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Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr.

(plant–parasite interaction/disease resistance/cosmid cloning/ice nucleation)

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ABSTRACT **A genomic library of *Pseudomonas syringae* pv. *glycinea* race 6 DNA was constructed in the mobilizable cosmid vector pLAFR1 and maintained in *Escherichia coli* HB101. Completeness of the library was estimated by assaying clones for the expression of ice-nucleating activity in *E. coli*. Ice-nucleation activity was represented approximately once in every 600 clones. Six hundred eighty random race 6 cosmid clones were mobilized from *E. coli* by plasmid pRK2013 in individual conjugations to a race 5 strain of *P. s. glycinea*. A single clone (pPg6L3) was detected that changed the race specificity of race 5 from virulent (compatible) to avirulent (incompatible) on the appropriate soybean cultivars. The clone was also mobilized from *E. coli* into race 1 and race 4 strains of *P. s. glycinea*, and it conferred on these transconjugants the same host range incompatibility as the wild-type race 6 strain. The cosmid clone was mapped by restriction endonucleases, and two adjacent *Eco*RI fragments were identified by transposon Tn*5* mutagenesis to be important in determining race specificity. Southern blot analysis showed that the two *Eco*RI fragments are unique to race 6 and are not present in the other races tested. The cosmid clone pPg6L3 was also mobilized to *Pseudomonas fluorescens* and *Rhizobium japonicum*. However, neither these isolates nor *E. coli* harboring pPg6L3 elicited a hypersensitive reaction in soybean leaves.**

Pseudomonas syringae pv. *glycinea*, the causal agent of bacterial blight of soybean, has been the subject of several comparative biochemical and physiological experiments aimed at elucidating the mechanism(s) involved in disease resistance (reviewed in ref. 1). These studies have suggested that resistance to incompatible (avirulent) races of *P. s. glycinea* is phenotypically conferred by a host response commonly referred to as the hypersensitive reaction that is controlled by a single dominant gene present in the host (2). The hypersensitive reaction is a necrotic reaction of plant cells in the vicinity of an invading incompatible pathogen. These host cells also accumulate large amounts of antibiotic phytoalexins called glyceollins. The available evidence indicates, but does not prove, that the phytoalexins cause the restriction of bacterial multiplication observed in hypersensitive, resistant leaves (1).

The expression of disease resistance in plants, including the hypersensitive-type resistance, results from the interplay of discrete genes in both the host and the pathogen (3) and appears to be due to a specific early recognition event (4). Infection of plants with compatible (virulent) races, on the other hand, does not lead to a resistant reaction; instead, the pathogen multiplies rapidly in the host. The genetic data have led to the gene-for-gene complementarity hypothesis first formulated by Flor (5). According to this model, the phenotype of hypersensitive disease resistance occurs only when dominant genes in the pathogen at avirulence loci interact with dominant alleles of complementary disease-resistance genes in the plant. Pathogen biotypes exhibiting various combinations of avirulence genes can be differentiated by inoculation of several host-resistant genotypes; those pathogen strains that give unique patterns are grouped into races (see Table 2).

We chose bacterial blight of soybean as a model system to study the molecular basis of race specificity because it potentially represented a gene-for-gene system in which natural variability in the host for resistance and the pathogen for virulence had been established. To date, nine races of *P. s. glycinea* have been identified that give unique patterns of hypersensitive resistant or susceptible reactions when inoculated in various soybean cultivars (6, 7). Although the gene-for-gene concept is firmly established for many fungal–host interactions, definitive proof for its existence in bacterial–plant interactions has thus far been impossible because of the lack of a useful genetic system to study the inheritance of avirulence genes in bacterial phytopathogens. Limited genetic crosses in soybean have identified a single dominant gene (*Rpg1*) for resistance to race 1 of *P. s. glycinea* in the cultivars Norchief and Harosoy, suggesting that a gene-for-gene relationship may exist in this particular host–pathogen interaction (2).

Our work has been directed to the development of a genetic system for *P. s. glycinea* by constructing genomic libraries of several races of the pathogen in the cosmid vector pLAFR1 (8) and mobilizing a DNA library maintained in *Escherichia coli* to various races of *P. s. glycinea*. We herein report the molecular identification and partial characterization of a race-specificity gene from race 6 of *P. s. glycinea*. The molecular genetic evidence supports the validity of the gene-for-gene hypothesis in bacterial–plant interactions and serves as a basis for further research on the molecular mechanisms that determine plant–pathogen specificity and expression of disease resistance in higher plants.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Transposons. The sources of bacterial strains and their relevant characteristics are shown in Table 1. Rifampicin-resistant (Rif^r) isolates of *P. s. glycinea* were obtained by spreading fresh cells (5×10^9/ml) onto King's medium B (Rif) (8) agar supplemented with rifampicin (100 mg/liter). After 3–4 days of incubation at 28°C, 3–20 Rif^r colonies grew on the agar. These cells were streaked onto King's medium B (Rif) plates to obtain single-colony isolates. Each Rif^r isolate was confirmed with respect to race identity by the host-response pattern on the

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Abbreviations: Rif^r, rifampicin-resistant; Psg1, Psg4, Psg5, and Psg6, *Pseudomonas syringae* pv. *glycinea* races 1, 4, 5, and 6; kb, kilobase(s).

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Table 1. Bacterial strains, plasmids, and transposons

	Genotype or relevant characteristics	Source (ref.)
	Strains	
P. s. glycinea (Psg1)	Race 1 (A2159) Rif^r	N. Keen (7)
P. s. glycinea (Psg4)	Race 4 (A29-2) Rif^r	N. Keen (7)
P. s. glycinea (Psg5)	Race 5 Sm^r Rif^r	N. Keen (6)
P. s. glycinea (Psg6)	Race 6 Rif^r	N. Keen (6)
E. coli HB101	F^-, *hsdS2o* (r_B^-, m_B^-), *recA13*, *ara-14*, *proA2*, *lacY1*, *galK2*, *rpsL20*(Sm^r), *xyl-5*, *mtl-1*, *supE44*, λ^-	N. Panopoulos
E. coli DH1	F^-, *recA1*, *endA1*, *gryA96*, *thi-1*, *hsdR17*, (r_{K1},m_{1+}), *supE44*, λ^-	S. Long
P. fluorescens	Rif^r	N. Keen
R. japonicum 194	Rif^r	C. Napoli
	Plasmids	
pLAFR1	Tc^r, cosmid derivative of pRK290	S. Long (9)
pRK2013	$Km^r Tra^+$, helper plasmid	S. Long (10)
pPg6L3	Tc^r, contains race 6-specificity gene	This paper
pPg6L3::Tn*5-1*	Tn*5* insertion into *Eco*RI band 6	This paper
	Transposon	
λ467:λb221rex::Tn*5*	*cI857 O*am29 *P*am80	R. Riedel (10)

Sm^r, streptomycin resistant; Tc^r, tetracycline resistant; Km^r, kanamycin resistant.

appropriate soybean cultivars. All Rif^r isolates also had the same indigenous plasmid profile as the wild type (unpublished observations).

Growth Media and Antibiotic Levels. *P. s. glycinea* was routinely subcultured on King's medium B. *E. coli* strains were grown in either LB medium (9) or King's medium B. The following levels of antibiotics were used unless otherwise indicated: kanamycin, 10 mg/liter; rifampicin, 100 mg/liter; tetracycline, 10 mg/liter; streptomycin, 25 mg/liter. Antibiotics were purchased from Sigma.

Growth of Plants and Plant Inoculations. Soybean seeds were grown in 8-oz (228 g) paper cups (Maryland Cup) filled with a commercial potting medium at 24°C daytime (5000 lux) (16 hr) and 18°C nighttime (8 hr). In some experiments, plants were grown in a greenhouse. Freshly grown cells of *P. s. glycinea* were prepared by inoculating a tube containing 10 ml of King's medium B with a loopful of cells and allowing them to grow overnight at 28°C. Twenty-five-microliter samples of this suspension were adjusted to 5×10^8 cells per ml and then dispensed into sterile Microfuge tubes and used as inoculum.

Each race of *P. s. glycinea* was inoculated into all of the soybean cultivars shown in Table 2. A rapid inoculation technique was used through most of the work. Small wounds were made with a needle on the undersurface of fully expanded soybean primary leaves (10 wounds per leaf), with care taken to not punch entirely through the leaf. Then, 25 μl of bacterial suspension was infiltrated into the wounded areas with a disposable plastic Pasteur pipette such that a small water-soaked area surrounded the wound. Plants were then returned to the pre-inoculation environment and observed for 4–6 days for visible reaction. Hypersensitive resistant reactions were typified by visible host cell necrosis 24–48 hr after inoculation, with little or no subsequent change and no water-soaking. On the other hand, compatible or susceptible reactions were characterized by the absence of any visible host reaction for 48 hr, followed by the subsequent appearance of spreading water-congested areas around the wound areas in 5–6 days.

In addition, all four races of *P. s. glycinea* were confirmed by inoculating them onto two positions of a primary soybean leaf without wounding by use of a Hagborg apparatus as described (11). As above, visible plant reactions were scored daily for 4–6 days after inoculation.

Bacterial Populations in Inoculated Leaves and Glyceollin Levels. Tests were made to determine whether the visible hypersensitive plant reactions observed in response to bacteria containing cloned DNA sequences were associated with restriction of bacterial populations and phytoalexin accumulation. Leaves inoculated with the Hagborg device were analyzed for bacterial populations and glyceollin levels as described (11).

Recombinant DNA Techniques. Basic techniques used such as plasmid preparation, ligation conditions, preparation of competent cells, transformation protocol, plasmid miniscreening, gel electrophoresis, construction of restriction maps, Southern transfer, and hybridization of filters are described in ref. 12.

Total DNA Isolation from *P. s. glycinea*. The following is a modification of the procedure described by Marmur (13). DNA was isolated from 100 ml of *P. s. glycinea* cell suspension shaken overnight at 28°C in King's medium B. Cells were then suspended in 100 ml of TES buffer (10 mM Tris·HCl, pH 8/1 mm EDTA/50 mM NaCl) and lysed by the addition of 8 ml of 20% (wt/vol) $NaDodSO_4$. Twelve milliliters of 3 M sodium acetate was added and DNA was precipitated by the addition of 64 ml of isopropanol. The precipitated DNA was suspended in TE buffer (10 mM Tris·HCl, pH 7.6/1 mm EDTA) and purified by CsCl/ethidium bromide ultracentrifugation in a Beckman VTi 50 rotor for 18 hr at 50,000 rpm. The fluorescent DNA band was collected, ethidium bromide was removed by extraction with isopropanol saturated with NaCl, and CsCl was removed by dialysis against TE buffer.

Construction of Genomic Libraries. Genomic libraries were constructed in the cosmid vector pLAFR1 according to published procedures (9).

Plate Matings. Triparental matings (10) were carried out by mixing 100 μl of Rif^r *P. s. glycinea* at 5×10^9 cells per ml with 25 μl each of HB101 (pLAFR1 clones) and HB101 (pRK2013) (each at 5×10^8 cells per ml). The mixture was spread onto a single Petri dish containing King's medium B and incubated at 28°C for 16 hr. The cells were retrieved by adding 2 ml of sterile water to the agar surface and preparing a turbid bacterial suspension by mixing with a glass rod. The mixture was plated onto a Petri dish of King's medium B containing tetracycline and rifampicin and incubated at 28°C. One transconjugant colony from each cross was restreaked on the same medium and a single colony was isolated.

In some experiments, 25 conjugations were conducted on a single Petri plate by inoculating the HB101 (pLAFR1 clones) cells onto King's medium B in a small patch, then adding 10 μl of a mixture of Rif^r *P. s. glycinea* and HB101 (pRK2013) cells to the patch, mixing, and incubating as

above. A single resultant transconjugant from each patch was restreaked as above.

Restriction Enzyme Analysis and Ligations. Restriction endonucleases and T4 DNA ligase were purchased from Bethesda Research Laboratories or New England Biolabs, and reactions were carried out as recommended by the manufacturers.

Transposon Tn*5* Mutagenesis of pPg6L3 and Mobilization of Random Mutagenized Clones to *P. s. glycinea*. Phage λ::Tn*5* mutagenesis of HB101 (pPg6L3) was carried out according to Ruvkun and Ausubel (14). A mixture of kanamycin-resistant colonies was scraped from the initial selection plate and these mutagenized pPg6L3 clones were mobilized *en masse* to *P. s. glycinea* race 5 using the helper plasmid HB101 (pRK2013) (13). Transconjugants were selected on King's medium B containing tetracycline, rifampicin, and kanamycin, and the resultant single colonies were then inoculated individually onto the soybean cultivar Harosoy and the plant reactions were scored. Clones that gave an altered plant reaction were further processed by recovering the mutagenized cosmid clone from the *P. s. glycinea* race 5 transconjugant by the "boil miniscreen" method. Because indigenous plasmids in race 5 interfered with analysis of the cosmid clone, the plasmid DNA was used to transform competent *E. coli* DH1 cells with selection for tetracycline resistance. The plasmid DNA was isolated from *E. coli* DH1 and used for restriction enzyme analysis.

Southern Blot Analysis of pPg6L3. Total DNA of races 1, 4, 5, and 6 was digested to completion with *Eco*RI and electrophoresed in 0.7% agarose (as above). The DNA was transferred to nitrocellulose and individually probed with ^{32}P-labeled nick-translated electroeluted *Eco*RI fragments 6, 7, 1, and 3 (see Figs. 2 and 3). Hybridizations were carried out for 16 hr in 50% formamide/32 mM NaCl/3.3 mM sodium citrate at 42°C. The filters were washed with 1.3 mM NaCl/0.13 mM sodium citrate/0.1% NaDodSO$_4$ at 65°C for 2 hr. Filters were dried and autoradiography was carried out at −80°C using Kodak XAR-5 film and DuPont Cronex Lightning Plus intensifying screens.

RESULTS

Race Reactions of *P. s. glycinea* on Soybean Cultivars. Although nine races of *P. s. glycinea* have been reported (6), we have found that not all the races react as reported in the literature. We chose to use only races 1, 4, 5, and 6, because they gave consistent and highly differentiated responses when inoculated onto seven different soybean cultivars (Table 2). We also determined that the varieties Hardee and Peking give unique responses to these four races that were not reported in ref. 6.

Mobilization of pLAFR1 to *P. s. glycinea* Races 1, 4, 5, and 6. The first step in developing a genetic system in *P. s. glycinea* was to test the efficiency of mobilizing pLAFR1 to the various races. *P. s. glycinea* race 5 (hereafter called Psg5) was the most efficient recipient in conjugation experiments (10^{-4} transconjugants per recipient cell) while the other three races were less efficient ($<10^{-7}$ transconjugants per recipient cell). For this reason, Psg5 was chosen as the recipient for detecting race-specificity genes from a genomic cosmid library made from *P. s. glycinea* race 6 (hereafter called Psg6).

Construction of a pLAFR1 Psg6 DNA Bank. Through empirical methods, we have determined that isolation of "clean" DNA is an essential step for constructing genomic libraries. Isolation of DNA by methods not using CsCl/ethidium bromide ultracentrifugation has resulted in poor pLAFR1 libraries. In a typical experiment, we isolated 5×10^5 transductants per μg of DNA and determined that >90% of the clones contained inserts. Twenty-four random tetracycline-resistant transductants were assayed by digesting miniscreen DNA with *Eco*RI. The average size of the insert was ≈25 kilobases (kb).

Estimation of Gene Frequency in the Library. To determine the frequency of any random gene in the library, we chose to assay an easily scorable gene. This was done by determining the occurrence of ice-nucleating clones by the freeze-replica assay (15). Because Psg6 is able to cause ice nucleation (6), we scored for the presence and expression of this gene in the HB101 cosmid clones. More than 1800 tetracycline-resistant transductants were screened and three ice-nucleation-positive clones were found.

Identification of the Race 6 Avirulence Gene. Psg5 causes a compatible reaction on the cultivars Harosoy and Peking while Psg6 causes an incompatible reaction (Table 2). Because the gene-for-gene hypothesis suggests that specificity is for incompatibility (3), we predicted that a race-specificity gene from Psg6 would be detected by mobilization of the race 6 library into Psg5 and selection of any clone(s) that caused a visible hypersensitive reaction on soybean cultivars Harosoy and Peking. To test the possibility that specificity might be for compatibility, cultivars Flambeau and Norchief were also inoculated and screened for any clones in Psg5 that caused a compatible reaction. None of the latter were found, but a single pLAFR1 clone of the 680 tested gave an incom-

Table 2. Phenotypes of wild-type *P. s. glycinea* isolates and transconjugants containing the race-specificity cosmid clone pPg6L3 when inoculated into soybean leaves

	Compatibility of soybean cultivar						
P. s. glycinea race	Acme	Chippewa	Flambeau	Hardee	Harosoy	Norchief	Peking
Psg1 (wild type)	+	−	+	−	−	−	+
Psg1 (pPg6L3)	−	−	+	−	−	−	−
Psg1 (pPg6L3::Tn*5-1*)	+	−	+	−	−	−	+
Psg4 (wild type)	+	+	+	+	+	+	+
Psg4 (pPg6L3)	−	−	+	+	−	+	−
Psg4 (pPg6L3::Tn*5-1*)	+	+	+	+	+	+	+
Psg5 (wild type)	−	−	−	+	+	−	+
Psg5 (pPg6L3)	−	−	−	+	−	−	−
Psg5 (pPg6L3::Tn*5-1*)	−	−	−	+	+	−	+
Psg6 (wild type)	−	−	+	+	−	+	−

Visible plant reactions after inoculation were scored as follows: +, compatible (susceptible) reaction involving no visible plant reaction for ≈48 hr followed by water-soaking and chlorosis symptoms; −, incompatible (resistant) reaction with considerable host cell necrosis observed around the infection site at 24 hr, with no appearance of subsequent compatible symptoms.

patible phenotype on cultivars Harosoy and Peking. This recombinant cosmid was designated pPg6L3 and consisted of pLAFR1 with a 27.2-kb insert of Psg6 DNA.

Several tests were performed to ensure that the observed hypersensitive reactions were caused by Psg5 (pPg6L3) and not a contaminant bacterium. Wild-type Psg5 has a characteristic indigenous plasmid that is not present in Psg6. This plasmid pattern was also found in Psg5 (pPg6L3), indicating that the transconjugant was indeed Psg5 (data not shown). The pLAFR1 clone, pPg6L3, was then conjugated from *E. coli* to race 1 and race 4 isolates of *P. s. glycinea* (Psg1 and Psg4). These transconjugants were inoculated onto soybean leaves to observe visible changes in host response. The pPg6L3 clone converted Psg4, which is normally compatible on all tested soybean cultivars, to precisely the Psg6 phenotype (Table 2). Miniscreening again showed that the transconjugant had an indigenous plasmid pattern indistinguishable from that of wild-type race 4 and not race 6. In addition, Psg1 (pPg6L3) was characterized by incompatible reactions on all soybean cultivars resistant to either race 1 or race 6 (Table 2) but not on cultivar Flambeau, which is compatible with both wild-type races.

Bacterial Populations and Glyceollin Accumulation. Additional experiments were performed to determine the effect of the avirulence gene clone (pPg6L3) on bacterial populations and glyceollin accumulation in various races of *P. s. glycinea* inoculated into soybean leaves (Table 3). As expected, the phenotypic change from virulence to avirulence was always accompanied by a factor of 10 reduction in bacterial growth after 48 hr. When the reactions were allowed to continue for 5 days, growth was reduced by a factor of 10^3–10^4 in races containing pPg6L3 on the appropriate host cultivar (data not shown). Thus, the change from virulence to avirulence was always associated with rapid appearance of the hypersensitive reaction, reduction of bacterial growth, and the accumulation of glyceollins.

Restriction Enzyme Mapping and Tn*5* Mutagenesis. The restriction enzyme map of pPg6L3 is shown in Fig. 1, and the *Eco*RI fragments responsible for race 6 avirulence were identified by Tn*5* mutagenesis. Of 180 Tn*5*-mutagenized clones screened, 4 were identified in which the race-specificity gene was inactivated as shown by loss of the race 6 phenotype when Psg5 (pPg6L3::Tn*5*) was inoculated into soybean plants. Restriction enzyme mapping showed that three Tn*5* insertions occurred in *Eco*RI fragment 6 (0.95 kb) and one mapped in the adjacent *Eco*RI fragment 7 (0.56 kb) (Fig. 1).

Table 3. Effect of pPg6L3 on bacterial growth and glyceollin accumulation in inoculated soybean leaves

Race	Cultivar	Reaction	Growth, cfu × 10^9/g leaf tissue	Glyceollin, μg/g leaves
Psg4	Acme	S	33.0 ± 4.7	30
Psg4 (pPg6L3)	Acme	R	4.6 ± 0.8	153
Psg6	Acme	R	3.4 ± 1.0	130
Psg4	Harosoy	S	31.0 ± 7.9	38
Psg4 (pPg6L3)	Harosoy	R	2.8 ± 1.8	190
Psg6	Harosoy	R	3.4 ± 0.8	140
Psg4	Flambeau	S	28.0 ± 9.8	33
Psg4 (pPg6L3)	Flambeau	S	21.0 ± 5.2	42
Psg6	Flambeau	S	21.0 ± 8.2	39

Bacterial growth (cfu, colony forming units) and glyceollin concentrations were determined after 48 hr as described (9). Bacterial growth level results are means ± SEM of three separate experiments, each with two replications. S, susceptible reaction; R, resistant reaction.

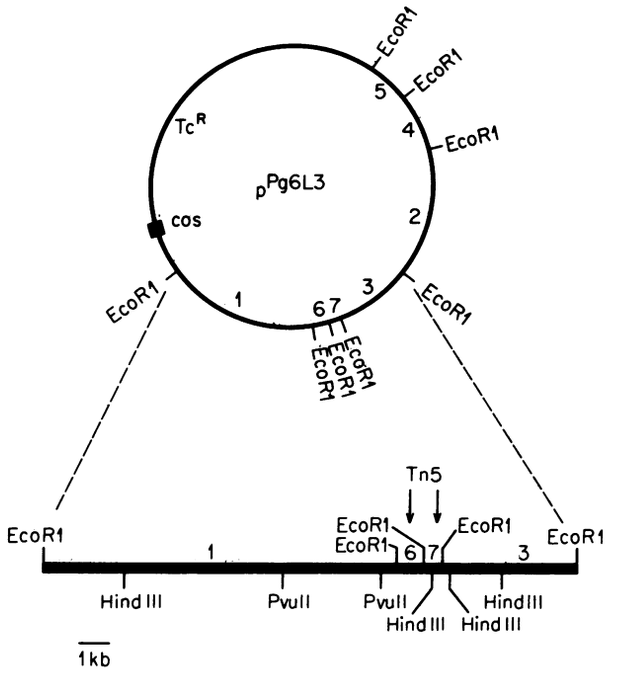

FIG. 1. Restriction map of the cosmid clone (pPg6L3) and a smaller segment of *P. s. glycinea* DNA containing the region coding for race-specific incompatibility as determined by transposon Tn*5* mutagenesis. Cosmid pPg6L3 consists of the vector pLAFR1 and 27.2 kb of *P. s. glycinea* DNA (*Eco*RI fragments 1–7). *Eco*RI fragments 1, 6, 7, and 3 are enlarged to show the region of the cosmid clone associated with the phenotype of race-specific incompatibility. Tn*5* insertions into *Eco*RI fragments 6 and 7 resulted in loss of the race-specific incompatibility. These four bands were individually isolated by electroelution and used as ^{32}P-labeled nick-translated probes against all four races of *P. s. glycinea* (see Figs. 2 and 3).

Southern Blot Analysis of the Race 6 Avirulence Gene. The results of the Tn*5* mutagenesis suggested that the avirulence gene might be contained in the adjacent *Eco*RI fragments 6 and 7. Using these fragments as probes against the DNA of all four races, we found that at least part of the race 6 avirulence gene is unique to race 6 and that *Eco*RI fragments 6 and 7 did not hybridize to races 1, 4, and 5 under the hybridization conditions tested (Fig. 2). Surprisingly, fragments 1 and 3 resulted in multiple hybridizations (Fig. 3). It is not yet known which regions of fragments 1 and 3 contain the multi-

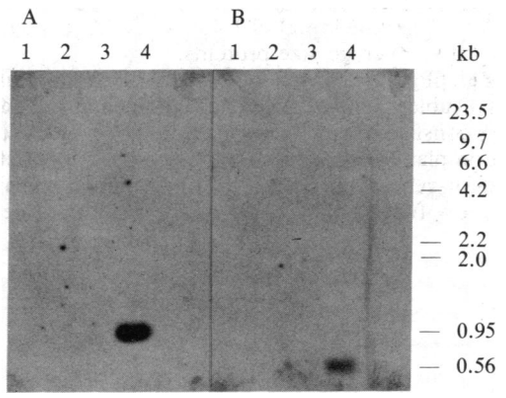

FIG. 2. Southern blot analysis of four races of *P. s. glycinea* DNA digested with *Eco*RI. Lanes: 1, Psg1; 2, Psg4; 3, Psg5; 4, Psg6. The four races were probed with ^{32}P-labeled nick-translated *Eco*RI fragments 6 (*A*) and 7 (*B*) (Fig. 1). In both blots, a hybridizing band was only seen with Psg6 (lane 4), the race from which the DNA was cloned.

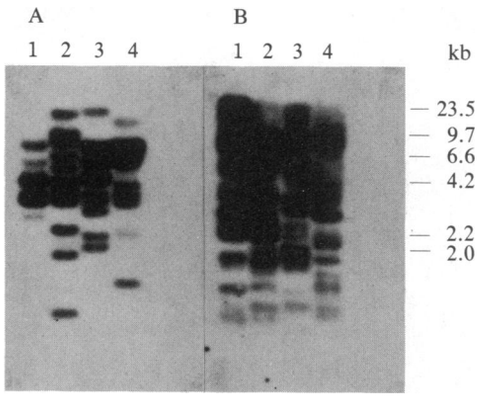

FIG. 3. Southern blot analysis of four races of *P. s. glycinea* DNA digested with *Eco*RI. Lanes: 1, Psg1; 2, Psg4; 3, Psg5; 4, Psg6. The four races were probed with a ^{32}P-labeled nick-translated *Eco*RI fragments 1 (*A*) and 3 (*B*) (Fig. 1). Multiple bands hybridizing to both *Eco*RI fragments were observed in all four lanes. The hybridization conditions were identical to Fig. 2. Preliminary experiments have shown that *Eco*RI fragments 1 and 3 do not cross-hybridize with each other. In addition, the precise locations of the regions responsible for the multiple hybridizing bands have yet to be determined.

ple hybridizing sequences. The significance of this cannot be readily explained, but this pattern of hybridization is often seen with multiple copies of indigenous insertion sequences (16).

Lack of Expression of the Avirulence Gene in Symbiotic and Saprophytic Bacteria. The pPg6L3 clone was modified into the saprophyte *Pseudomonas fluorescens* and one of the fast growing strains of *Rhizobium japonicum* (data not shown). These transconjugants did not elicit a hypersensitive reaction in the leaves of any soybean cultivar listed in Table 2.

DISCUSSION

The use of a cosmid library of genomic DNA from *P. s. glycinea* should allow the isolation of any gene from this pathogen for which an appropriate screen or selection is available. The capability of mobilizing *P. s. glycinea* libraries from *E. coli* into various races of *P. s. glycinea* and other bacteria in the conjugational range of pLAFR1 makes this an attractive system for future experiments. In this paper, we have reported that a cosmid clone (pPg6L3) from race 6 of *P. s. glycinea* induces three other races to mimic the host range incompatibility of the wild-type race 6. The Tn*5* mutagenesis experiments further suggest that *Eco*RI fragments 6 and 7 account for the race 6 specificity (Fig. 1). It is probable that these two fragments, a combined total of 1.4 kb of DNA, code for one or at most two average size proteins.

Although pPg6L3 changes race specificity from virulent to avirulent (Table 2), the opposite has not been observed. This finding is consistent with the available genetic and biochemical data on plant–pathogen interactions and is predicted by the gene-for-gene hypothesis (3). The availability of pPg6L3 should allow further subcloning of the avirulence gene sequence and permit eventual investigation of the nature of its protein product. Ellingboe (3) has hypothesized that a direct interaction occurs between a pathogen race-specificity gene product and the product of the complementary disease-resistance gene in the plant. Others have proposed that the product of the pathogen race-specificity gene is not itself directly involved in plant recognition of incompatible pathogen races and suggested that glycosyl transferases that determine the structures of cell surface carbohydrates are the recognitional elements (for review, see ref. 1). We are now in a position where these possibilities can be addressed experimentally.

Although the recombinant cosmid pPg6L3 led to a hypersensitive resistant reaction in soybean leaves when conjugated into races 1, 4, and 5 of *P. s. glycinea*, no hypersensitive response was observed when *E. coli*, *P. fluorescens*, or *R. japonicum* cells bearing the cosmid were infiltrated into soybean leaves. Whether this results from lack of expression of pPg6L3 in these bacteria or to other factors has yet to be investigated.

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