Transfer, Mapping, and Cloning of *Pseudomonas syringae* pv. *syringae* Plasmid pCG131 and Assessment of Its Role in Virulence

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ABSTRACT

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Pseudomonas syringae pv. *syringae* strain HS191 is a corn pathogen harboring plasmid pCG131. We determined that plasmid-free derivative strains still produced the phytotoxin, syringomycin. Plasmid pCG131 was cleaved with restriction endonuclease *Bam*HI or *Sal*I and these fragments were cloned by using the broad host range vector, pRO1614. These recombinant plasmids were transformed into PSO100, a plasmid-free, toxin-producing derivative of HS191. Plant pathogenicity tests with the collection of chimeric plasmid-containing strains in *Zea mays* suggested that pCG131 may contribute to the virulence of strain HS191. A selective marker for carbenicillin resistance was added to the plasmid pCG131 by the transposition of Tn*1*. Plasmid pCG131::Tn*1* (pCG133) conjugally transferred intraspecifically to mutants of HS191 and to the SD19 strain of *P. syringae* pv. *syringae*. Toxin-negative recipients used for these tests were not converted to toxin production by the acquisition of plasmid pCG131.

Additional key words: conjugation, transposon labeling.

Pseudomonas syringae pv. *syringae* is a pathogen of many agricultural crops and may be involved in significant reductions of crop yield and quality (17). Furthermore, it is distinctive among pseudomonads in having an unusually broad plant host range: it is pathogenic to more than 40 plant genera (20). Most strains of *P. syringae* pv. *syringae* isolated from naturally infected plants produce the phytotoxin designated as syringomycin (SR) (19) which appears to be a virulence factor. Many of the SR-producing (SR^+) strains contain plasmids (3,6,18). Plasmids either are required for, or have been implicated in, the pathogenicity of several bacterial phytopathogens (1,4,13,21) but not others (2,3,18).

The ability of a bacterial strain to produce a toxin may be necessary but insufficient to cause disease. A pathogenic bacterium generally must grow in or on a host for disease to occur; growth of the pathogenic bacterium is critical for disease development. Consequently, conditions that inhibit pathogen growth are limiting factors. In a previous report (5), the holcus spot disease of maize appeared to be associated with the presence of plasmid pCG131 in *P. syringae* pv. *syringae* strain HS191; a later report questioned this relationship (7).

The objectives of this study were to clarify the relationship between plasmid pCG131 and toxin production by strain HS191, to assess the contribution of the plasmid to virulence in plant tissue, and to determine if the plasmid could be transferred by conjugation.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The relevant properties of the bacterial strains and plasmids used in this study are listed in Table 1.

Media. Minimal medium (VBG) and complex medium (TN) were described previously (14). VBG was supplemented with amino acids to individual concentrations of 0.5 mM. Medium NBY (22) was used for cultivation of bacteria before plant assays for virulence.

When antibiotics were used for the selection or characterization of transconjugants or transformants, media were supplemented as follows: carbenicillin (Cb) (Geopen; Pfizer, Inc., New York, NY), 1 mg/ml or tetracycline (Tc) (Sigma Chemical Co., St. Louis, MO) 25 μg/ml.

Bacterial matings. Bacterial cells used for matings were grown overnight on TN agar. A turbid suspension was made from these plates in TN broth (25 ml) which was incubated with shaking (250 rpm) at 23 C until the late log phase of growth (OD 1.0 at 425 nm) had been reached. Suspensions of donor and recipient cultures were mixed 1:1 from the TN broth cultures and incubated 2 hr at 23 C. Following this incubation, the mixed suspensions were concentrated 10-fold by centrifugation at ambient temperature and the pellets were resuspended in 0.01 M potassium phosphate buffer (pH 7.0), diluted, and plated onto selective medium containing the appropriate antibiotic. Colonies were counted after 48 and 72 hr of incubation at 23 C.

Transformation. Bacterial cells were grown in TN broth at 23 C and transformed as described by Olsen et al (16). Transformants prepared for strain construction or molecular cloning experiments were plated onto TN agar supplemented with antibiotic and incubated at 23 C.

Preparation of DNA. Plasmid DNA of pCG131, pRO1601, or pRO1610 was prepared as described previously (5). For molecular cloning, pCG131 DNA was prepared as described by Hansen and Olsen (9). The vector, plasmid pRO1614, was prepared by using a

modification of the method of Guerry et al (8). All DNA used for cloning experiments was purified by centrifugation in CsCl isopycnic gradients containing ethidium bromide (9). A miniaturized version of the procedure of Hansen and Olsen (9) was used for preliminary analysis of transformants from the cloning experiments to compare the size of their plasmids with the vector. Such transformants were grown overnight on TN agar, scraped from the agar surface with an inoculating loop, and added to 2 ml of phosphate buffer (0.01 M, pH 7.0). An aliquot (1.5 ml) of the cell suspension with an OD of 1.5–2.0 at 425 nm was transferred into a 1.5-ml microcentrifuge tube. The suspension was pelleted by centrifugation in an Eppendorf model 5412 centrifuge (Brinkmann Instruments Inc., Westbury, NY). The pellet was resuspended in 202 μl of TS buffer (8), 15 μl of lysozyme (10 mg/ml, Sigma), and 75 μl of 0.5 M EDTA (pH 8.0) (8). The mixture was vortexed (1 min) and incubated at 4 C for 10 min. After incubation, 75 μl of 20% sodium dodecyl sulfate (SDS) in TE buffer (8) and 75 μl of 3 N NaOH were added to the sample. The tubes were then heat pulsed at 55 C for 5 min and mixed quickly by several inversions. After cellular lysis, the mixture was neutralized by the addition of 150 μl of 2 M Tris (pH 7) (8) and mixed by inversion. To this mixture, 97.5 μl of 20% SDS and 187.5 μl of 5 M NaCl were added and mixed by inversion. The tubes were then placed in a dry ice-methanol bath for 5 min and centrifuged at 4 C for 10 min. The supernatant was removed by aspiration and transferred to another 1.5-ml polypropylene tube. Then, 40 μl of 2 M sodium acetate (pH 5) and 800 μl of absolute ethanol were added to the supernatant; mixing was done by inversion. Tubes were incubated in a dry ice-methanol bath for 15 min and centrifuged at 4 C. The precipitate was dried under vacuum and resuspended in TO buffer (8). The DNA was electrophoresed with appropriate plasmid standards as previously described (16). The electroelution of restriction fragments from agarose gels was as described by Maniatis et al (12).

Enzymes. Restriction endonuclease digestion and ligation were done as suggested by the supplier (Bethesda Research Laboratories, Rockville, MD). Other aspects of molecular cloning were as described by Olsen et al (16).

Virulence assessment of chimeric plasmid-containing strains. Bacterial strains (Table 1) were grown to mid-exponential phase in NBY broth, centrifuged at low speed, and suspended in 12.5 mM phosphate buffer (pH 7.1) to give an OD of 0.2–0.3 at 425 nm. Strains with recombinant plasmids were maintained on NBY agar supplemented with Cb (500 μg/ml); the remainder were maintained on NBY agar. Prior to use, 6 ml of cells were added to 600 ml of distilled water, along with 6 ml of 1% Triton X-100 as a wetting agent, to give a final concentration of $\sim 10^6$ colony-forming units per milliliter. The field corn cultivar PX-69 (Northrup King Co., Modesto, CA) or the sweet corn cultivar Golden Cross Bantam were grown in a controlled-environment growth chamber to the three- to four-leaf stage, as previously described (5). Three to five plants per pot, sometimes replicated twice per test, were vacuum infiltrated with inoculum in each test as described previously (5). Plants were assessed for ratings of bacterial pathogenicity and virulence 7–10 days after inoculation. The rating system used is described in Table 2.

Mutagenesis. Bacterial mutants were obtained by exposure of cells (2×10^9 colony-forming units per milliliter) to 50 μg/ml of 1-methyl-3-nitro-1-nitrosoguanidine (NTG) (Sigma) in 0.15 M sodium citrate buffer (pH 5.4) for 15 min at 23 C. Following this treatment, the cells were centrifuged at ambient temperature and the pellet was resuspended in VBG supplemented with 0.02% vitamin-free casamino acids (Difco), 0.05 mM adenine (Sigma), and 0.05 mM uracil (Sigma). The resuspended cells were incubated for 2 hr at 23 C. Mutants were selected from survivors of an NTG treatment that killed about 99.9% of the cells. The auxotrophic mutants were characterized by plating on VBG agar and VBG agar supplemented with amino acids to a final concentration of 0.5 mM, except isoleucine and valine (each at 0.25 mM) and tyrosine (0.1% final concentration).

TABLE 1. Bacterial strains of *Pseudomonas syringae* pv. *syringae* and plasmids used in this study

Bacterial strains or plasmids	Relevant characteristics[a]	Reference or source
Strains		
HS191	SR^+, contains pCG131	(5)
AO111	SR^-, strain HS191 cured of pCG131	(5)
PSO100	SR^+, strain HS191 cured of pCG131	This study
PSO102	*his-2*, SR^+, PSO100	This study
PSO105	*cys-5*, SR^-, PSO100	This study
PSO109	*ura-9*, SR^-, HS191	This study
PSO110	*leu-10*, SR^+, HS191	This study
PSO111	SR^-, HS191	This study
PSO112	*pro-12*, SR^-, PSO100	This study
PSO1001	PSO100(pRO1614), Cb^r, Tc^r	This study
PSO1002	PSO100(pRO1614:pCG131*Bam*HI-A), Cb^r	This study
PSO1003	PSO100(pRO1614:pCG131*Bam*HI-B), Cb^r	This study
PSO1004	PSO100(pRO1614:pCG131*Sal*I-A), Cb^r	This study
PSO1005	PSO100(pRO1614:pCG131*Sal*I-B), Cb^r	This study
PSO1006	PSO100(pRO1614:pCG131*Sal*I-C), Cb^r	This study
PSO1007	PSO100(pRO1614:pCG131*Sal*I-D), Cb^r	This study
PSO1008	PSO100(pRO1614:pCG131*Sal*I-E), Cb^r	This study
PSO1009	PSO100(pRO1614:pCG131*Sal*I-F), Cb^r	This study
PSO10010	PSO100(pRO1614:pCG131*Sal*I-G),Cb^r	This study
PSO1021	PSO102(pCG133), Cb^r, pCG133 = pCG131::Tn*1*	This study
PSO1051	PSO105(pCG133), Cb^r	This study
PSO1101	PSO110(pRO1601/pCG131), Cb^r	This study
PSO1102	PSO110(pRO1610/pCG131), Cb^r	This study
SD19	rifampin resistant, SR^-, wild-type isolate	J. Otta[b]
Plasmids		
pRO1601	pRO1600::Tn*1*	(16)
pRO1610	pRO1600::Tn*1*	This study
pRO1614	Cb^r, Tc^r, cloning vector	(16)

[a] Marker abbreviations. Bacterial strains: *cys*, cysteine; *his*, histidine; *leu*, leucine; *pro*, proline; *ura*, uracil; SR^+, syringomycin production (+), nonproduction (−); Cb^r, carbenicillin resistance; Tc^r, tetracycline resistance.
[b] South Dakota State University, Brookings.

RESULTS

Curing of plasmid pCG131. A previously described nonpathogenic, plasmid-free strain derived from *P. syringae* pv. *syringae* HS191, strain AO111 (5), may have been a pleiotropic mutant since it was produced by exposure to acridine orange, a potent mutagen. Therefore, we sought to produce plasmid-free isolates by the more gentle method of mild heat treatment. Thus, strain HS191 was grown in TN broth in a closely regulated water bath shaker at 30 ± 0.2 C. Under these conditions, strain HS191 grew at approximately one-half its optimal rate. From cultures growing overnight under these conditions, samples were plated on TN agar and incubated at 23 C. After incubation for 48 hr, 14 colonies were selected for further analysis. The isolates were then grown and lysed as described in Materials and Methods. Agarose gel electrophoresis of DNA samples showed that six of the 14 isolates had lost plasmid pCG131. These and plasmid-containing isolates from the 30 C TN broth incubations were then tested for production of SR by using the bioassay described by Sinden et al (19); all isolates retained SR production. Therefore, growth at 30 C, unlike exposure to acridine orange treatment, provided plasmid-free isolates that retained toxin production. In addition, no other phenotypic traits, eg, auxotrophy or growth on various carbon sources, were altered as a consequence of prior growth at 30 C. One such isolate, designated PSO100, typical of isolates that apparently had lost pCG131, was used for further studies.

To facilitate our studies on the relationship between toxin production, pathogenicity, and plasmid content, a collection of mutant strains were produced from the parental strain, HS191, and its plasmid-free derivative, PSO100, by exposure of growing cultures to NTG as described in Materials and Methods. From this treatment we obtained mutants typified by those shown in Table 1.

Transfer of plasmid pCG131. A comparison of strains HS191 and PSO100 for metabolic activities and antibiotic resistances showed no unique phenotypic characteristics associated with plasmid pCG131. Therefore, to test the transferability of pCG131, we added a selective marker for carbenicillin resistance (Cb^r) as described previously for another *Pseudomonas* plasmid, FP2 (15). Two pRO1600::Tn*1* plasmids, with Tn*1* transposed into different sites, designated pRO1601 (16) and pRO1610 (Table 1) were used as transposon donors. Plasmids pRO1601 and pRO1610 were transformed into strain PSO110 to construct the aggregate plasmid-containing strains. Strain PSO110 containing pRO1601 or pRO1610 were designated as PSO1101 and PSO1102, respectively. The two aggregate plasmid-containing strains were tested for mobilization of Cb^r to PSO102 by selecting for the acquisition of Cb^r by PSO102 as shown in Table 3. The data show similar mobilization frequencies for either of the donors used. Typical transconjugants from these matings were also examined for plasmid content in relation to the parental aggregate plasmid-containing strains and these results are shown in Fig. 1. Lanes A and B in Fig. 1 show plasmid pCG131 in the parental strain HS191 and its auxotrophic derivative PSO110, respectively. The PSO110 transformant, PSO1102, shows the addition of pRO1610 (Fig. 1, lane C, lower band). A transconjugant from a PSO1102 × PSO102 (lane D, Fig. 1) mating is shown in lane E of Fig. 1. The upper band here is larger than pCG131 shown in lane C reflecting the acquisition of Tn*1* (4.8 kb) by pCG131 from the transposon donor, pRO1610. This plasmid was designated pCG133. We surmise from the survey gel that Tn*1* had transposed onto pCG131 to form pCG133. This observation was confirmed by subjecting pCG131 and pCG133 to restriction enzyme digestion with *Bam*HI. Plasmid pCG131 contained only two *Bam*HI sites (Fig. 2, lane A) while pCG133 showed three *Bam*HI sites and an increase in size of 4.8 kb (Fig. 1, lane E). The difference in the physical size and an additional *Bam*HI site indicated that the transposon was located on pCG133. Strain PSO1021 was then mated with strain PSO105 which is

TABLE 2. Virulence of chimeric plasmid-containing *Pseudomonas syringae* pv. *syringae* strains[a]

Strain	Reference	Trial no. and plant cultivar		
		Trial 1 PX69	Trial 2 GCB	Trial 3 GCB
HS191	(3)	4	3.5	2
AO111	(3)	0	0	0
PSO100	This study	2	2	2
PSO1001	This study	ND[b]	2.5	2
PSO1002	This study	4	2.5	2
PSO1003	This study	1	4	1
PSO1004	This study	2	4	3
PSO1005	This study	3	4	1
PSO1006	This study	4	3	3
PSO1007	This study	3	1.5	3
PSO1008	This study	3	3.5	4
PSO1009	This study	3	3.5	2
PSO10010	This study	2	3	3.5
Buffer control		0	0	0

[a] Virulence rating 0 to 5 scale. 0 = no disease-specific injury; 1 = injury mild with no leaf collapse, lesions not coalesced; 2 = moderate disease with some lesion coalescence, with no more than one leaf collapsed; 3 = severe disease, with lesions all coalesced, leaves withered; 4 = very severe disease, with lesions all coalesced, pronounced water-soaked appearance; 5 = dead plants, with only necrotic tissue in inoculated sites.
[b] ND = not determined.

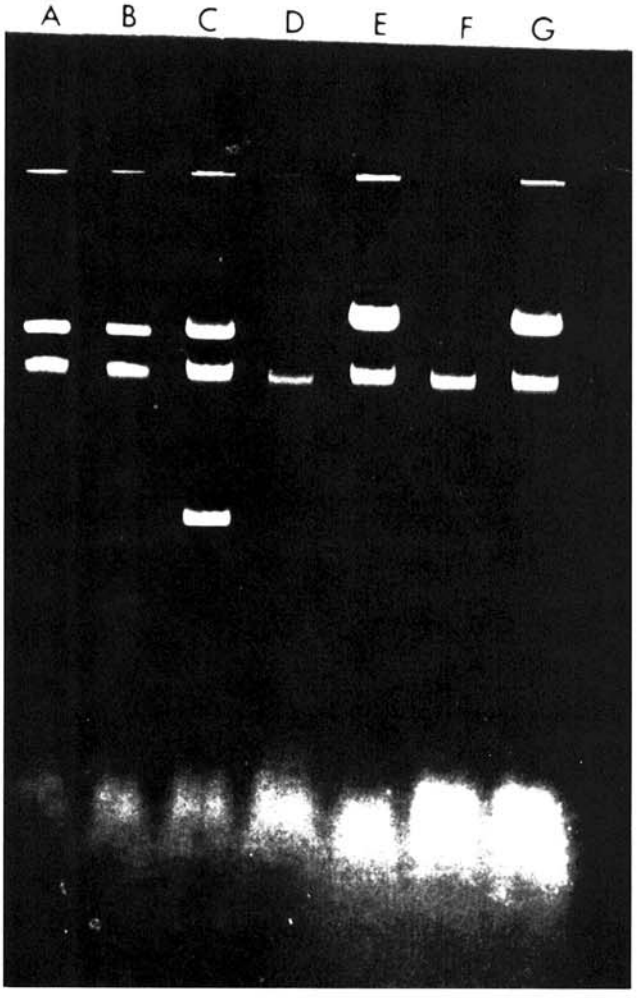

Fig. 1. Agarose gel electrophoresis of DNA from *Pseudomonas syringae* pv. *syringae* strain HS191 and its derivatives. Electrophoresis of DNA was in 0.7% agarose at 100V for 2 hr. Bands are identified top to bottom. Lane A: HS191; pCG131, 51.2 kb, and chromosomal DNA. Lane B: PSO110; pCG131 and chromosomal DNA. Lane C: PSO1102; pCG131, chromosomal DNA, and pRO1610, 7.9 kb. Lane D: PSO102; chromosomal DNA. Lane E: PSO1021; pCG133, 56 kb and chromosomal DNA. Lane F: PSO105; chromosomal DNA. Lane G: PSO1051; pCG133 and chromosomal DNA.

shown in lane F and the frequency of transfer is shown in Table 3. A transconjugant from this mating, PSO1051 is shown in Fig. 1, lane G. Its size is the same as the donor, PSO1021, indicating no further changes in plasmid pCG133 subsequent to its serial transfer.

The data presented in Table 3 indicated that the pCG131::Tn*1* derivative, pCG133, is transfer proficient. However, all these matings were accomplished by using isogenic strains that produced SR. We considered the possibility that genes encoding toxin production might be located on both plasmid pCG131 and the chromosome. In this case, PSO100, although cured of plasmid pCG131, would still be a producer of SR. To evaluate this possibility, the atoxigenic mutant AO111 and a naturally occurring atoxigenic strain, SD19, were used as recipients in conjugal experiments with pSO1021 as the donor. Also, included as recipients were the pCG131 containing isolates PSO109(SR^-) and PSO110(SR^+). Mating experiments showed no change in the SR phenotype of transconjugants as a consequence of the acquisition of pCG133. This is also the case for recipients in which plasmid pCG131 was displaced by the acquisition of plasmid pCG133 (confirmed by electrophoresis of lysates [*unpublished*]).

TABLE 3. Conjugal transfer of *Pseudomonas syringae* pv. *syringae* plasmid pCG131 and its derivative pCG133[a]

Donor	Plasmid(s)	Recipient	Transconjugants per donor
PSO1101	pRO1601/pCG131	PSO102	4.5×10^{-5}
PSO1102	pRO1610/pCG131	PSO102	4.5×10^{-4}
PSO1021	pCG133[b]	PSO105	1.0×10^{-3}

[a] Mating conditions for the transfer of plasmids were as described in Materials and Methods. Selection was for carbenicillin resistant transconjugants on VBG agar medium containing the appropriate amino acid supplementation.
[b] pCG133 = pCG131::Tn*1*.

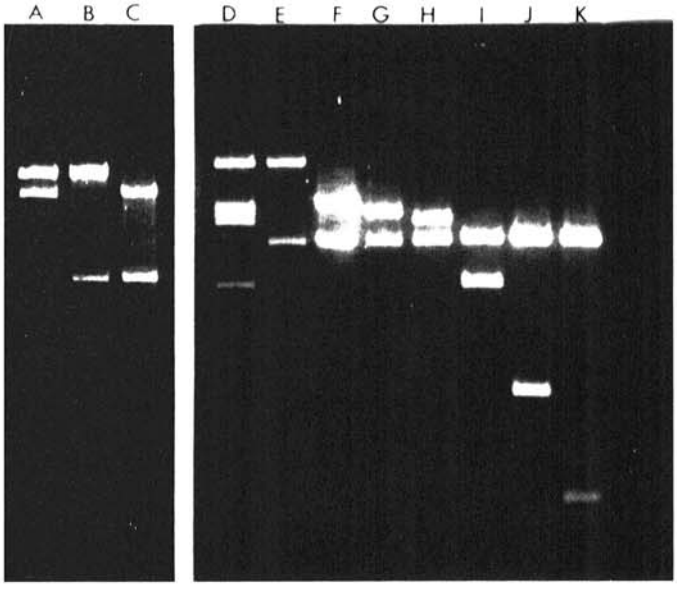

Fig. 2. Agarose gel electrophoresis of pRO1614:pCG131 recombinant plasmids. The origin and derivation of plasmids are described in the text. Electrophoresis of DNA was in 0.7% agarose gels constructed and run as described previously (16). Lanes A–C were cleaved with *Bam*HI. Lanes D–K were cleaved with *Sal*I. Bands are identified from top to bottom. Lane A: pCG131; fragments *Bam*HI-A and *Bam*HI-B. Lane B: pRO1614:pCG131*Bam*HI-A; *Bam*HI-A and pRO1614. Lane C: pRO1614:pCG131*Bam*HI-B; *Bam*HI-B and pRO1614. Lane D: pCG131; *Sal*I-A-G (fragment *Sal*I G, weak; see lane K). Lane E: pRO1614:pCG131*Sal*I-A. Lane F: pRO1614:pCG131*Sal*I-B. Lane G: pRO1614:pCG131*Sal*-C. Lane H: pRO1614:pCG131*Sal*I-D. Lane I: pRO1614:pCG131*Sal*-E. Lane J: pRO1614:pCG131*Sal*-F. Lane K: pRO1614:pCG131*Sal*-G. Lower band in lane E common to lanes E through K is vector pRO1614; remaining fragments in lanes E through K represent fragments *Sal*I-A through G, respectively.

Physical mapping of pCG131. To facilitate the choice of restriction endonuclease enzymes to be used in subsequent molecular cloning experiments, pCG131 DNA was cleaved with restriction endonucleases. The number of cleavage sites for each enzyme used was *Eco*RI, 15; *Hind*III, 11; *Bgl*II, 10; *Sal*I, 7; *Kpn*I, 3; *Bam*HI, 2; *Sst*I, 2; and *Pst*I, 0. Based on these results and the distribution of the sizes of the cleavage fragments observed, we concluded that gene-banks derived from either *Bam*HI or *Sal*I cleavage of pCG131 would be appropriate for further studies. The physical map of these sites in relation to those for *Kpn*I and *Sst*I is shown in Fig. 3. As can be observed from the figure, the two *Bam*HI cleavage sites are within *Sal*I sites at 0/51.2 kb and 31.3 kb on the restriction map and therefore these *Sal*I fragments should provide possible expression of loci interrupted by *Bam*HI cleavage. Furthermore, the distribution of sizes for the *Sal*I fragments allows their unambiguous identification in relation to each other.

Molecular cloning of pCG131. The broad host range cloning vector, pRO1614, described previously (16) replicates in *P. syringae* pv. *syringae* strain HS191. However, transforming DNA derived from bacterial strains other than HS191 or its mutant derivatives resulted in low recovery of transformants. Because of this low recovery and possible restriction activity, all vector DNA was extracted from an HS191 derivative strain, PSO1001, that had been constructed for this purpose. Our initial attempts to clone pCG131 fragments produced by digestion with restriction endonuclease, *Sal*I, produced several transformants containing only a few of the pCG131 fragments. To clone the remaining *Sal*I fragments, pCG131 DNA was digested with *Sal*I and the desired fragments were electro-eluted from an agarose slab gel after electrophoresis, concentrated individually by ethanol precipitation, and then ligated into the vector, pRO1614, that had also been cleaved with *Sal*I. Transformants derived from these experiments were selected for Cb^r and showed insertional inactivation of tetracycline resistance. Several colonies derived from each DNA

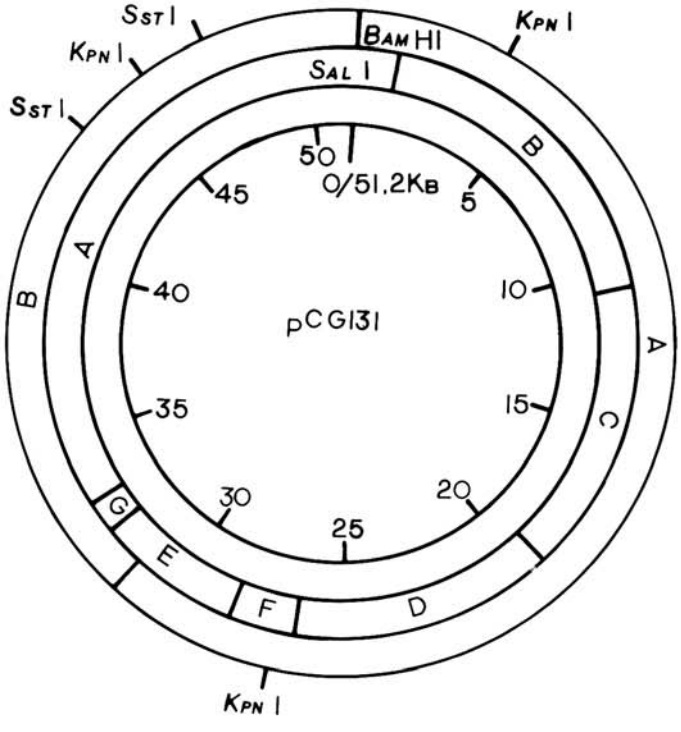

Fig. 3. Circular restriction map of *Pseudomonas syringae* pv. *syringae* plasmid pCG131. The total length is 51.2 kb. Position "O" has been arbitrarily chosen at the middle of a *Bam*HI site. *Kpn*I and *Sst*I restriction sites are indicated on the outer ring. The figure shows the restriction maps for the endonucleases *Bam*HI and *Sal*I, which are indicated in concentric rings. The numbers on the inside of the inner ring refer to kb coordinates.

suspension containing a pCG131 fragment were purified and resolved as described in Materials and Methods. The two recombinant plasmids that contained the *Bam*HI cleavage-fragments of plasmid pCG131 were obtained and analyzed as described above. Later, recombinant plasmid DNA was purified and cleaved with enzymes used for the initial cloning. The results are shown in Fig. 2. Lane A shows pCG131 cleaved with *Bam*HI yielding two fragments, 31.3 and 19.9 kb, respectively. Lanes B and C show the *Bam*HI-produced recombinant plasmids which individually contain each of the *Bam*HI fragments and were designated pRO1614:pCG131 *Bam*HI-A and pRO1614:pCG131 *Bam*HI-B, respectively. The lower band common to both lanes is the cloning vector, pRO1614, as a linear *Bam*HI fragment derived from the recombinant plasmids. Lane D shows plasmid pCG131 cleaved with restriction endonuclease, *Sal*I, producing seven fragments (19.2, 9.5, 8.6, 7.4, 3.9, 1.8, and 0.75 kb). Lanes E through K show DNA from recombinant plasmids which individually contain each of the *Sal*I fragments. The lower band in lane E which is common to all the lanes E through K is the vector pRO1614, as a linear *Sal*I fragment derived from the recombinant plasmids. The *Sal*I-produced recombinant plasmids were designated pRO1614:pCG131 *Sal*I-A through G (largest fragment to the smallest). The recombinant plasmids displayed in Fig. 2, thus, have fragments resembling the individual fragments present in pCG131 as shown in lane A for *Bam*HI cleavage or lane D for *Sal*I cleavage. The bacterial recipient for these cloning experiments was strain PSO100, a plasmid-free SR^+ strain described earlier. This collection of SR^+ strains (Table 1) containing part of pCG131 was then used, with appropriate controls, to determine possible contributions to virulence in corn plants by pCG131 as described below.

Virulence of strains containing pCG131 cloned fragments. Data presented in Table 2 demonstrates that the presence of some of the pCG131 fragments increased the virulence of the chimera-containing strains over that observed with the plasmid-free isolate PSO100. Strains PSO1006 and PSO1008, which contain the *Sal*I-C and *Sal*I-E fragments, respectively, showed increased virulence relative to PSO100 in both cultivars (Table 2). Strains PSO1004 and PSO10010, which contain *Sal*-A and *Sal*-G fragments, respectively, showed increased virulence in cultivar GCB and not in PX69 (Table 2). Strains harboring chimeric plasmids which contained *Sal*-B, D, and F fragments, respectively, showed more variable results with both cultivars. Strains harboring chimeric plasmids with several of the *Sal*-I fragments (ie, *Sal*-C and *Sal*I-E) showed increased virulence. The two strains containing the *Bam*HI chimeric plasmids (PSO1002 and PSO1003) showed inconsistent and variable results. It would be expected that since the *Sal*I fragments are within the *Bam*HI-A and B fragments, that strains harboring fragments A or B would produce disease comparable to that expressed by strains containing a sequence within the larger fragments. Variability of pathogenicity was not due to instability of chimeric plasmids in strain PSO100, since no carbenicillin-sensitive isolates of the chimeric plasmid-containing strains were obtained in the course of this study.

DISCUSSION

Our studies show that the plasmid pCG131 of *P. syringae* pv. *syringae* and its derivatives were conjugative, and that the pCG131 may play a role in virulence. Production of SR was shown to be independent of the presence of pCG131. The diverse collection of mutants obtained here showed no relationship between plasmid content and toxin production. The loss of SR production in mutants previously produced by exposure to acridine orange (5) may have occurred as a consequence of exposure to the mutagenic effect of acridine orange. Therefore, it appears unlikely that SR production is encoded by pCG131 or that the presence of the plasmid affects toxin production.

The decreasing virulence of wild-type strain HS191 in plant tests probably was due to continued transfer throughout the series of experiments; at least some of the plants infected with chimeras expressed virulence comparable to the original wild-type strain in each test. It should be noted that while cultivar homogeneity can be assumed for selected genes, there is not necessarily homogeneity for unselected traits, such as holcus spot susceptibility or resistance.

Although it is unknown how the plasmid may contribute to virulence, there is at least some evidence that it may encode for changes found in the outer membrane of the cell (11). In a recent report, Hurlbert and Gross (11) have shown that both strains AO111 and PSG100 (here designated PSO100) lack a 68,000 molecular weight outer membrane protein. They suggested that pCG131 may encode for this protein and repress the synthesis of two other proteins (molecular weights 78,000 and 63,000). Hignett and Quick (10) have also suggested that an outer membrane protein affects virulence of another phytopathogenic pseudomonad. Further analysis of chimera-containing strains will determine if any of the cloned segments coded for the proteins described by Hurlbert and Gross (11).

Molecular cloning of pCG131 was effective in creating chimeric plasmids that replicated in *P. syringae* pv. *syringae*. Such techniques should prove useful in further analyses of both plasmid and chromosomal traits.

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