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Identification of DNA Sequences Involved in Host Specificity in the Pathogenesis of *Pseudomonas solanacearum* Strain T2005

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Two genomic libraries were constructed in *Escherichia coli* from DNA of *Pseudomonas solanacearum* wild-type strains T2003 (pathogenic to potato, but nonpathogenic to peanut) and T2005 (pathogenic to peanut) by using a broad host range vector pLAFR1. Transferring clones from the strain T2003 library into a strain T2005 derivative recipient, followed by plant pathogenicity tests, revealed no presumptive avirulence gene in the nonpathogenic *P. solanacearum* donor strain. However, transferring clones from the strain T2005 library to a T2003 derivative recipient resulted in the identification of two transconjugants that can cause wilting on peanut, indicating that the host range of the original nonpathogenic (to peanut) strain T2003 can be extended to peanut by introducing two cosmid clones from the strain T2005 library. These two cosmid clones were designated pGX1234 and pGX1236, and restriction enzyme mapping showed that they overlapped in a region containing two *Eco*RI fragments (12.8 and 2.4 kb). The 12.8-kb *Eco*RI fragment was subcloned into pLAFR1 vector and shown to retain the ability to extend the host range of *P. solanacearum* transconjugants to peanut when transferred to strain T2003. The evidence presented here shows that at least some genes for host specificity in strain T2005 are carried in the 12.8-kb DNA fragment.

Additional keywords: genomic library.

Bacterial wilt caused by *Pseudomonas solanacearum* is one of the major plant diseases of worldwide economic importance (Buddenhagen and Kelman 1964). The biochemical bases of this disease have been extensively studied in order to elucidate the interaction between the host plant and bacteria (Kelman 1954; Staskawicz *et al.* 1983; Hendrick and Sequeira 1984; Drigues *et al.* 1985; Morales and Sequeira 1985).

Plant pathogens have variable limits of host specificity. For some pathogens the host range is narrow, but with others it is wide. The nature of factors determining host specificity in the pathogen is largely unclear. To investigate the molecular and biochemical events that determine race-cultivar specificity, Staskawicz *et al.*(1984) isolated and cloned an avirulence gene from a soybean blight pathogen, *P. syringae* pv. *glycinea*. Transferring the avirulence gene of race 6 to race 4 recipients can convert the transconjugants to the race-specific incompatibility pattern of race 6. Similar avirulence genes were identified in *Xanthomonas campestris* pv. *malvacearum* (Gabriel *et al.* 1986), *X.c.* pv. *vesicatoria* (Swanson *et al.* 1988), and *P.s.* pv. *glycinea* (Staskawicz *et al.* 1987). One avirulence gene (*avr*A) from race 6 of *P.s.* pv. *glycinea* has been completely sequenced (Napoli and Staskawicz 1987). Identification of these genes suggests the existence of a gene-for-gene interaction determining host-pathogen relationships and the negative effect of a particular gene pair on plant disease development Flor 1971; Ellingboe 1984; Crute 1986).

In *P. solanacearum* three races have been designated (Buddenhagen *et al.* 1962; Lozano and Sequeira 1970). Race 1 has a very wide host range, race 2 affects bananas, and race 3 affects certain solanaceous plants. Within each race are numerous pathotypes and strains differing in geographical origin. The host range covered by a certain race of *P. solanacearum* can involve not only plant species, as in the cases of some fungi and bacterial pathogens in which the race-cultivar interaction has been well studied (Day 1974), but also many different plant genera and families.

Transposon mutagenesis was used in *P. solanacearum* to isolate mutants of factors involved in pathogenesis (Staskawicz *et al.* 1983; Mills 1985; Boucher *et al.* 1985; Morales *et al.* 1985). Evidence was obtained that many pathogenicity genes were located in a megaplasmid (Boucher *et al.* 1986). Genes that determine pathogenicity, encode some enzyme activities, and are responsible for inducing hypersensitive reaction (HR) have been cloned recently (Boucher *et al.* 1987; D. P. Roberts, T. P. Denny, and M. A. Schell, unpublished; Xu *et al.* 1988).

In the genetic studies of *P. solanacearum*, cosmid vectors with a broad host range were used to facilitate the identification of corresponding genes by complementation (Xu *et al.* 1988; Boucher *et al.* 1987; Roberts *et al.*, unpublished). In this paper we report the use of this approach to identify DNA sequences that are involved in host-specificity and that exert a positive effect on plant disease development. However, attempts to clone a putative avirulence gene from *P. solanacearum* T2003, pathogenic to potato but nonpathogenic to peanut, was unsuccessful.

MATERIALS AND METHODS

Bacterial strains and plasmids. Table 1 lists the bacterial strains and plasmids used. Strains of *P. solanacearum* were derivatives of the wild-type strains T2005 and T2003, which were originally isolated by He *et al.* (1983).

Media and culture conditions. *P. solanacearum* was routinely grown in GBM medium containing (g/L): Difco Bacto-Peptone, 5; Difco yeast extract, 2; beef extract, 2; sucrose, 6; at 30° C. TZC medium, which contains 0.005% 2,3,5-triphenyl-2H-tetrazolium chloride, was described by

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Kelman (1954). For genomic DNA isolation of *P. solanacearum*, nutrient yeast glycerol agar (NYGA) containing a small amount of utilizable carbohydrates (Turner *et al.* 1984) was used. The culture conditions for *E. coli* strains were as described by Maniatis *et al.* (1982). The following antibiotic concentrations were used for *P. solanacearum*: kanamycin (Km), 25 μg/ml, tetracycline (Tc), 10 μg/ml, and rifampicin (Rif), 50 μg/ml.

Conjugation and plasmid transfer. pGX1234, pGX1236, and their derivatives constructed with the vector pLAFR1 (Friedman *et al.* 1982) were transferred from *E. coli* donors to *P. solanacearum* recipients by triparental matings, with pRK2013 (Ruvkun and Ausubel 1981) as the helper plasmid. Transconjugants were selected for Tc^R, and *E. coli* donor cells were counterselected using rifampicin.

DNA isolation. Genomic DNA isolation from strains T2003 and T2005 was performed as described by Boucher *et al.* (1985), except that NYGA medium (Turner *et al.* 1984) was used to reduce the amount of extracellular polysaccharide. The resultant DNA was dissolved in 10 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and reprecipitated by 0.1 M spermine as described by Daniels *et al.* (1984). Small- and large-scale isolations of plasmid DNA from *E. coli* were essentially as described by Birnboim and Doly (1979) and Maniatis *et al.* (1982).

Construction of genomic libraries of strains T2005 and T2003. Genomic DNA was partially digested with *Eco*RI by incubating 60 μg of DNA samples divided into three equal aliquots, with 0.1, 0.2, and 0.3 enzyme units per μg of DNA, separately, for 15 min at 37° C. The digests were pooled and loaded on a 1% low-melting-point agarose (BRL) gel.

Electrophoresis was carried out in 1× GGB buffer (0.04 M Tris, 0.02 M NaAc, 1 mM EDTA, pH 8.3) at 1.5 V/cm for 24 hr. Gel segments containing DNA bands between 20–40 kb were excised, and DNA was reisolated as described by Maniatis *et al.* (1982). The fractionated genomic DNA and the vector pLAFR1 DNA (a gift from M. J. Daniels) were ligated with T4 DNA ligase, packaged, and transfected into the *E. coli* ED8767 recipient as described by Grosveld *et al.* (1981). DNA cloning and manipulation were as described by Maniatis *et al.* (1982) and Hopwood *et al.* (1985).

Table 1. Bacterial strains and plasmids

Strain or plasmid	Relevant attributes	Reference
Plasmid		
pLAFR1	Tra^- Mob^+ Tc^R IncP replicon	Friedman *et al.* 1982
pRK2013	Tra^+ Mob^+ Km^R ColEl replicon	Figurski and Helinski 1979
pGX1234	Clone from T2005 pLAFR1 library	This work
pGX1236	Clone from T2005 pLAFR1 library	This work
pGX1237	2.4-kb *Eco*RI fragment of pGX1234, subcloned in pLAFR1	This work
pGX1252	12.8-kb *Eco*RI fragment of pGX1234 subcloned in pLAFR1	This work
Escherichia coli		
ED8767	*rec*A *met sup*E *sup*F *hsd*S	Murray *et al.* 1977
HB101	*rec*A13 *rps*L *pro*A2 *leu* B6 *thi*-1	Boyer and Roulland-Dussoix 1969
Pseudomonas solanacearum		
T2003	Wild type, isolated from potato	He *et al.* 1983
T2005	Wild type, isolated from peanut	He *et al.* 1983
T2014	T2003 *rif*-3	This work
T2015	T2005 *rif*-2	This work
T2100	T2014 (pGX1234)	This work
T2102	T2014 (pGX1236)	This work
T2103	T2014 (pGX1237)	This work
T2122	T2014 (pGX1252)	This work

DNA-DNA hybridization. DNA fragments and undigested DNA were electrophoresed in agarose gel and transferred to nylon filters (Biodyne) as described by Southern (1975) and Wahl *et al.* (1979). Radioactively labeled DNA was prepared by nick translation (Rigby *et al.* 1977). Hybridization was carried out in 3× saline sodium citrate (SSC), 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), and 100 μg/ml of denatured salmon sperm DNA at 65° C for 16 hr. The filters were washed in 0.1× SSC and 0.5% SDS at 65° C.

Pathogenicity tests on peanut. Peanut seeds (*Arachis hypogaea* L. cv. Yue-You No. 1) were surface-sterilized and thoroughly rinsed with sterile water, then incubated at 30° C on GBM plates for 2 days to test for sterility and allow for germination. Germinated seeds were transplated separately in small bottles filled with water-soaked sand that had been sterilized for 2 hr. The seeds were covered with 0.5 cm of sand. Peanut seedlings were grown in the greenhouse at 28–30° C under supplemental lighting ($8–10 \times 10^3$ lux) provided by a Bio-Lux (Nanjing, China) and cool-white fluorescent tubes.

About 7–10 days after germination, the plants (10–12 cm in height) were inoculated by stem-injection. Inoculum (80–100 μl) containing 10^7–10^8 ml^{-1} bacteria was injected with a 0.5-ml syringe into plant stems at 3 cm above the base. Inoculated plants were placed in the greenhouse under the conditions described.

Fourteen days after inoculation, the severity of disease symptoms was ranked on the following scale: 0, no symptoms; 1, slight browning around the injection site; 2, brown area extended with stem stunting; 3, stem dark brown and stunting with occasional or partial wilting; 4, plant wilted and dead.

The ability to induce a HR on tobacco leaves was tested as described by Boucher *et al.* (1985).

RESULTS

Reaction of *P. solanacearum* strain inoculated on peanuts. Strains T2003 (race 3) and T2005 (race 1) were initially isolated from potato and peanut host plants, respectively, from the southern part of China (He *et al.* 1983). We used strains T2003 and T2005 because they gave consistent, clear-cut responses when inoculated into peanut plants (Table 2). Strain T2005 caused peanut seedlings to wilt rapidly 3–5 days after inoculation. However, under the same conditions, peanut seedlings inoculated with strain T2003 showed no differences from uninoculated controls, even 2 wk after inoculation (Fig. 1). Strain T2005 caused a typical HR in tobacco leaves 24 hr after infiltration, but tobacco leaves infiltrated with strain T2003 suffered only a slow collapse around the infiltrated area after 48 hr. No HR occurred when strain T2003 was infiltrated into peanut leaves.

Behavior of pLAFR1 in *P. solanacearum* and construction of strains T2003 and T2005 genomic libraries. The cosmid pLAFR1 is a broad host range vector derived from plasmid RK2, which can be transferred conjugatively with the helper plasmid strain pRK2013 (Figurski and Helinski 1979) from *E. coli* donors to a number of gram-negative bacteria, including some Pseudomonads (Ditta *et al.*

1980; Friedman *et al.* 1982; Staskawicz *et al.* 1984). In triparental matings between *P. solanacearum* T2014 and T2015 carrying chromosomal mutations for resistance to rifampicin, *E. coli* (pLAFR1) (donor), and *E. coli* (pRK2013) (helper), pLAFR1 was found to be transferred to *P. solanacearum* at a rather high frequency (around 10^{-2}–10^{-3}). Therefore, such transfer can be easily carried out by the patch cross method (Johnston *et al.* 1978). pLAFR1 appeared to be stably maintained in *P. solanacearum* transconjugants. After two subcultures of *P. solanacearum* transconjugants, which contained pLAFR1 under a nonselective condition, more than 95% of the bacteria remained Tc^R.

To construct a DNA library, a relatively small amount of genomic DNA (~60 μg) was needed, using the procedure described. After ligation of *P. solanacearum* strains T2003 and T2005 size-fractionated genomic DNA with linear, alkaline phosphatase-treated pLAFR1 DNA, transduction of *E. coli* ED8767 with packaged DNA yielded 8×10^3–10^4 Tc^R colonies per μg of *P. solanacearum* genomic DNA added to the ligation mixture.

About 2,000 of these colonies from both *P. solanacearum* strains T2003 and T2005 libraries were individually isolated and stored in 20% glycerol at −20° C. DNA were isolated from 24 clones chosen at random from each library and digested with *Eco*RI, followed by agarose gel electrophoresis. All samples showed 1–10 insert bands, with an average number of 4.2 for T2003 and 4.8 for T2005 libraries. The sum of these was in the range of 21.5–31.8 kb, and the average size was 25.8 kb for the T2003 library and 27.8 kb for the T2005 library.

Transfer of T2003 library clones to *P. solanacearum* T2005 derivative recipient. *P. solanacearum* strain T2005 (race 1) caused a rapid wilting on peanut (cv. Yue-You No. 1), whereas strain T2003 (race 3) was unable to induce disease symptoms under the same conditions (Table 2), a situation similar to the compatible and incompatible reactions proposed by the gene-for-gene hypothesis. Therefore, it was thought that DNA clones containing avirulence genes possibly could be detected by transferring the T2003 library into recipient strain T2005 and selecting any clones that could alter the pathogenic properties of T2005 on peanut.

1,632 clones from T2003 gene library were individually transferred from *E. coli* to pathogenic strain T2015, a Rif^R derivative of T2005 (Table 1). All of the Rif^R and Tc^R transconjugants were then individually inoculated into peanut seedlings. None of the transconjugants were shown to change the recipient from pathogenic to nonpathogenic to peanut, although a few of the transconjugants were less severe compared with the wild-type pathogenic strain. However, these less severe reactions were not reproducible. They were not investigated further.

Transfer of *P. solanacearum* T2005 library clones to T2003 derivative recipient. Nearly 2,000 clones from T2005 gene library were individually transferred from *E. coli* to T2014, a Rif^R derivative of T2003 that is pathogenic to potato but nonpathogenic to peanut (Table 1). All Rif^R and Tc^R transconjugants were individually inoculated into peanut seedlings. The majority of these transconjugants remained nonpathogenic, but two transconjugants, T2100 and T2102, showed the alteration from nonpathogenic to pathogenic to peanut. Peanut plants infected with strains T2100 and T2102 showed a rapidly extending stem browning and wilted 7–10 days after inoculation, although symptom development was 3–5 days delayed compared with the wild-type pathogenic strain T2005 (Fig. 1). Sometimes a small number of the plants infected with T2100 and T2102 remained stunted, with dark browning of the stem and a height of about one-half that of the control plant. Plant wilting was not observed at the scoring time (14 days after inoculation), although some of the stunted plants wilted later. The severity of disease symptoms caused by T2100 and T2102 is summarized in Table 3. These observations indicate that a *P. solanacearum* strain that is pathogenic to potato can extend its host range to peanut by introduction of a particular cosmid clone.

Two recombinant cosmids showing these effects were isolated from *P. solanacearum* T2005 gene library and were designated pGX1234 and pGX1236.

Bacteria were reisolated from the wilted peanut plants infected with strains T2100 and T2102. They were found to be Tc^R and to harbor plasmids that are identical to pGX1234 and pGX1236, respectively, as shown by restriction enzyme (*Eco*RI and *Bam*HI) digestion.

Table 2. Plant responses 2 wk after inoculation with *P. solanacearum* strain T2003 and T2005

Strain	Original host	Race	Plant responses[a]		
			Potato	Peanut	Tobacco
T2003	Potato	3	W/D	0	C
T2005	Peanut	1	W/D	W/D	HR

[a] 0, no symptoms; W/D, plant wilted and dead; C, infiltrated area chlorotic; HR, hypersensitive reaction.

Fig. 1. Disease symptoms produced in peanut seedlings by stem-injection with suspensions of *Pseudomonas solanacearum*. **A,** wild-type pathogenic strain T2005, showing wilting at early stage. **B,** T2122, the plant is wilting. **C,** T2122, showing extended stem browning, plant wilting is about to occur. **D,** Nonpathogenic strain T2014, plant remains normal. **E,** T2100, the plant is wilting. Plants were photographed 10 days after inoculation.

Table 3. Severity of disease symptoms caused by *P. solanaceaum* transconjugants on peanut

Strain	Average disease index[a]	Pathogenicity rating[a]
T2003	0	0
T2005	4	H
T2014	0	0
T2014 (pLAFR1)	0	0
T2014 (pGX1234)	3.1–3.4	H
T2014 (pGX1236)	2.9–3.1	M/H
T2014 (pGX1237)	1.8–2.5	L/M
T2014 (pGX1252)	3.2–3.5	H

[a] Results based on average severity record of 5–10 plants 14 days after inoculation. Severity was ranked as described in text. H, high (3.1–4); M, medium (2.1–3); L, low (0.1–2); 0, none (0).

Mapping of pGX1234 and pGX1236. *Eco*RI digestion showed that pGX1234 contained eight insert DNA fragments of 12.8, 7.7, 3, 2.4, 1.6, 1.4, 0.7, and 0.6 kb and that pGX1236 contained three fragments of 12.8, 7.7, and 2.4 kb, in addition to the 21.6-kb pLAFR1 vector band (Fig. 2). It is interesting to find that three *Eco*RI bands (12.8, 7.7, and 2.4 kb) are common in size to pGX1234 and pGX1236.

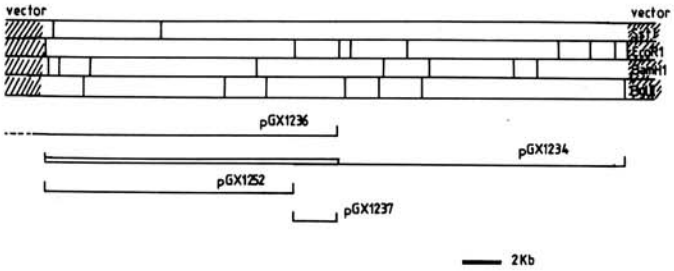

Fig. 2. Restriction enzyme map of pGX1234. Symbols are: E, *Eco*RI; B, *Bam*HI; Bg, *Bgl*II; S, SstI. The overlapped region between pGX1234 and pGX1236 is indicated by the hollow bar in pGX1234. The map of pGX1236 is incomplete. Subclones were constructed with pLAFR1 (not shown in the map).

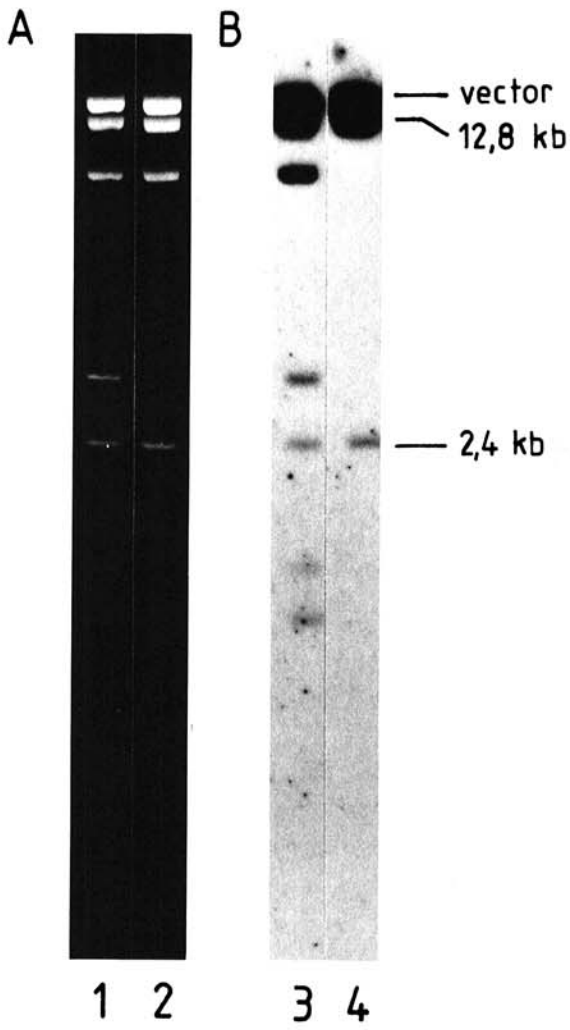

Fig. 3. Restriction enzyme digestion and DNA-DNA hybridization analysis of pGX1234 and pGX1236. **A,** Restriction enzyme digestion (*Eco*RI) pattern visualized in a 0.7% agarose gel. Lane 1, pGX1234; lane 2, pGX1236. **B,** Southern blot analysis of pGX1234 and pGX1236 after probing with ^{32}P-labeled nick-translated pGX1234 DNA. Lane 3, pGX1234; lane 4, pGX1236, showing that only two *Eco*RI fragments (12.8 and 2.4 kb) were hybridized.

By restriction enzyme digestion, subcloning, and DNA hybridization between the subclones, a restriction map of pGX1234 was prepared (Fig. 2).

A restriction map of pGX1236 was also prepared. By probing *Eco*RI-digested pGX1236 with ^{32}P-labeled pGX1234 DNA, it was found that only two *Eco*RI bands (12.8 and 2.4 kb) showed hybridization (Fig. 3). Obviously pGX1234 and pGX1236 overlap in the two *Eco*RI-fragment regions (Fig. 2). Because both pGX1234 and pGX1236 have the potential to extend the host range of strain T2014 to peanut, the logical explanation is that some DNA sequences involved in host specificity are located in the 12.8-kb and 2.4-kb *Eco*RI fragments that are common to pGX1234 and pGX1236.

Subcloning of pGX1234. To determine if the DNA sequences involved in host specificity are located in the overlapped region of pGX1234 and pGX1236, subclones containing each single 12.8- and 2.4-kb *Eco*RI fragment were constructed with pLAFR1 by digesting pGX1234 DNA with *Eco*RI, followed by self-ligation and examination by agarose gel electrophoresis. Subclones containing each single 12.8- and 2.4-kb *Eco*RI insert fragment were designated pGX1252 and pGX1237, respectively (Table 1). pGX1252 and pGX1237 were mobilized into strain T2014, which resulted in the transconjugants T2122 and T2103, respectively (Table 1).

Pathogenicity tests were carried out by inoculating T2122 and T2103 on peanut seedlings. Disease symptoms observed in peanut seedlings infected with T2122 were similar to those of T2100 and T2102. Plants wilted 7–10 days after T2122 inoculation (Fig. 1), a delay of 3–5 days compared with the wild-type strain T2005. Peanut seedlings inoculated with T2103 showed slight stem browning around the injection site only (Table 3).

These data clearly show that pGX1252 and pGX1237 may carry some DNA sequences responsible for host specificity in pathogenesis of *P. solanacearum* T2005. However, the symptoms caused by T2014 (pGX1237) were generally much less severe compared with those caused by T2014 (pGX1252). Therefore, the 2.4-kb fragment was not studied in detail.

DISCUSSION

We have shown in this study that the host range of *P. solanacearum* can be extended by introducing a cosmid clone containing DNA sequences involved in host specificity. A region containing these DNA sequences has been located in a 12.8-kb DNA fragment by subcloning. Transfer of this fragment to a *P. solanacearum* strain, which is pathogenic to potato but nonpathogenic to peanut, resulted in a transconjugant that acquired the ability to cause wilting on peanut seedlings. This clearly shows that at least some genes involved in host specificity of *P. solanacearum* T2005 are carried in the 12.8-kb DNA fragment.

In a parallel experiment we were unable to identify clones containing presumptive avirulence genes from the *P. solanacearum* strain that is nonpathogenic to peanut. The number of clones (1,632) tested should be enough to represent 99.9% of *P. solanacearum* genomic DNA based on the bacterial genome size determination by DNA renaturation studies (Bak *et al.* 1970). Therefore, this failure may be an indication that such genes do not exist or that detection of such genes is difficult in a less clear race-species system.

Avirulence genes have been isolated in some bacterial

pathogens (Staskawicz *et al.* 1984; Gabriel *et al.* 1986; Staskawicz *et al.* 1987; Swanson *et al.* 1988). All were found in cases in which the compatibility of race-cultivar interaction was clear. The avirulence gene interacted with the resistance gene in the host plant and resulted in an incompatible reaction, indicating the negative function of a gene on disease development. The results presented here show that plant disease development may also be initiated by the positive function of a particular DNA sequence. Because races in *P. solanacearum* are divided not by race-cultivar interaction but by strain-host interaction (which covers plant species, genus, and even family), the new finding may present another example of a complicated host-pathogen interaction.

It is generally agreed that a substantial number of genes in the pathogen are involved in the disease process. In *P. solanacearum* pathogenicity genes have been proven to be carried in a megaplasmid (Boucher *et al.* 1985). By probing the cosmid clone pVir2 (Boucher *et al.* 1986) containing part of the pathogenicity region of the megaplasmid in *P. solanacearum* with cosmid clone pGX1234 digested with *Eco*RI, no hybridization was found, indicating no homology between these two different DNA sequences (data not shown). However, DNA homologous with pVir2 was found in the genomic DNA of strain T2005, indicating the possibility that similar pathogenicity genes to pVir2, in addition to the genes carried in pGX1234, may be effective in the wilt development. This is in agreement with recent findings that the pathogenicity genes of *P. solanacearum* are probably not involved in host-specificity (Xu *et al.* 1988).

The *P. solanacearum* transconjugant containing pGX1234 was able to cause peanut wilting, but the occurrence of wilting was delayed, and the disease index was always less compared with strain T2005, which is naturally pathogenic to peanut. This may reflect the fact that genes other than those in pGX1234 are needed to cause disease development as severe as the one observed with the peanut strain. In fact, a number of clones were found to cause minor symptoms when transferred to the nonpathogenic recipient by plant screening. Because these minor symptoms were more variable, it was difficult to obtain a reliable response. These clones were not studied in detail and were often overlooked in the screening procedure.

In the plant test, similar phenotypic effects caused by T2014 (pGX1234) and T2014 (pGX1252) were found. This means that the overall functional effect of a piece of DNA is not the simple addition of its components. The reasons for this are not clear.

It is known that plants affected by *P. solanacearum* not only wilt but also show some morphological changes possibly caused by alteration in auxin balance (Buddenhagen and Kelman 1964). The levels of indole-3-acetic acid (IAA) were found to be increased greatly in susceptible plants infected with *P. solanacearum* (Sequeira and Kelman 1962). It is interesting that in some cases, *P. solanacearum* transconjugants containing pGX1234 caused stem stunt when inoculated on peanut seedlings, in addition to other symptoms. We do not know if this was caused by the increased level of IAA in the infected plant.

Although we have identified a *P. solanacearum* DNA sequence that appears to carry genes for host specificity, the nature of the putative genes encoded by this DNA sequence is unknown. It is probable that the pathogen must enter into the host plant before gene function can be initiated, because no symptoms were found in peanut seedlings inoculated with cell-free supernatant of cultures of *P. solanacearum* transconjugants T2100, T2122, and even the wild pathogenic strain T2005 (data not shown). Experiments are underway to identify and characterize these genes by Tn*5* mutagenesis, DNA sequencing, and analysis of gene products.

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