8585 was obtained from the cross IR8/BJ1/IR22/CR98-7216.

^c TN-1 = Taichung Native-1 rice cultivar.

^d Experiments were repeated twice.

^e Similar reactions were obtained with BXOs 4, 7, 9, 10, 12, 25, 28, 33, and 34.

^f Similar reactions were obtained with BXO5 and BXO17.

22). These two cultivars are being used in developing bacterial leaf blight-resistant cultivars in India. IET8585, a derivative of BJ1, is one such cultivar and exhibits resistance to most of the strains used in this study. The resistance genes *xa-5* and *xa-13* are present in BJ1, while *xa-5* and *Xa-7* are found in DV85 (17). Besides these genes, it has been suggested that other major and minor genes for disease resistance may be present in these two cultivars. It remains to be determined which gene(s) in these cultivars are providing resistance against strains within the BXO1 lineage.

ADDENDUM

Note added in proof. In addition to the results described above, we have analyzed an additional 19 strains that were collected in 1996 from seven different locations in India. Four of these locations had not been sampled earlier; two of the new locations are in the western state of Gujarat. None of the sites sampled earlier were located in this state. All 19 strains belonged to the BXO1 lineage. Their haplotype was identical to that of BXO1 when *avrXa10* was used as a probe and exhibited >95% similarity to the BXO1 haplotype when *IS1112* was used as a probe.

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