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Cloning and Characterization of an Avirulence Gene from *Xanthomonas campestris* pv. *oryzae*

Segenet Kelemu and Jan E. Leach

Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan 66506-5502 U.S.A.
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A genomic library of a strain of *Xanthomonas campestris* pv. *oryzae* race 2 was constructed in the cosmid vector pSa747 and transduced into *Escherichia coli* HB101. Race 2 strains are incompatible with rice cultivar Cas 209, which carries the *Xa-10* gene for resistance. Randomly selected cosmid clones in *E. coli* were individually mobilized to a strain of *X. c.* pv. *oryzae* race 6, which is compatible with Cas 209. The resulting transconjugants were screened for incompatibility on Cas 209. A clone (pSK8-4) was identified that altered the strain of race 6 from compatible to incompatible with Cas 209, but did not affect the interaction phenotypes with rice cultivars lacking *Xa-10*. The clone also changed a strain of race 1 that was compatible on Cas 209 to incompatible. In Cas 209 leaves, the multiplication of race 1 transconjugants was significantly lower than that of the race 1 strain itself. The race-specific activity of pSK8-4 was contained in a 2.5-kb *Eco*RI fragment. Southern blot analysis showed that sequence homology to this *Eco*RI fragment was present in all tested races of *X. c.* pv. *oryzae*, other *X. campestris* pathovars, and other *Xanthomonas* species. A *Pseudomonas* species that is pathogenic to rice lacked sequence similarity by DNA hybridization.

Additional keywords: bacterial leaf blight of rice, gene-for-gene hypothesis.

Flor (1955, 1959) first formulated the gene-for-gene concept, which states that for each host gene for resistance there is a corresponding pathogen gene for avirulence. The concept predicts that disease resistance occurs when a dominant avirulence gene in the pathogen interacts with a corresponding dominant host resistance gene. The validity of the gene-for-gene concept has been demonstrated or suggested in a number of host-pathogen interactions using classical genetic analyses (Flor 1946, 1947, 1955, 1959; Bagga and Boone 1968; Moseman 1959), mathematical analytical methods (Person 1959; Noronha-Wagner and Bettencourt 1967; Toxopeus 1956), and recombinant DNA techniques (Gabriel *et al.* 1986; Staskawicz *et al.* 1984; Staskawicz *et al.* 1987; Swanson *et al.* 1988).

Bacterial blight of rice, caused by *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye, is one of the most destructive diseases of rice in Asia (Ou 1985). Eleven genes conferring resistance in seedlings and adult rice plants (or both) have been defined by classical genetic analysis (for review, see Mew 1987). Races of *X. c.* pv. *oryzae* that have patterns of incompatible and compatible reactions on rice cultivars with distinct resistance genes to the pathogen have been identified (Mew 1987). This host-pathogen interaction pattern is evidence that the gene-for-gene concept operates in the *X. c.* pv. *oryzae*-rice system. If it does, then the various races of the pathogen must possess at least 11 avirulence genes corresponding to the 11 resistance genes in the host. Although classical genetic analysis helped to identify genes conditioning resistance in rice cultivars, a useful genetic system to study the inheritance of avirulence genes in *X. c.* pv. *oryzae* has only been developed recently (Kelemu and Leach 1987). In this study, we report the cloning and characterization of a dominant avirulence gene (*avr10*) from a race 2 strain of *X. c.* pv. *oryzae* that corresponds with a dominant resistance gene (*Xa-10*) in rice cultivar Cas 209. A preliminary report has been published (Kelemu and Leach 1988).

Current address of S. Kelemu: Department of Plant Pathology, Cornell University, Ithaca, NY 14853 U.S.A.
Address correspondence to J. E. Leach.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. The sources and relevant characteristics of the bacterial strains and plasmids used in this study are shown in Table 1. *Escherichia coli* strains were cultured on Luria medium (Miller 1972) or in nutrient broth (NB, Difco Laboratories, Detroit, MI) at 37° C. *X. c.* pv. *oryzae* strains were cultured routinely on peptone-sucrose agar (PSA; Tsuchiya *et al.* 1982) medium or modified Miller's minimal medium (M4). M4 contained per liter: 10.5 g K_2HPO_4, 4.5 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.5 g sodium citrate, 200 mg $MgSO_4 \cdot 7H_2O$, 5 μg thymine hydrochloride, 100 mg L-methionine, 100 mg nicotinic acid, 10 g glucose, 1 g L-glutamic acid, and 15 g agar at 28° C. The concentrations of antibiotics incorporated into media were: kanamycin (Km), 50 μg/ml; chloramphenicol, 10 μg/ml; cephalexin (Cp), 20 μg/ml; rifampicin (Rif), 20 μg/ml; and spectinomycin (Sp), 50 μg/ml.

Rif-resistant (Rif^r) strains were selected using a gradient plate technique (Szybalski 1952). Czapek-Dox agar (Cz)-selected strains of *X. c.* pv. *oryzae* were isolated by streaking wild-type strains on Czapek-Dox broth (Difco Laboratories) supplemented with 15 g/L of agar and 2

g/L of glutamic acid. After incubation for 2–3 wk at 28° C, bacteria from the few colonies observed were restreaked on fresh Cz until single colonies were obtained within 4 to 7 days (usually three to five cycles of streaking on the medium). Cz-selected strains were maintained on Cz medium. Complementation to proline prototrophy was assessed in *E. coli* HB101 on AG medium (Lichtenstein and Draper 1985) supplemented with leucine and thymine at 100 μg/ml each.

Recombinant DNA techniques. Total DNA isolation, alkaline lysis for plasmid isolation, transformation, preparation of competent cells, alkaline phosphatase treatment, gel electrophoresis, ligation, and restriction map construction were as described by Maniatis *et al.* (1982). Restriction enzymes, calf intestinal alkaline phosphatase, T4 DNA ligase, nick translation kits, and *in vitro* packaging kits were purchased from Bethesda Research Laboratories (BRL, Gaithersburg, MD) or Promega Biotech (Madison, WI). Reactions were conducted as recommended by the manufacturers.

For Southern blot analysis, total bacterial DNA was digested to completion with restriction endonucleases, and fragments were separated on 0.7 or 1.5% agarose gels. DNA was transferred to GeneScreenPlus membranes (Du Pont, Wilmington, DE) and hybridized with pSK8-4 that had been ^{32}P-labeled by nick translation. Hybridizations were performed for 18 hr in a solution containing 1 M NaCl, 0.2 M sodium phosphate buffer (PB, pH 7.0), 0.1% sodium dodecyl sulfate (SDS; Bio-Rad, Richmond, CA), and 300 μg/ml denatured salmon sperm DNA at 65° C with shaking. The probed membranes were washed in 300 mM NaCl, 30 mM sodium citrate, 0.1% SDS, 5 mM PB for 1 hr, and then in 75 mM NaCl, 7.5 mM sodium citrate, 0.1% SDS, 3 mM PB for 1 hr at 65° C. Filters were sealed in plastic bags, and autoradiography was conducted at −80° C using Du Pont Cronex film and Du Pont Cronex Lightning Plus intensifying screens.

Construction of genomic DNA library. Total genomic DNA from a race 2 strain of *X. c.* pv. *oryzae*, PXO86^{Rif}, was partially digested with *Eco*RI. Vector pSa747 was

Table 1. Bacterial strains and plasmids used in this study

Designation	Relevant characteristics[a]	Source or reference
Escherichia coli HB101	F-, *recA13*, *proA2*, Str^r	Boyer and Roulland-Dussoix 1969
E. coli C2110	*his*, *rha*, *polA1*	Figurski and Helinski 1979
Xanthomonas campestris pv. *oryzae*		
PXO61^{Cz}	Race 1, Cz-selected strain of PXO61	This study
PXO99^{Cz}	Race 6, Cz-selected strain of PXO99	This study
PXO86^{Rif}	Race 2, Rif^r strain of PXO86	This study
PXO103	Race 2	IRRI[b]
PXO63	Race 2	IRRI
PXO112	Race 5	IRRI
PXO145	Race 5	IRRI
PXO123	Race 6	IRRI
PXO124	Race 6	IRRI
PXO118	Race 6	IRRI
PXO115	Race 6	IRRI
PXO20	Race 1	IRRI
PXO143	Race 3	IRRI
PXO113	Race 4	IRRI
pv. *vesicatoria* UF81-23		R. Stall, Univ. of Florida, Gainesville
pv. *malvacearum* KXM28		L. Claflin, KSU[c]
pv. *oryzicola* BLS175		IRRI
pv. *translucens* XT115		L. Claflin, KSU
pv. *secalis* XT104		L. Claflin, KSU
pv. *vasculorum* NCPPB206		L. Claflin, KSU
pv. *holcicola* KXH86		L. Claflin, KSU
pv. *campestris* KXCC1		N. Tisserat, KSU
X. albilinians ATCC 33915		L. Claflin, KSU
Pseudomonas spp. CIAT1171		Vera Cruz *et al.* 1984
Plasmid		
pSa747	Km^r, Sp^r, cosmid, IncW replicon	Tait *et al.* 1983
pSa325	Ap^r, Cm^r, helper, derivative of pSa	Tait *et al.* 1983
pUCD623	Cm^r, Ap^r, Tc^r, contains Tn*4431*	Shaw *et al.* 1988
pSK8-4	2.5 kilobase (kb) fragment in pSa747, contains *avr10*	This study
pSK11-33	32-kb fragment in pSa747, contains a putative *avr* gene	This study
pBS8-4	2.5-kb *avr10* fragment in pBluescript KS+ (Stratagene)[d]	This study
pSK8-4::Tn*4431*	2.5-kb *avr10* fragment containing Tn*4431*	This study

[a] Rif^r, Tc^r, Km^r, Ap^r, Sp^r, Str^r, and Cm^r indicate resistance to rifampicin, tetracycline, kanamycin, ampicillin, spectinomycin, streptomycin, and chloramphenicol, respectively. Rif^r or Czapek-Dox agar (Cz)-selected strains that were used had the same race-specificity pattern on the differential rice cultivars as did the wild-type strains from which they were derived.
[b] The International Rice Research Institute, Los Banos, the Philippines.
[c] KSU, Kansas State University, Manhattan.
[d] Stratagene is located in La Jolla, CA.

digested to completion with *Eco*RI and treated with calf intestinal alkaline phosphatase. The partially digested genomic DNA was then ligated to the vector. Recombinant cosmids were packaged into lambda phage *in vitro* and transduced into *E. coli* HB101. Km^r, Sp-sensitive (Sp^s) colonies were selected.

Bacterial conjugation. Triparental matings (Ditta *et al.* 1980) were performed by mixing 150 μl of late-logarithmic cultures (17 hr) of Cz-selected *X. c.* pv. *oryzae* at 10^{10} colony forming units (cfu) per milliliter with 20 μl each of *E. coli* HB101(pSa747) and HB101(pSa325) (5-hr-old cultures; 10^8 cfu/ml) in microtiter dish wells. Bacterial strains were grown in NB without antibiotics for mating experiments. The 190 μl mating mixture was incubated at 28° C for at least 16 hr. Transconjugants were selected on M4 or Cz containing Km at 28° C for 10–15 days. Transconjugants were selected from each mating and streaked on PSA containing Km and Cp to isolate pure single colonies.

Plant assays. Seeds of the rice differential cultivars Cas 209, IR8, IR20, IR1545-339, and DV85, which possess the bacterial blight resistance genes *Xa-10, Xa-11, Xa-4, xa-5*, and *Xa-7, xa-5*, respectively, were obtained from the International Rice Research Institute (Los Banos, the Philippines). Greenhouse-grown rice plants (28 days after seeding) were inoculated with bacterial suspensions of 5 × 10^9 cfu/ml (grown in 200 μl of NB in microtiter wells) using the double-needle technique (Barton-Willis *et al.* 1989). Inoculated plants were incubated in a growth chamber with a 13-hr photoperiod (28° C) followed by 11 hr of darkness at 22° C. Symptoms (lesion lengths) were assessed 8–10 days after inoculation.

Monitoring bacterial populations in rice leaves. Cas 209 leaves were inoculated as described above with suspensions of PXO61^{Cz}, PXO86^{Rif}, and PXO61^{Cz}(pSK8-4). To monitor bacterial multiplication in the plants, five leaves per treatment were sampled at 0 (immediately after inoculation), 3, 6, and 9 days after inoculation. Each of the five leaves was individually ground with a mortar and pestle in 1–5 ml of sterile distilled water. Serial dilutions were plated on the medium containing appropriate antibiotics and 75 μg/ml of cycloheximide (to suppress fungal contamination). Colonies of bacteria were counted after 4–5 days at 28° C.

Tn*4431* mutagenesis of pSK8-4. Tn*4431* mutagenesis of the cosmid clone pSK8-4 was conducted in *E. coli* C2110 (Table 1) as described by Shaw *et al.* (1988).

RESULTS

Cosmid stability in *X. c.* pv. *oryzae*. The presence of the cosmid pSa747 in *X. c.* pv. *oryzae* had no apparent effect on bacterial growth in culture or the pathogenicity patterns of strains PXO61^{Cz} or PXO99^{Cz} on the five rice differential cultivars (data not shown). In addition, pSa747 was stable in these strains of *X. c.* pv. *oryzae* in the absence of selection pressure in culture and until full disease expression in rice plants (8–10 days). Integration of pSa747 into the genome was not observed in *X. c.* pv. *oryzae*, in contrast to *X. c.* pv. *malvacearum* (Gabriel *et al.* 1986).

Construction of a race 2 genomic DNA library. One thousand five hundred Km^r *E. coli* HB101 colonies were individually selected. All were Sp^s, indicating insertional inactivation of the Sp gene. Restriction enzyme analysis of 30 randomly selected colonies revealed random DNA fragmentation and an average DNA insert size of 30 kilobases (kb).

Four clones from 1,500 Km^r and Sp^s transductants complemented *E. coli* HB101 to proline prototrophy. This number concurs with the prediction that, with an insert size of 30 kb, about 611 clones are needed to represent each gene of *X. c.* pv. *oryzae* at least once with a 99% probability, if the genome size of *X. c.* pv. *oryzae* approximated that of *E. coli* (4,000 kb) and DNA fragmentation was random (Clarke and Carbon 1976).

Identification and cloning of an avirulence gene. Five hundred clones in *E. coli* HB101 were individually mobilized by conjugation into PXO99^{Cz} (race 6, Table 1). The transconjugants were screened on Cas 209 (four plants per transconjugant). A clone, pSK8-4, containing a 2.5-kb DNA insert was identified which, when introduced into PXO99^{Cz}, changed the interaction with Cas 209 from compatible to incompatible (Fig. 1, Table 2). A second clone, pSK11-33 (35-kb DNA insert), also altered the interaction phenotype of transconjugants with Cas 209. However, transconjugants containing pSK11-33 caused

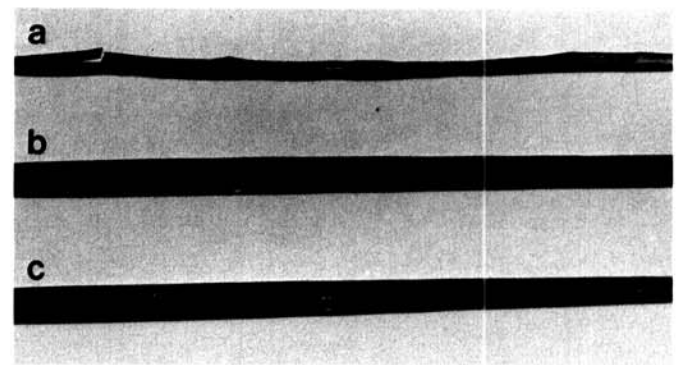

Fig. 1. Phenotypic interactions of strains of *Xanthomonas campestris* pv. *oryzae* (**a**) PXO61^{Cz}, (**b**) PXO61^{Cz}(pSK8-4), and (**c**) PXO86^{Rif} with rice cultivar Cas 209 nine days after inoculation.

Table 2. Interactions of strains of *Xanthomonas campestris* pv. *oryzae* and transconjugants containing cosmid clones from race 2 (PXO86^{Rif}) on five differential rice cultivars

Strain or transconjugant	Average lesion length (cm) per leaf of cultivar[a]				
	Cas 209	IR8	IR20	DV85	IR1545-339
PXO86^{Rif}	0.3a[b]	11.0ab	13.0d	0.4a	0.3a
PXO61^{Cz}	12.0c	10.4ab	3.5b	1.0ab	0.2a
PXO61^{Cz}(pSK8-4)	0.1a	9.4a	1.0a	0.5a	0.0a
PXO61^{Cz}(pSK11-33)	3.1b	10.5ab	3.0ab	2.7b	0.6a
PXO99^{Cz}	13.5c	12.0b	11.0c	10.0d	11.5b
PXO99^{Cz}(pSK8-4)	0.4a	11.0ab	11.0c	8.0c	11.5b
PXO99^{Cz}(pSK11-33)	2.7b	10.0ab	12.5cd	10.0d	10.0b
L.S.D.$_{0.01}$	2.3	2.6	2.1	1.9	3.1

[a] Leaves were inoculated by the double-needle technique (Barton-Willis *et al.* 1989). Lesions were measured 9 days after inoculation. Value (cm) is the mean lesion length of five inoculated leaves. 0.0–5.0 cm = resistant; 5.1–15.0 cm = susceptible.

[b] Means of lesion lengths were compared for interactions between strains within a cultivar. Values followed by common letters are not significantly different according to Duncan's multiple range test.

lesions longer than those observed with PXO86^{Rif} or transconjugants containing pSK8-4 (Table 2).

Both PXO99^{Cz}(pSK8-4) and PXO99^{Cz}(pSK11-33) were compatible on IR8, a cultivar with no known resistance gene to the Philippine strains of *X. c.* pv. *oryzae* (Table 2). The fragments contained in pSK8-4 and pSK11-33 shared no homology based on Southern blot hybridization experiments (data not shown). Because pSK8-4 conferred a host response to PXO99^{Cz} similar to the response to the parental strain PXO86^{Rif}, the clone was selected for characterization and subsequent experiments. The gene(s) contained in pSK8-4 responsible for the incompatible phenotype in Cas 209 were termed *avr10*.

Repeated inoculations of the rice differentials (IR8, Cas 209, IR1545-339, IR20, and DV85) with transconjugants containing pSK8-4 revealed that only the interaction on Cas 209 was altered (Table 2). The clone pSK8-4 isolated from transconjugants of *X. c.* pv. *oryzae* before and after plant inoculation was identical in size (2.5 kb) to that isolated from *E. coli* HB101. Southern blot hybridizations of *Eco*RI-digested genomic DNA from strain PXO86^{Rif} confirmed the presence of a homologous 2.5-kb fragment in the genome (described below).

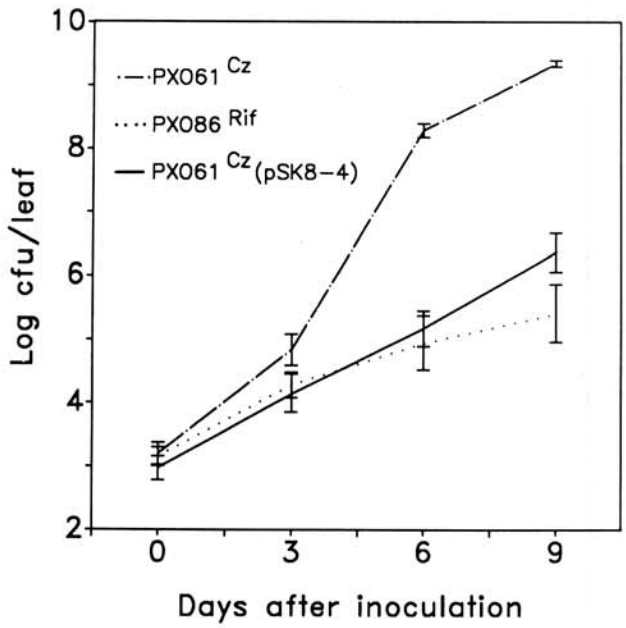

Fig. 2. Multiplication of strains of *Xanthomonas campestris* pv. *oryzae* PXO86^{Rif} (·····, race 2, incompatible), PXO61^{Cz} (— · — · —, race 1, compatible), and PXO61^{Cz}(pSK8-4) (——) in leaves of rice cultivar Cas 209. Values are means ± standard errors of the means for five replications of each treatment. Similar results were obtained from a second experiment.

Bacterial populations in inoculated Cas 209 leaves. The multiplication of PXO61^{Cz}, PXO86^{Rif}, and PXO61^{Cz}(pSK8-4) was monitored in Cas 209 leaves at 0, 3, 6, and 9 days after inoculation. The incompatible phenotype conferred by *avr10* resulted in lower bacterial numbers of PXO61^{Cz}(pSK8-4), *in planta*, than the numbers of PXO61^{Cz} in compatible interactions (Fig. 2). Six days after inoculation, bacterial numbers were about 10^4 cfu per leaf lower in rice leaves containing PXO61^{Cz}(pSK8-4) than in leaves containing PXO61^{Cz}.

Restriction enzyme mapping of pSK8-4 and transposon mutagenesis. To assist in restriction mapping, the *Eco*RI fragment contained in pSK8-4 was cloned into pBluescript KS+ (Stratagene, La Jolla, CA), constructing the plasmid pBS8-4. The fragment contained two *Pst*I, two *Pvu*II, four *Bgl*I, and two *Sal*I restriction enzyme sites (Fig. 3).

The clone pSK8-4 was subjected to mutagenesis with Tn*4431* (Shaw *et al.* 1988). Plasmids containing tetracycline resistance markers were mobilized into strain PXO61^{Cz} of *X. c.* pv. *oryzae*, and transconjugants were screened on Cas 209 for loss of the avirulence phenotype. A clone, pSK8-4::Tn*4431*, when introduced into PXO99^{Cz}, caused no avirulence phenotype on Cas 209 (lesion length, 12.3 ± 3.1, mean ± SD). The Tn*4431* was contained within the 0.4-kb *Pst*I-*Pvu*II fragment in pSK8-4 (data not shown). Attempts at marker exchange mutagenesis with pSK8-4::Tn*4431* were unsuccessful; therefore, inactivation of the genomic copy of *avr10* in PXO86^{Rif} (race 2) was not shown.

Restriction endonuclease analysis of the six *X. c.* pv. *oryzae* races. Twenty-three strains representing the six races of *X. c.* pv. *oryzae* were analyzed using pSK8-4 as a probe. All strains possessed sequence similarity with pSK8-4 (for example, Fig. 4). DNA of *X. c.* pv. *oryzae* did not hybridize with DNA of vector pSa747 (data not shown). All strains of races 1 and 4 that were tested contained one *Eco*RI DNA fragment (about 9 kb) which hybridized to pSK8-4 (Fig. 4). Two *Eco*RI fragments (2.5 and about 8 kb) from strains of races 2, 5, and 6 consistently hybridized with the probe (Fig. 4). Race 3 strains were variable in their hybridization pattern, with some being like those of races 2, 5, and 6 (Fig. 4, lane e) and others being like those of races 1 and 4 (data not shown). Plasmids isolated from PXO112, PXO40, PXO124, and PXO80 did not hybridize with pSK8-4 (data not shown).

Digestion of genomic DNA with *Eco*RI did not reveal polymorphisms that were correlated with *avr10* activity, that is, strains of race 2 (incompatible to Cas 209) and

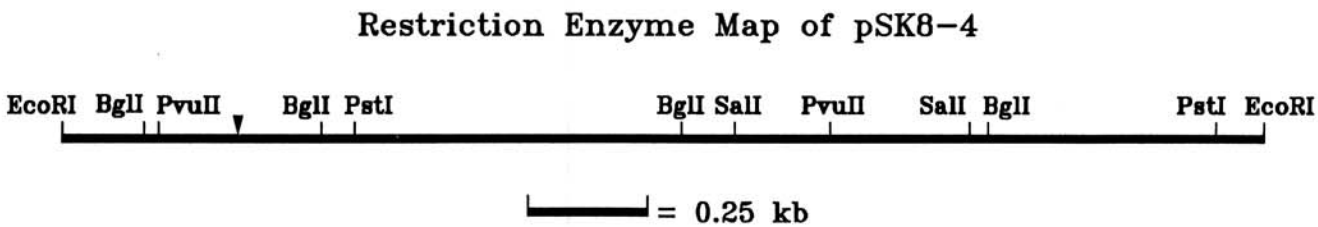

Fig. 3. Restriction enzyme map of the 2.5-kilobase (kb) *Eco*RI fragment of pSK8-4. The arrow indicates the fragment insertionally inactivated by Tn*4431*.

race 6 (compatible to Cas 209) revealed identical patterns after probing with pSK8-4 (Fig. 4, lanes b, c, d, h, and i; Fig. 5, lanes j and l). To determine if DNA from various races of *X. c.* pv. *oryzae* had restriction fragment length polymorphisms (RFLPs) that correlated with phenotypic expression of the *avr10* gene, genomic DNA from strains of race 1 (PXO61^{Cz}, PXO35, and PXO84), race 2 (PXO86^{Rif}, PXO126, and PXO63), and race 6 (PXO99^{Cz}, PXO114, and PXO116) was digested with *Bgl*I, which has four restriction sites within the cloned *Eco*RI fragment (Fig. 3), and probed with pSK8-4. The genomic DNA was treated with *Bgl*I alone or in double digests with *Eco*RI. Fragments less than 0.2 kb were not detected in these analyses. Four of the five fragments that resulted from double digestion with *Eco*RI and *Bgl*I (750, 640, 570, and 370 base pairs [bp]) were identical in all strains after hybridization with pSK8-4 (Fig. 5, lanes e–h). Three of those fragments (750, 640, and 570 bp) were revealed by digestion with *Bgl*I (Fig. 5, lanes b–d). A *Bgl*I fragment of about 2.8 kb present in DNA from race 2 and race 6 strains was not observed in DNA from race 1 strains. Race 1 strains contained a *Bgl*I fragment of about 2.5 kb that was not observed in race 2 and race 6 strains.

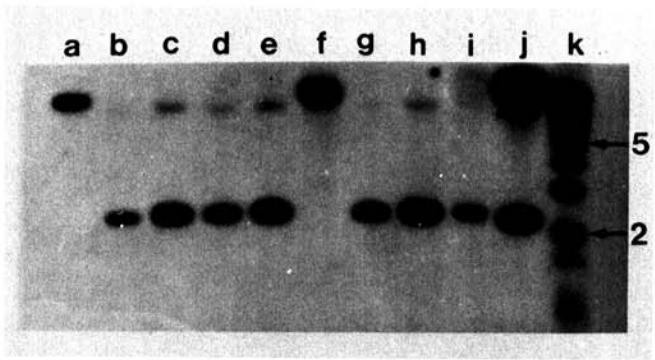

Fig. 4. Southern blot analysis of *Eco*RI-digested DNA from strains of different races of *Xanthomonas campestris* pv. *oryzae*. DNA from strains (races) of *X. c.* pv. *oryzae* are in the following lanes: a, PXO20 (1); b, PXO86^{Rif} (2); c, PXO103 (2); d, PXO63 (2); e, PXO143 (3); f, PXO113 (4); g, PXO145 (5); h, PXO123 (6); i, PXO118 (6); and j, pSK8-4. In lane k is a BRL (Bethesda Research Laboratories) 1-kilobase ladder. The probe was ^{32}P-labeled pSK8-4.

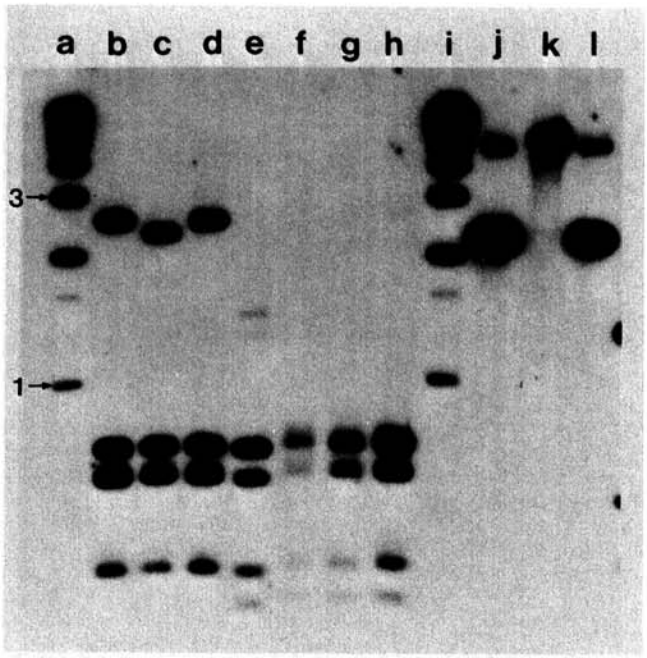

Fig. 5. Restriction analysis of DNA from strains of different *Xanthomonas campestris* pv. *oryzae* races probed with ^{32}P-labeled pSK8-4. DNA was digested with *Bgl*I (lane b, PXO86^{Rif}; lane c, PXO61^{Cz}; and lane d, PXO99^{Cz}); *Bgl*I and *Eco*RI (lane e, pBS8-4; lane f, PXO86^{Rif}; lane g, PXO61^{Cz}; and lane h, PXO99^{Cz}); and *Eco*RI (lane j, PXO86^{Rif}; lane k, PXO61^{Cz}; and lane l, PXO99^{Cz}). Lanes a and i contain a BRL 1-kilobase ladder.

Southern blot analysis of other pathovars of *X. campestris*. Strains from 16 other pathovars of *X. campestris* (Pammel) Dowson also contained DNA sequences homologous to pSK8-4 (for example, Fig. 6). DNA from two other species of *Xanthomonas, X. albilinians* (Ashby) Dowson (Fig. 6, lane l) and *X. fragariae* Kennedy and King (data not shown), also hybridized with pSK8-4. The rice brown blotch pathogen, a *Pseudomonas* spp. (Vera Cruz *et al.* 1984), did not hybridize with pSK8-4 (Fig. 6, lane m).

DISCUSSION

We have cloned from *X. c.* pv. *oryzae* PXO86^{Rif} a 2.5-kb DNA fragment containing a functional gene, *avr10*, which controls bacterial elicitation of resistance in rice cultivars carrying the *Xa-10* gene. In the absence of a typical hypersensitive response (Parry and Callow 1986; Barton-Willis *et al.* 1989), the change from a susceptible (compatible) to a resistant (incompatible) response is measured as reduced lesion lengths (Ou 1985) and lower final bacterial numbers per leaf (Barton-Willis *et al.* 1989). The presence of cosmid pSK8-4, which contains *avr10*, in a strain of race 1 (PXO61^{Cz}) resulted in both shorter lesions (Table 2) and lower final bacterial numbers (Fig. 2), specifically in cultivar Cas 209. The response in other cultivars was not altered (Table 2). The phenotypic expression of *avr10* was shown in race 1 (PXO61^{Cz}) and race 6 (PXO99^{Cz}) strains. Therefore, the expression of the gene is not strain-specific.

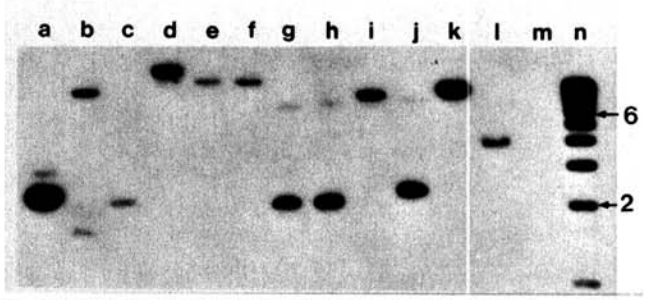

Fig. 6. Southern blot analysis of *Eco*RI-digested DNA from different pathovars of *Xanthomonas campestris* probed with ^{32}P-labeled pSK8-4. Lanes: a, pSK8-4; b, *X. c.* pv. *vesicatoria*; c, *X. c.* pv. *malvacearum*; d, *X. c.* pv. *oryzicola*; e, *X. c.* pv. *translucens*; f, *X. c.* pv. *secalis*; g, *X. c.* pv. *vasculorum*; h, *X. c.* pv. *holcicola*; i, *X. c.* pv. *campestris*; j, *X. c.* pv. *oryzae* PXO86^{Rif}, race 2; k, *X. c.* pv. *oryzae* PXO61^{Cz}, race 4; l, *X. albilinians*; m, *Pseudomonas* spp.; and n, BRL 1-kilobase ladder.

Race 1 merodiploids containing inactivated *avr10* (pSK8-4::Tn*4431*) were pathogenic on Cas 209. It would be predicted, based on the gene-for-gene hypothesis, that the mutation of the dominant avirulence allele for which the strain was heterozygous (merodiploid) would make the strain pathogenic on the cultivar carrying the corresponding resistance gene (Flor 1959). Therefore, our study offers additional compelling evidence that resistant interactions between *X. c.* pv. *oryzae* and rice follow a gene-for-gene type interaction.

Sequences homologous to pSK8-4 were present in strains of all races of *X. c.* pv. *oryzae* as shown in the Southern blot experiments (Fig. 4). RFLP analysis of genomic DNA from strains of race 2 (incompatible to Cas 209) and races 1 and 6 (both compatible to Cas 209) revealed differences between the patterns from race 1 strains and those from strains of races 2 and 6 (Fig. 5). However, there were no differences in patterns generated by DNA from race 2 (incompatible to Cas 209) and race 6 (compatible to Cas 209) strains, even in the small fragments (370–750 bp) generated by *Eco*RI and *Bgl*I digestions. Therefore, the RFLP analysis revealed no polymorphisms that correlated with expression of the *avr10* phenotype. This suggests the presence of recessive or nonfunctional alleles for *avr10* in *X. c.* pv. *oryzae*.

Similarly, Gabriel *et al.* (1986) used RFLP analysis to compare two *X. c.* pv. *malvacearum* strains, one containing three active *avr* genes and another lacking active *avr* genes. Comparison of the hybridization intensities and positions of fragments that hybridized with a cosmid clone containing all three *avr* genes revealed only minor differences between the strains. The authors suggested that, since there were no major DNA rearrangements over the 32-kb stretch of DNA, alleles of the *avr* genes were present in the virulent strain.

In contrast, Swanson *et al.* (1988) found that a DNA fragment containing the $avrBs_1$ locus only hybridized to DNA from races of *X. c.* pv. *vesicatoria* which were avirulent on the pepper cultivar ECW10R. So far, $avrBs_1$ is the only avirulence gene from *X. campestris* that is described as plasmid-borne. This is of interest because it raises the possibility that $avrBs_1$ may have been acquired from other species or pathovars through plasmid transfer. To the best of our knowledge, *avr10* is not plasmid-borne (data not shown).

Other pathovars of *X. campestris* also contained DNA sequences that hybridized to pSK8-4 (Fig. 6). Kobayashi *et al.* (1989) reported that a 5.6-kb DNA fragment cloned from *Pseudomonas syringae* pv. *tomato* caused a hypersensitive response in certain soybean cultivars when introduced into *P. s.* pv. *glycinea*. The 5.6-kb fragment contained an avirulence gene homologous to *avrA*, the avirulence gene first cloned from *P. s.* pv. *glycinea* (Staskawicz *et al.* 1984), and in addition, the fragment hybridized to identical size fragments of other pathovars of *P. syringae*. pSK8-4, a 2.5-kb fragment, did not hybridize to identically sized *Eco*RI fragments in other pathovars of *X. campestris*. In fact, the patterns generally differed from the two patterns characteristic of races of *X. c.* pv. *oryzae* (Fig. 6). It is possible that the hybridization to DNA from other pathovars was to sequences flanking *avr10*. Therefore, we cannot conclude that *avr10* is present or, if present, functional in other pathovars.

A second clone, pSK11-33, was identified that also changed virulent race 1 and race 6 strains (lesion size 8–14 cm) to avirulent (lesion size 2–4 cm) on Cas 209. The host response was quantitatively different from that observed after inoculation with transconjugants containing pSK8-4 (lesion size 0–0.5 cm). In addition, pSK8-4 and pSK11-33 share no sequence homology. Because Cas 209 is not a near-isogenic cultivar, it is possible that pSK11-33 contains a second avirulence gene corresponding to a resistance gene other than *Xa-10* in Cas 209 which was not detected by classical genetic analysis. If this is true, then cloned avirulence genes may prove to be powerful tools with which to detect resistance genes in segregating breeding populations.

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