

# Analysis of the Interaction Between *Xanthomonas oryzae* pv. *oryzae* and the Rice Cultivars IR24 and IRBB21

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## ABSTRACT

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*Xanthomonas oryzae* pv. *oryzae* causes bacterial blight of rice, and interactions between this pathogen and its host occur in a gene-for-gene manner. In contrast to other resistance genes, the dominant locus *Xa-21* confers resistance to all Indian and Philippine races of *X. o. oryzae* that have been examined. In this study, multiplication of *X. o. oryzae* and subsequent disease development were comparable in cultivar IRBB21 possessing *Xa-21* and in the susceptible rice cultivar IR24 when plants

were inoculated prior to 21 days postemergence. Resistance conveyed by *Xa-21* was evident in 21-day-old plants, and the level of resistance increased with plant maturity. Cultivar IR24, which is used as a susceptible control in screening trials, displayed resistance to bacterial blight that was expressed only in adult plants. DNA homologous to the avirulence gene *avrBs2* from *Xanthomonas campestris* pv. *vesicatoria* was present in all Philippine races of *X. o. oryzae*. Inactivation of the *avrBs2*-homologous region by transposon mutagenesis did not affect the fitness of *X. o. oryzae* and did not alter the response elicited by this bacterium on IRBB21, indicating that this locus does not confer *avrXa21* activity.

Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae*, has become an increasingly important disease problem in rice production. Genetic resistance is the most effective and economical measure for the control of this disease, and breeding for bacterial blight resistance is an important component of rice improvement programs (15). Resistance to bacterial blight has been identified in several cultivars, and examples of both quantitative and major gene resistance to *X. o. oryzae* have been described for rice (15,17).

Molecular genetic analysis of the *X. o. oryzae*-rice interaction has demonstrated that the resistance conferred by single dominant loci in the host occurs in a race-specific manner (6). An incompatible plant response is elicited when a rice cultivar that possesses a specific resistance gene is inoculated with a strain of *X. o. oryzae* that carries the corresponding avirulence gene. Recently, a dominant gene for resistance to bacterial blight, designated *Xa-21*, was identified in the wild rice *Oryza longistaminata* and was introduced into the rice cultivar IR24 by backcrossing (10). In contrast to previously identified bacterial blight resistance genes, *Xa-21* conveys resistance to all Indian and Philippine races of *X. o. oryzae* that have been evaluated (7,10).

Although many avirulence genes are restricted to particular races of a given bacterial species, the avirulence gene *avrBs2* is present and highly conserved among all strains of *Xanthomonas campestris* pv. *vesicatoria* that have been examined (16). In addition to its role in the elicitation of a resistance response on pepper cultivars possessing the *Bs2* resistance gene, *avrBs2* also contributes to the fitness of this bacterium in association with its plant host. Kearney and Staskawicz (9) demonstrated that mutations in the *avrBs2* locus resulted in the inability of *X. c. vesicatoria* to multiply to wild-type levels in normally susceptible pepper cultivars. Pepper cultivars carrying the *Bs2* resistance locus are resistant to all strains of *X. c. vesicatoria* because of the distribution of *avrBs2* throughout the population of this bacterial pathogen.

In addition to conservation of the *avrBs2* locus among strains of *X. c. vesicatoria*, homologous sequences are distributed among

several plant-pathogenic members of the genus *Xanthomonas*, including *X. o. oryzae* (9). Although functional copies of this avirulence gene are present in several *X. campestris* pathovars, the activity of the *avrBs2* homologue in *X. o. oryzae* and its distribution in the population of this pathogen have not been examined. On the basis of previous reports that *Xa-21* confers resistance to all Philippine and Indian strains of the bacterial blight pathogen (7,10) as well as the widespread presence of *avrBs2*-homologous sequences among pathovars of *X. campestris* (9), our initial objective was to determine whether or not the *avrBs2* homologue in *X. o. oryzae* conveyed *avrXa21* activity. During our initial experiments, the response of rice cultivars to inoculation with *X. o. oryzae* suggested that the resistance conferred by *Xa-21* may not be functional under certain experimental conditions. Therefore, additional investigations were conducted to determine the effect of plant age on the interaction between *X. o. oryzae* and the rice cultivar IRBB21.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, bacteriophage, and media.** The bacterial strains, plasmids, and phage used in this study are listed in Table 1. *X. o. oryzae* was cultured in peptone-sucrose (PS) medium (22) at 28 C. For isolation of *X. o. oryzae* from plant tissue, 1.5% agar was added to PS medium. Strains of *Escherichia coli* were cultured in Luria-Bertani medium (13) or terrific broth (21). Antibiotics were used in selection media at the following concentrations ( $\mu\text{g/ml}$ ): cephalixin, 30; cycloheximide, 100; kanamycin, 50; nalidixic acid, 50; rifampicin, 30; spectinomycin, 100; and streptomycin, 100.

**Recombinant DNA techniques.** Isolation of plasmid DNA from *E. coli* was performed as described by Birnboim and Doly (5). Restriction endonuclease digestions were performed as recommended by the supplier (Bethesda Research Laboratories, Gaithersburg, MD). Isolation of genomic DNA, transfer of DNA to nitrocellulose, labeling of DNA with  $^{32}\text{P}$ -dCTP by nick translation, hybridization, electrophoresis, transformation of *E. coli*, and conjugative transfer of plasmids to *X. o. oryzae* were conducted essentially as described by Maniatis (14) or Ausubel et al (1). Colony

blots were performed as described by Maas (13) and were washed after hybridization under high stringency conditions (11).

**Identification of sequences homologous to *avrBs2* in *X. o. oryzae*.** The plasmid p81533 contains a 2.3-kb *SphI* DNA fragment from *X. c. vesicatoria* that has *avrBs2* activity (9). A 1.9-kb *HindIII* fragment that is internal to *avrBs2* can be used as a specific probe for the gene (B. Staskawicz, personal communication). Plasmid p81533 was digested with *HindIII*, and fragments were separated on a 0.7% agarose gel. The 1.9-kb *HindIII* fragment was cut from the agarose gel and electroeluted into TE (10 mM Tris and 1 mM EDTA) buffer. The DNA was labeled with <sup>32</sup>P-dCTP by nick translation and used in colony hybridizations to probe a genomic library of *X. o. oryzae* PXO86 constructed in the cosmid pHMI (6). Plasmid DNA was isolated from *E. coli* strains that hybridized with the *avrBs2*-specific probe in colony blots. Plasmid DNA was digested with *SphI* and probed with the 1.9-kb *HindIII* fragment in Southern hybridizations to confirm the presence of *X. o. oryzae* DNA homologous to *avrBs2*.

**Tn5 mutagenesis.** Clones of *X. o. oryzae* DNA in pHMI were mutagenized in *E. coli* strain TB1 with  $\lambda$ B20::Tn5 (20). Conjugative transfer of mutated plasmids to *E. coli* strain C2110 was conducted by triparental matings with strains possessing presumptive Tn5 insertions in cosmid clones as the donor strains and pRK2073 as the helper plasmid. Transconjugates were selected on Luria-Bertani agar containing kanamycin, nalidixic acid, and spectinomycin. Plasmid DNA was isolated from pooled transconjugates and transformed into *E. coli* strain S17-1. Plasmid DNA was isolated from individual colonies and digested with *HindIII* or *SphI*, separated by agarose gel electrophoresis, and probed with Tn5 or the 1.9-kb *HindIII* fragment specific for *avrBs2*. Mutagenized clones possessing a Tn5 insertion within the *avrBs2*-homologous region were utilized in marker-exchange mutagenesis.

**Marker-exchange mutagenesis.** Mutated plasmids were introduced into *X. o. oryzae* strains PXO86R and PXO99A in biparental matings. Transconjugates were grown for several generations in PS broth without selection for resistance to spectinomycin, and then cultures were plated onto PS agar containing kanamycin.

Individual colonies were selected and replica plated onto PS agar amended with kanamycin or spectinomycin. Total DNA was isolated from kanamycin-resistant, spectinomycin-sensitive colonies. DNA was digested with *HindIII*, separated by agarose gel electrophoresis, transferred to nitrocellulose, and probed with pHMI, the 1.9-kb *avrBs2*-specific DNA fragment, and Tn5 to ensure that the mutated *avrBs2*-homologous sequence had integrated into the chromosome of *X. o. oryzae*.

**Plant materials and inoculation.** The rice cultivar IR24 is susceptible to all Philippine races of *X. o. oryzae*. Cultivars IRBB10 and IRBB21 are near-isogenic lines of IR24 and possess the *Xa-10* and *Xa-21* resistance loci, respectively (7,10,23). Cultivar IRBB10 is resistant to races of *X. o. oryzae* that possess the avirulence gene *avrXa10* (6) and was used as the resistant control in inoculations with strain PXO86R.

Seed were planted and incubated in the greenhouse at a day-night temperature regime of 30 and 25 C without supplemental lighting. *X. o. oryzae* strains PXO99A and PXO86R were grown in PS broth for 24 h. Cells were collected by centrifugation, washed with sterile water, and resuspended in a volume of sterile water to obtain a cell density of approximately 10<sup>9</sup> cfu/ml. Plants were inoculated with the leaf-clip method (8) or by localized infiltration of *X. o. oryzae* cell suspensions with a needleless syringe into leaves of 10-day-old rice plants as described by Reimers and Leach (18). Lengths of lesions induced by *X. o. oryzae* after leaf-clip inoculation of 11-, 17-, 21-, 31-, 41-, 51-, 61-, and 71-day-old plants were assessed 14 days postinoculation. Twenty leaf samples were assayed per cultivar-strain combination. Infiltrated leaves of 10-day-old rice plants were scored for water-soaking (susceptible) or the hypersensitive (resistant) responses 48 h postinoculation. Experiments were repeated twice.

**Colonization of leaf tissue by *X. o. oryzae*.** The effect of plant age on the multiplication of *X. o. oryzae* strain PXO99A in leaves of the rice cultivars IR24 and IRBB21 was assessed. Plants were inoculated at 11, 21, 41, or 61 days postemergence with the leaf-clip method as described above. For each rice cultivar, three inoculated leaves were randomly harvested at 0, 4, 8, 12, and 16 days postinoculation. The leaves were ground individually with

TABLE 1. Bacterial strains, plasmids, and phage used in this study

	Relevant characteristic <sup>c</sup>	Source or reference
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>		
PXO86R	Race 2, Rif <sup>R</sup>	3
PXO86-13.19	PXO86R::pMM13.19 marker-exchange mutant, Km <sup>R</sup>	This study
PXO86-31.10	PXO86R::pMM31.10 marker-exchange mutant, Km <sup>R</sup>	This study
PXO99A	Race 6, grows on 200 $\mu$ M 5-azacytidine	6
PXO99-67.1	PXO99A::pMM67.1 marker-exchange mutant, Km <sup>R</sup>	This study
PXO99-69.11	PXO99A::pMM69.11 marker-exchange mutant, Km <sup>R</sup>	This study
<i>Escherichia coli</i>		
C2110	Nal <sup>R</sup> , <i>polA1</i> , <i>rha</i> , <i>his</i>	12
S17-1	294, <i>recA</i> , chromosomally integrated RP4 derivative, Sm <sup>R</sup> , Tc <sup>R</sup>	19
TB1	JM83, <i>lacZ</i> , <i>hsdR</i>	2
Plasmids		
p81533	pUC118 clone containing a 2.35-kb <i>SphI</i> fragment from <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> with <i>avrBs2</i> activity	B. Staskawicz, University of California, Berkeley
pHMI	<i>cos</i> , <i>parA</i> , IncW, Sp <sup>R</sup> , Sm <sup>R</sup>	R. Innes, Indiana University
pRK2073	Tra <sup>+</sup> , Sp <sup>R</sup>	4
pXO15-10	pHMI clone containing <i>X. o. oryzae</i> DNA homologous to <i>avrBs2</i> from <i>X. c. vesicatoria</i>	This study
pXO16-43	pHMI clone containing <i>X. o. oryzae</i> DNA homologous to <i>avrBs2</i> from <i>X. c. vesicatoria</i>	This study
pMM13.19	pXO15-10 containing a Tn5- <i>lac</i> insertion in the <i>avrBs2</i> homologous region	This study
pMM31.10	pXO15-10 containing a Tn5- <i>lac</i> insertion in the <i>avrBs2</i> homologous region	This study
pMM67.1	pXO15-10 containing a Tn5- <i>lac</i> insertion in the <i>avrBs2</i> homologous region	This study
pMM69.11	pXO15-10 containing a Tn5- <i>lac</i> insertions in the <i>avrBs2</i> homologous region	This study
Phage		
Tn5-B20	<i>lac</i> fusion, Km <sup>R</sup>	19

<sup>c</sup>Rif<sup>R</sup> = rifampicin resistant; Km<sup>R</sup> = kanamycin resistant; Nal<sup>R</sup> = nalidixic acid resistant; Sm<sup>R</sup> = streptomycin resistant; Tc<sup>R</sup> = tetracycline; Sp<sup>R</sup> = spectinomycin resistant.

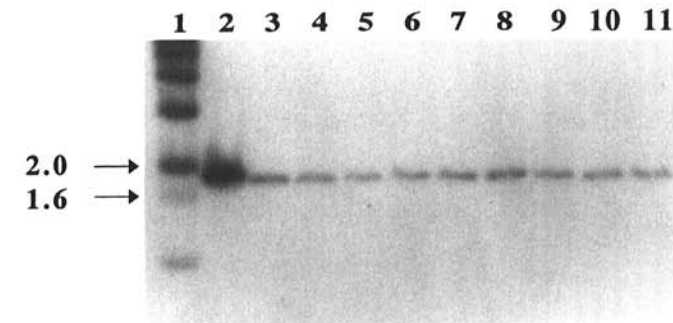
a mortar and pestle. The resulting leaf homogenate was serially diluted with sterile water and plated onto PS agar containing cycloheximide and cephalixin. The plates were incubated at 28 C, and colonies were counted 3 days after plating. All experiments were conducted two times.

The activity of the *avrBs2*-homologous sequence in *X. o. oryzae* was determined by monitoring the multiplication of strains PXO99A and PXO86R and their respective mutants in the cultivar IR24 after leaf-clip inoculation. Population densities of *X. o. oryzae* were determined as described above at 0, 1, 3, 6, and 9 days after inoculation of 51-day-old rice plants. Plant response to bacterial infiltration of 10-day-old rice plants was assessed 48 h postinoculation. In addition, lengths of lesions induced by PXO99A and mutants possessing insertions in the *avrBs2*-homologous region were assessed 14 days after leaf-clip inoculation of IR24 and IRBB21.

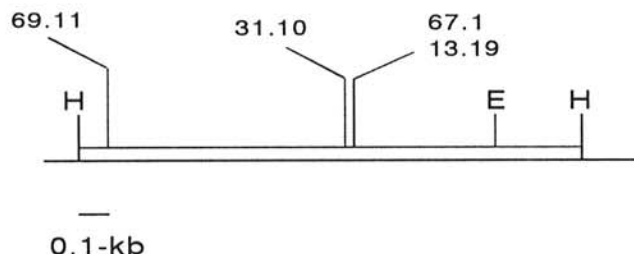
## RESULTS

**Identification and activity of *avrBs2*-homologous sequences in *X. o. oryzae*.** Total DNA from Philippine strains of *X. o. oryzae*, representing races 1–8, was probed with the 1.9-kb *Hind*III fragment from *X. c. vesicatoria* specific for *avrBs2* in Southern hybridizations. In each case, the *avrBs2*-specific probe hybridized to a fragment from *X. o. oryzae* that was similar in size to the 1.9-kb fragment from *X. c. vesicatoria* (Fig. 1). Two overlapping cosmid clones, pXO15-10 and pXO16-43, from the *X. o. oryzae* strain PXO86 genomic library possessed sequences homologous to *avrBs2*, as determined by colony blots and Southern hybridization analysis.

The cosmid clone pXO15-10 was subjected to transposon mutagenesis, and the positions of the insertions were determined by



**Fig. 1.** Southern blot analysis of *Hind*III-digested genomic DNA from Philippine strains of *Xanthomonas oryzae* pv. *oryzae* probed with a  $^{32}$ P-labeled 1.9-kb *Hind*III fragment from *Xanthomonas campestris* pv. *vesicatoria* that is specific for *avrBs2*. Lane 1, 1-kb ladder; lane 2, *avrBs2*-specific 1.9-kb *Hind*III fragment from *X. c. vesicatoria*; lane 3, *X. o. oryzae* strain PXO132 (race 1); lane 4, *X. o. oryzae* strain PXO86 (race 2); lane 5, *X. o. oryzae* strain PXO79 (race 3); lane 6, *X. o. oryzae* strain PXO176 (race 3); lane 7, *X. o. oryzae* strain PXO71 (race 4); lane 8, *X. o. oryzae* strain PXO80 (race 5); lane 9, *X. o. oryzae* strain PXO99 (race 6); lane 10, *X. o. oryzae* strain PXO54 (race 7); and lane 11, *X. o. oryzae* strain PXO211 (race 8).

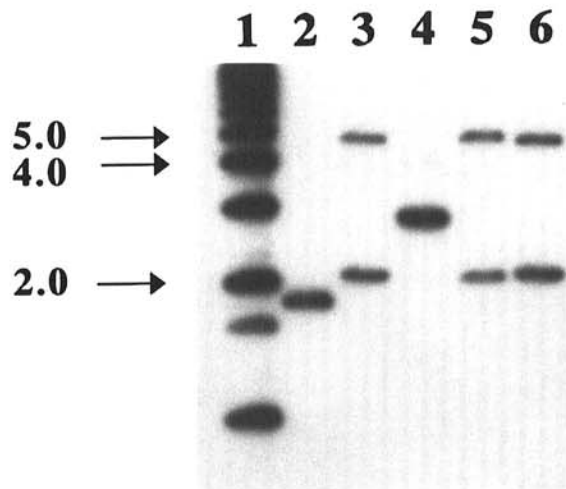


**Fig. 2.** Restriction and physical map of the *avrBs2*-homologous sequence of *Xanthomonas oryzae* pv. *oryzae*. Sites of Tn5-B20 insertions and the designation of the corresponding *X. o. oryzae* mutants are shown. E = *Eco*R1, and H = *Hind*III.

restriction enzyme mapping (Fig. 2). Several mutated clones containing insertions in the 1.9-kb *Hind*III fragment were identified and used in marker exchange mutagenesis to introduce the Tn5 into the chromosomes of strains PXO99A and PXO86R (Fig. 3). Insertion of the mutated clone into the chromosome of *X. o. oryzae* was confirmed by the absence of a hybridization signal when mutants were probed with pHMI. In addition, while the *avrBs2*-specific probe hybridized with a *Hind*III fragment of approximately 1.9 kb from PXO99A, insertion of Tn5 into the *avrBs2*-homologous region created *Hind*III fragments of approximately 2.1 and 4.9 kb in strains PXO99-67.1, PXO86-13.19, and PXO86-31.10 and fragments of 2.9 and 4.1 kb in strain PXO99-69.11, which hybridized with this probe (Fig. 3). The faint signal resulting from hybridization of the 1.9-kb *avrBs2*-specific probe to the 4.1-kb *Hind*III fragment of strain PXO99-69.11 may result from the fact that this DNA fragment possesses less than 100 bp of DNA from the *avrBs2*-homologous region in *X. o. oryzae*. The mutants and their respective parental strains were used in inoculations of 10- and 51-day-old plants of the rice cultivars IR24 and IRBB21. A water-soaking (susceptible) response was observed within 48 h postinoculation on both IR24 and IRBB21 after infiltration of 10-day-old plants with PXO99A, PXO86R, or their respective mutants. Lengths of lesions produced by PXO99A, PXO86R, and derivatives possessing Tn5 insertions in the *avrBs2*-homologous region were not significantly different on either rice cultivar after inoculation of 51-day-old plants (Table 2). Likewise, multiplication of the parental strains and of their corresponding mutants was similar on the rice cultivar IR24 (Fig. 4).

**Disease symptom development.** A water-soaking reaction was observed on all rice cultivars examined when leaves of 10-day-old seedlings were infiltrated with a cell suspension of PXO99A and on cultivars IR24 and IRBB21 inoculated with PXO86R. Infiltration of PXO86R into leaves of IRBB10 resulted in the induction of a hypersensitive resistance response that was detected within 48 h postinoculation.

The rice cultivars IR24, IRBB21, and IRBB10 responded differentially to leaf-clip inoculation with *X. o. oryzae* strain PXO99A, and the response, in terms of lesion lengths, by any given cultivar varied with plant age. Lengths of lesions induced by strain PXO99A on 11- and 17-day-old plants did not differ significantly among the three rice cultivars. However, the development of a resistant reaction in the cultivar IRBB21 was apparent for plants that were inoculated at 21 days postemergence; lesion lengths on this cultivar



**Fig. 3.** Southern blot analysis of genomic DNA from *Xanthomonas oryzae* pv. *oryzae* strain PXO99A and derivatives of PXO99A and PXO86R containing Tn5 insertions in the *avrBs2* homologous region. DNA was digested with *Hind*III, and the blot was probed with a  $^{32}$ P-labeled 1.9-kb *Hind*III fragment from *Xanthomonas campestris* pv. *vesicatoria* that is specific for *avrBs2*. Lane 1, 1-kb ladder; lane 2, *X. o. oryzae* strain PXO99A; lane 3, *X. o. oryzae* strain PXO99-67.1; lane 4, *X. o. oryzae* strain PXO99-69.11; lane 5, *X. o. oryzae* strain PXO86-13.19; and lane 6, *X. o. oryzae* strain PXO86-31.10.



were significantly less than those observed on either IR24 or IRBB10 (Fig. 5). The relative susceptibility-resistance of the three cultivars was similar for plants inoculated at 21, 31, or 41 days postemergence. Plants inoculated at 51 days postemergence had significantly smaller lesions than had been observed when 21-, 31-, or 41-day-old plants were inoculated with *X. o. oryzae*. Lesions induced by strain PXO99A on all cultivars at subsequent inoculation periods were smaller than those detected at the previous inoculation period. The lengths of lesions observed on cultivars IR24 and IRBB10 inoculated at 61 and 71 days postemergence are considered to represent a moderately resistant or resistant reaction to the bacterial blight pathogen on the basis of previous classification schemes (17).

**Population dynamics of *X. o. oryzae* in the cultivars IR24 and IRBB21.** As was observed for lengths of lesions induced by *X. o. oryzae*, plant age had a significant effect on the population dynamics of this bacterial pathogen in rice leaves. When plants were inoculated 11 days postemergence, the population dynamics of strain PXO99A were similar in leaves of both IR24 and IRBB21.

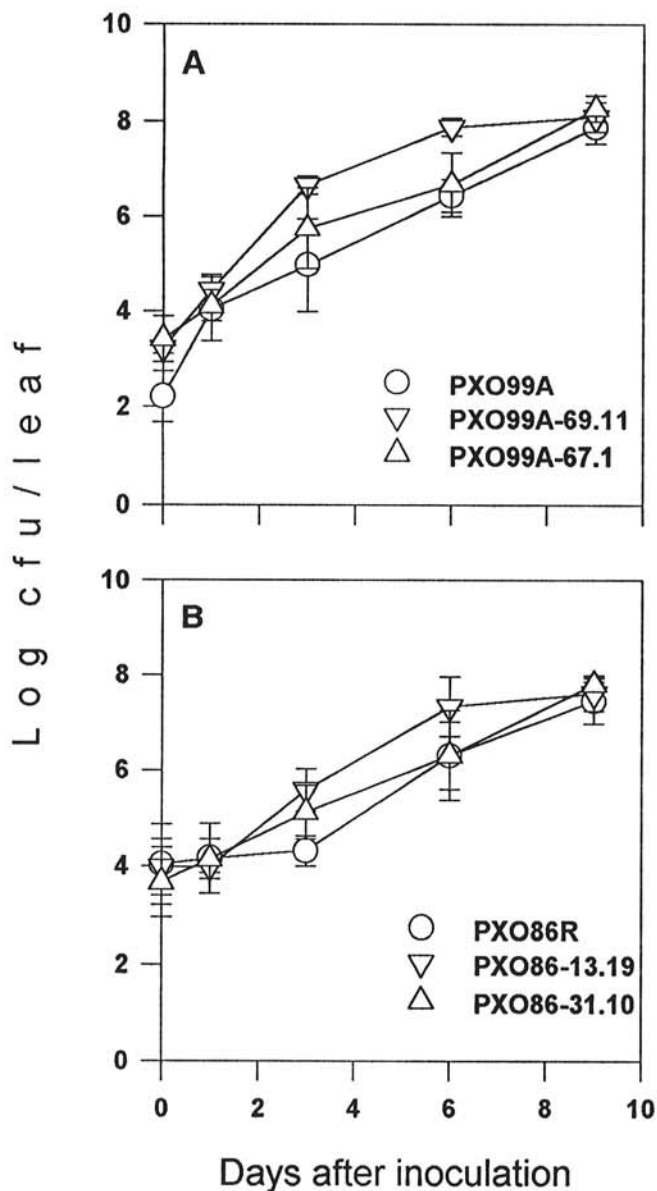


Fig. 4. Populations of *Xanthomonas oryzae* pv. *oryzae* strains A, PXO99A and B, PXO86R and corresponding mutants possessing Tn5 insertions in the *avrBs2*-homologous region in leaves of the rice cultivar IR24. Leaves were clip inoculated with a bacterial suspension of  $10^9$  cfu/ml. Values are means from three repetitions, and standard errors of mean population size are indicated. Similar results were obtained in two independent experiments.

Strain PXO99A multiplied to a maximum population of  $10^8$  cfu per leaf within 4 days postinoculation, and thereafter the population remained stable or declined gradually (Fig. 6A). The population dynamics of PXO99A in IRBB21 inoculated 21 days postemergence were similar to those observed in leaves of 11-day-old plants inoculated with *X. o. oryzae*, multiplying to a maximum population of  $10^8$  cfu per leaf and then remaining stable (Fig. 6B). In contrast, strain PXO99A continued to multiply in leaves of IR24 inoculated at 21 days postemergence until approximately 12 days postinoculation, and its population was approximately two orders of magnitude larger than that detected in the cultivar IRBB21. Multiplication of PXO99A in leaves of 41-day-old rice plants was comparable to that observed in 21-day-old plants with the exception that maximum bacterial numbers detected in leaves of IR24 were nearly two orders of magnitude larger than had been observed in 21-day-old plants (Fig. 6C). Multiplication of *X. o. oryzae* after inoculation of 61-day-old plants was significantly restricted in comparison with that observed in 41-day-old plants. Populations of strain PXO99A in both IR24 and IRBB21 at 16 days postinoculation were three orders of magnitude smaller than had been detected in leaves of 41-day-old plants at 16 days postinoculation (Fig. 6C and D).

## DISCUSSION

The rice resistance gene *Xa-21* was previously reported to convey resistance to all tested Philippine and Indian strains of *X. o.*

TABLE 2. Lengths of lesions induced by *Xanthomonas oryzae* pv. *oryzae* strains PXO99A and PXO86R and mutants possessing transposon insertions in the *avrBs2*-homologous region on the rice cultivars IR24 and IRBB21 after leaf-clip inoculation of 51-day-old rice plants

Strain	Lesion length (cm) <sup>y</sup>	
	IRBB21	IR24
PXO99A	3.3 a	11.2 a
PXO99A-67.1	3.8 a	10.4 a
PXO99A-69.11	3.4 a	10.0 a
PXO86R	1.7 b	ND <sup>z</sup>
PXO86-31.10	2.2 b	ND
PXO86-13.19	1.6 b	ND

<sup>y</sup> Means in the same column followed by the same letter are not significantly different ( $P = 0.05$ ) based on Fischer's protected least significant difference.

<sup>z</sup> Not determined.

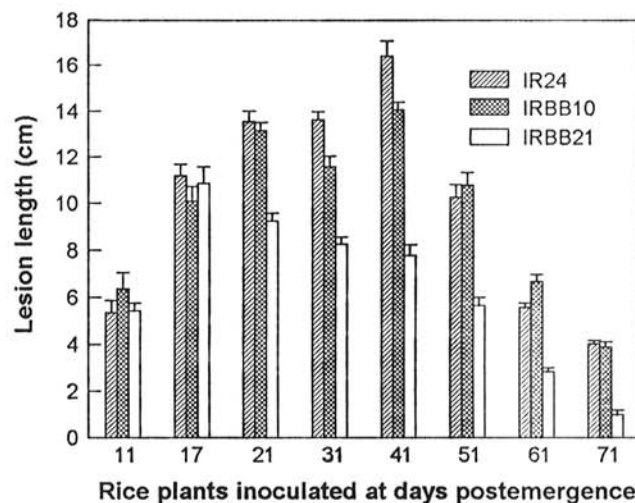


Fig. 5. Lengths of lesions induced by *Xanthomonas oryzae* pv. *oryzae* strain PXO99A on rice cultivars IR24, IRBB10, and IRBB21. Leaves (20 per cultivar) of 11-, 17-, 21-, 31-, 41-, 51-, 61-, and 71-day-old plants were clip inoculated with a bacterial suspension of  $10^9$  cfu/ml. Lesion lengths were assessed 14 days postinoculation. Values are means from three independent experiments. Standard errors of mean lesion length are indicated.

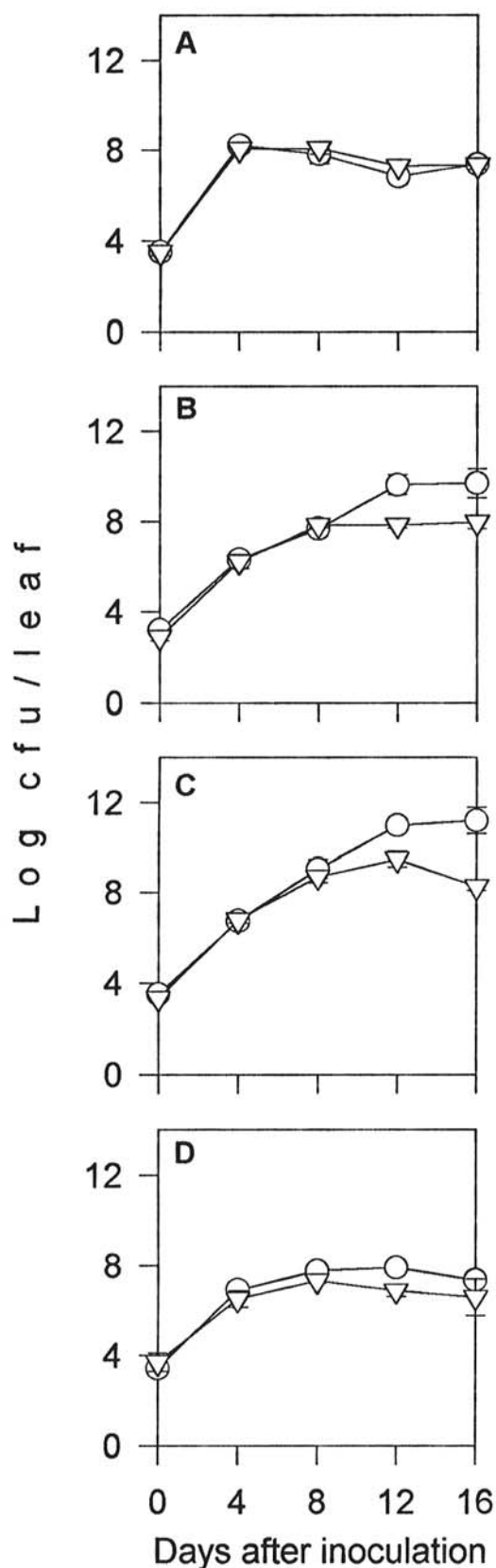


Fig. 6. Populations of *Xanthomonas oryzae* pv. *oryzae* strain PXO99A in leaves of the rice cultivars IR24 (○) and IRBB21 (∇). Leaves were clip inoculated with a bacterial suspension of  $10^9$  cfu/ml at A, 11, B, 21, C, 41, or D, 61 days postemergence. Values are means of three repetitions. Standard errors of mean population size are indicated. Similar results were obtained in two independent experiments.

*oryzae* (7,10). Khush et al (10) indicated that the level of resistance conferred by this locus was very high as determined after leaf-clip inoculation of rice plants. In contrast to these studies, we observed that rice plants possessing *Xa-21* were susceptible to the bacterial blight pathogen during the initial 2-3 wk following plant emergence. The response of 10-day-old plants to infiltration of leaves with *X. o. oryzae* PXO99A or PXO86R was comparable for both the susceptible (IR24) and resistant (IRBB21) cultivars; spreading water-soaked lesions were observed on leaves within 48 h postinoculation. Likewise, lengths of lesions induced by *X. o. oryzae* PXO99A were similar on both cultivars when plants were inoculated prior to 3 wk postemergence. Reimers and Leach (18) demonstrated that resistance-susceptibility to *X. o. oryzae* could be distinguished among 10-day-old seedlings on the basis of bacterial multiplication in rice leaves. In this study, there were no differences in multiplication of PXO99A in 11-day-old plants of IR24 and IRBB21, which indicates that resistance conferred by *Xa-21* is not expressed in rice seedlings.

Barton-Willis et al (3) demonstrated that multiplication of *X. o. oryzae* in 21-day-old plants of susceptible and resistant rice cultivars was comparable until bacterial numbers reached  $10^7$  to  $10^8$  cfu per leaf. Thereafter, bacterial multiplication in resistant plants was negligible in comparison with that in susceptible cultivars. In this study, similar results were obtained when 21-day-old plants were inoculated with *X. o. oryzae*. Populations of strain PXO99A in the resistant cultivar IRBB21 stabilized at  $10^8$  cfu per leaf, while maximum populations of strain PXO99A in leaves of the susceptible cultivar IR24 were approximately  $10^{10}$  cfu per leaf at 16 days postinoculation.

As plants matured, the apparent level of resistance conveyed by *Xa-21* increased as defined by lesion length and maximum populations of *X. o. oryzae* at 16 days postinoculation. Plants exhibited a moderate level of resistance when inoculated at 21 and 41 days postemergence, while plants inoculated at 51 days postemergence or later exhibited increased levels of bacterial blight resistance. Similarly, the cultivar IR24 demonstrated a moderate level of adult plant resistance, which was expressed in plants inoculated at 61 days postemergence or later. Maximum populations of *X. o. oryzae* and lengths of lesions induced by this pathogen in IR24 declined significantly as plants matured. The population dynamics of strain PXO99A in leaves of IR24 inoculated at 61 days postemergence were similar to those observed in the resistant cultivar IRBB21 when inoculated at 41 days postemergence. The results obtained for IRBB10 and IRBB21 also support the existence of mature plant resistance in IR24. These near-isogenic lines were generated with IR24 as the backcross parent, and thus all three cultivars possess a similar genetic background, with the exception of the specific resistance loci *Xa-10* and *Xa-21*. Each of these rice cultivars demonstrated a similar increase in resistance to the bacterial blight pathogen as plants matured, which was expressed as reduced bacterial populations and/or lesion lengths.

These findings have significant implications for breeding programs directed toward the development of improved rice cultivars that possess resistance to bacterial blight. Several improved rice cultivars, including IRBB10 and IRBB21, have been generated with IR24 as the recurrent parent. In screening progeny for resistance to *X. o. oryzae*, IR24 has been utilized as the susceptible host plant. This procedure is suitable provided that screening for resistance is conducted prior to the booting stage when adult plant resistance is expressed in IR24. The results of this study also demonstrate the possible risk of screening for disease resistance during a single phase of plant development rather than at multiple growth stages. While IRBB21 possessed a high level of resistance to *X. o. oryzae* in adult plants, seedlings were susceptible to bacterial blight, and plants possessed only a moderate level of resistance through the fifth-leaf stage of plant growth (approximately 50 days postemergence) under the conditions of these experiments. Thereafter, IRBB21 expressed a high level of resistance to race 2 and race 6 strains of *X. o. oryzae*.

The variation in resistance of IRBB21 and IR24 to bacterial blight observed at different growth stages is in agreement with

previous work conducted with other rice genotypes (24). In general, rice cultivars that express a moderate level of resistance to *X. o. oryzae* at the seedling stage either maintain a similar level of resistance or become more resistant as the plant matures (15). This type of resistance describes that observed in this study for IRBB21 to both race 2 and race 6 strains of *X. o. oryzae*. An increase in resistance with growth stage is most pronounced in rice cultivars that express adult plant resistance (15). In this study, lesions induced by *X. o. oryzae* on IR24 inoculated at 71 days postemergence were as much as 72% smaller than those observed on 31-day-old plants. Although adult plant resistance usually is observed at leaf position 10 or 11 (24), resistance in IR24 was generally expressed at leaf position 6 or 7.

In previous studies, sequences homologous to *avrBs2* from *X. c. vesicatoria* have been identified in several other pathovars of *X. campestris* (9). The *avrBs2*-homologous sequences from *X. c. alfalfae*, *X. c. malvacearum*, *X. c. phaseoli*, *X. c. vignicola*, and *X. c. vitians* were shown to confer *avrBs2* activity. In addition, mutations in *avrBs2* reduced the ability of *X. c. alfalfae* to multiply in susceptible host tissues in a manner similar to that observed for *X. c. vesicatoria* (9). In this study, sequences homologous to *avrBs2* were identified in strains of all Philippine races of *X. o. oryzae*, and no polymorphisms were detected within this locus. All strains possessed similar *Hind*III and *Sph*I fragments of approximately 1.9 and 2.3 kb, respectively, that hybridized with the *avrBs2*-specific probe from *X. c. vesicatoria*. The widespread distribution of this sequence and the ability of the *Xa-21* locus to convey multirace resistance led to our investigation of whether the *avrBs2* homologue in *X. o. oryzae* conferred *avrXa21* activity. Mutations in the *avrBs2*-homologous sequence, when introduced into PXO99A or PXO86R, had no effect on the ability of these strains to multiply in the susceptible cultivar IR24 and did not alter the development of an incompatible interaction when inoculated onto the resistant cultivar IRBB21. Therefore, the *avrBs2*-homologous sequence does not confer *avrXa21* activity in *X. o. oryzae*. In addition, unlike several other *X. campestris* pathovars, the *avrBs2* homologue from *X. o. oryzae* does not appear to have an important role in the fitness of this bacterium during pathogenesis, since it is not required for full virulence on susceptible rice cultivars. Interestingly, the *avrBs2*-homologous sequence from *X. c. holcicola*, a pathogen of sorghum, did not have *avrBs2* activity (9). Thus, the *avrBs2* homologue from these two bacterial pathogens, which infect monocotyledonous plants, do not appear to function in the manner described for seven of eight *X. campestris* pathovars that are pathogenic toward dicotyledonous plant species. Further experiments involving the replacement of *avrBs2* in *X. c. vesicatoria* with the homologous sequence from *X. o. oryzae* and *X. c. holcicola* will be necessary to determine whether these sequences are functional or whether expression of the *avrBs2*-homologous sequence is suppressed in these bacteria.

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