Plasmid pDC190: Distribution Among *Erwinia stewartii* Strains and Nonassociation with Virulence

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ABSTRACT

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The correlation between the presence of pDC190 and the virulence of *Erwinia stewartii* to corn was investigated. Southern blot hybridizations with use of a pDC190::Mu pf7701 probe confirmed earlier observations that pDC190 replicons were present in all virulent strains. However, a virulent strain in which pDC190 had been labeled with Mu pf7701 was cured of the hybrid plasmid without affecting virulence. Furthermore, the virulence of DC336-1, a previously isolated mutant suspected of lacking pDC190 genes for virulence, was restored by a cloned chromosomal fragment. Therefore, even though highly conserved in *E. stewartii*, pDC190 does not appear to be essential for virulence on corn seedlings.

Knowledge of the plasmids of *Erwinia stewartii* and their relationship to the virulence of this bacterium to corn is important because they comprise 20–25% of its genome. SS104, a virulent strain of *E. stewartii*, has 11 plasmids, sizes 2.7, 2.8, 8.8, 16.7, 22, 29.5, 41, 43, 49, 68, and 210 megadaltons (Mdalton) (4). Although the functions of the plasmids in *E. stewartii* are still unknown, we previously noted (3) a correlation between 68- to 70-Mdalton plasmids and virulence. Of 31 virulent strains examined, only one, GC6, was missing a plasmid of this size, whereas six of eight avirulent strains lacked such a plasmid. Upon further examination, we found that GC6 total plasmid DNA contained seven *Bam*H1 fragments that were part of the 68-Mdalton plasmid in other virulent strains, and we speculated that these *Bam*H1 fragments were part of a 47-Mdalton deletion plasmid.

Coplin et al (4) observed that two avirulent mutants derived from SS104 by Garibaldi and Gibbins (7) were missing the 210-, 49-, 41-, and 22-Mdalton plasmids. In an attempt to genetically label any one of these plasmids, we induced replication of Mu pf7701 in SS104 and then selected for conjugal transfer of kanamycin resistance (Km^r) to one of the above mutants, DC350 (3). We expected DC350 would be a good recipient for only the missing plasmids and the procedure would select for insertion of Mu pf7701 into any of these plasmids that might be conjugative. Instead, a new plasmid of 89 Mdalton was observed in one of the transconjugants, and the resident 68-Mdalton plasmid, pDC190, was missing. Restriction analysis suggested that the new plasmid was a pDC190::Mu pf7701 hybrid containing a 2-Mdalton deletion. However, whether the new plasmid, designated pDC191, was actually derived from the copy of pDC190 in SS104 or from the one in DC350 was unclear; Mu pf7701 could have been transposed either before or after conjugal transfer, since zygotic induction of the Mu prophage probably occurred in the recipient.

To determine if pDC191 had a role in virulence, we transferred pDC191 by conjugation into two avirulent strains (DC150 and ES-4) and two virulent strains (DC336 and DC211) of *E. stewartii* (3). Although pDC191 did not restore virulence to the avirulent strains, a few of the transconjugants from the matings with the virulent recipients became avirulent. Examination of the plasmid DNA from the avirulent transconjugants revealed the presence of pDC191 and a new 51-Mdalton plasmid and the loss of the resident 68-Mdalton plasmid. The introduced plasmid (pDC191) and the resident plasmid (pDC190) were incompatible; since the two plasmids have identical replication functions, stable maintenance in the same cell would not be expected. Therefore, we suggested that pDC190 was a cointegrate plasmid that dissociated to form the 51-Mdalton plasmid in order to resolve incompatibility with pDC191. We further speculated that the avirulence of these strains could have been due to mutation of a locus for virulence on pDC191 (caused either by Mu pf7701 insertion or by prior mutation in DC350) and loss of the corresponding virulence genes from pDC190 when it dissociated. Alternatively, the missing portion of the resident plasmid could have integrated into the chromosome and interrupted a chromosomal locus for virulence.

In this study, the role of pDC190 in virulence was assessed by a number of complementary approaches. First, we confirmed previous observations on the presence of 68-Mdalton plasmids in virulent *E. stewartii* strains, particularly GC6, which appears to be missing this plasmid. Second, we reexamined the effect of displacing the resident pDC190 plasmid in SS104 with pDC191 and characterized the genetic changes in the avirulent transconjugants. Third, we characterized cloned DNA that restored virulence to an avirulent pDC191 transconjugant. Finally, we determined the virulence of a strain cured of pDC190.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains, plasmids, and bacteriophages are listed in Table 1. Virulent *E. stewartii* strains SW2, SW3, SW13, SW14, and SW18 were field isolates obtained by D. Coplin; virulent strains GC6 and LC were received from A. Karr; avirulent strain ES-4 was obtained from T. Woods; and avirulent strains SS10, SS11, and SS13 were received from A. Kelman.

Media and mating conditions. L-broth (9) (10 g of Bacto tryptone, 5 g of Bacto yeast extract, 5 g of NaCl per liter of distilled water) was used for all experiments and solidified with 1.5% Bacto purified agar for plate cultures. Semisolid media contained 0.7% agar. The following were used for antibiotic selective media: kanamycin, 20 μg/ml; nalidixic acid, 20 μg/ml; and streptomycin, 50 μg/ml. Matings were done as described by Coplin (2) on

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nitrocellulose filters at 30 C for 3 hr on L-agar; the donor-to-recipient cell ratio was 1:2.

Transformation of *Escherichia coli* with pDC191 and pDC192. A modification of the transformation procedure described by Scheif and Wensink (12) was used. Total plasmid DNA from either DC350-9 (containing pDC191) or SM192 (containing pDC192) in TE (10 mM Tris-HCl [pH 7.0], 2 mM EDTA) was added to 0.2 ml of competent HB101(Mu) cells. The approximate amounts of total plasmid DNA used were 1.5, 3.0, and 4.5 μg of DC350-9 and 2.25, 4.5, and 6.75 μg of SM192. Cells were allowed to take up the DNA for 25 min in an ice-water bath and then were heat-shocked at 37 C for 2 min and held at room temperature (22 C) for 10 min. One milliliter of L-broth was added to each tube, and the cells were incubated for 1 hr at 30 C before plating on L-agar containing kanamycin. Plates were incubated at 30 C.

Isolation of plasmid DNA. For large-scale extraction of plasmid DNA, the alkaline-denaturation method of Currier and Nester (6) as modified by Currier and Morgan (5) was used. Plasmid DNA was twice purified on CsCl-ethidium bromide gradients. CsCl-ethidium bromide gradients were centrifuged at 36,000 rpm in a Beckman Ti55 rotor for 48 hr at 15 C.

The alkaline extraction method of Birnboim and Doly (1) was used as a rapid miniscreen for plasmids. L-broth cultures (2.5 ml) were grown for 18 hr at 30 C with agitation, and the cells from 0.4 ml of the overnight culture were used for plasmid isolation. Purified DNA was resuspended in 40 μl of sterile distilled water and 10 μl of tracking dye (25 ml of 80% glyercol, 20 ml of TE buffer, 5 ml of 1% bromophenol blue in TE buffer), and a 10–15 μl aliquot was electrophoresed in a horizontal 0.5% agarose gel in TA buffer (40 mM Tris-HCl, 20 mM acetic acid, 2 mM EDTA, pH 8.0) at 5.0 V/cm for 4–5 hr. Plasmids from SS104 were used as molecular size standards (4).

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

Strains, plasmids	Characteristics	Source
Erwinia stewartii		
DC283	SS104 Nal[r]	D. L. Coplin
DC336	DC283::Mu *cts*62	D. L. Coplin et al (3)
DC336-1	DC336(pDC191)	D. L. Coplin et al (3)
DC350	104W13 Rif[r] (avirulent nonpigmented, from SS104)	L. N. Gibbins (7)
DC350-9	DC350(pDC191)	D. L. Coplin et al (3)
DC377	DC336 Met[-] Ile[-]	D. L. Coplin
RDF6011	DC336-1 cured of pDC191	This paper
RDF28303	SM192 cured of pDC192	This paper
SM192	DC283(pDC192)	S. L. McCammon et al (11)
SS104	Wild type	ICPB[a]
SW2	Wild type	D. L. Coplin
Escherichia coli		
HB101	F[-] *hsd*S20 *rec*A13 *ara*14 *pro*A2 *lac*Y1 *gal*K2 *rps*L20 *xyl*5 *mtl*1 *sup*E44	C. I. Kado
HB101(Mu)	HB101 (Mu *cts*62)	D. L. Coplin
DC988	C600 *hsd*M *hsd*R (Mu *cts*62)	D. L. Coplin
RDF101	HB101(pDC191)	This paper
RDF201	HB101(pDC192)	This paper
Plasmids		
pDC191	pDC190::Mu pf7701	D. L. Coplin et al (3)
pDC192	pDC190::Mu pf7701	S. L. McCammon et al (11)
pES1044	pVK100 clone containing 24-kb SS104 DNA insert; Tc[r]	D. L. Coplin et al (unpublished)
Bacteriophages		
Mu *cts*62	*cts*62	M. Howe
Mu pf7701	Mu *cts*62 pf7701 hPl#1, Km[r]	M. Howe[b]

[a]International Collection of Phytopathogenic Bacteria, M. P. Starr, curator.

[b]Thompson, C. J., and Howe, M. 1979. Tn5 induced mutations in bacteriophage Mu. Abstr. 544. Annu. Meet. Am. Soc. Microbiol.

Isolation of genomic DNA. Genomic DNA was isolated from *E. stewartii* using the growth and lysis conditions of Currier and Nester (6). Following lysis, DNA was extracted with phenol, precipitated with 95% ethanol, and purified by CsCl-ethidium bromide gradient centrifugation as previously described.

Restriction endonuclease digestions. Restriction endonucleases *Eco*R1 and *Hin*dIII were obtained from New England Biolabs; *Bam*H1, *Kpn*I, *Sma*I, and *Xba*I were purchased from Bethesda Research Laboratories. Digestions were performed according to manufacturers' recommendations, using 10–20 units of enzyme per microgram of DNA at 37 C, except for *Hin*dIII, which was incubated at 55 C. DNA was digested for 2–3 hr, at which time the reactions were stopped by heating at 65 C for 10 min. Restriction fragments were separated as previously described in 0.8% agarose gels in TA buffer at 1.55 V/cm for 12 hr. *Eco*R1 and *Hin*dIII restriction fragments of phage λ DNA were used as molecular weight standards.

Southern blot transfers, nick translation, and hybridizations. All blot transfers were made using the procedure of Southern (13) as modified by Wahl et al (14). Plasmid DNA was labeled with a nick translation kit from Bethesda Research Laboratories according to their recommendations, using either 80–100 μCi of [^{35}S]-dATP (specific activity = 406.0 Ci/mmol) or 100 μCi of [^{32}P]-dCTP (specific activity = >800 Ci/mmol). The labeled DNA was separated from unincorporated nucleotides by batch chromatography on an Elutip-d (Schliecher and Schuell) as directed by the manufacturer. Hybridization solutions and conditions have been described by Maniatis et al (10). For hybridizations using [^{35}S]-dATP labeled probes, all solutions contained 10 mM dithiothreitol to reduce background. Blots were prehybridized at 65 C for 4–6 hr. Probe DNA was denatured by boiling for 10 min, and $2-10 \times 10^6$ cpm of labeled, denatured probe was added per 100 cm^2 of nitrocellulose filter paper. Blots were hybridized for 12–14 hr at 65 C, then washed according to Maniatis et al (10). For autoradiography of [^{35}S]-dATP probed blots, filters were sprayed with En^{3}hance (New England Nuclear).

Elimination of pDC191 and pDC192 from *E. stewartii*. Ten-milliliter L-broth cultures of *E. stewartii* strains DC336-1(pDC191) and SM192(pDC192) were grown overnight with aeration. The cultures were diluted 10-fold and grown to about 1×10^9 cells/ml, and phage Mu pf7701 replication was induced at 42 C for 2 hr. The cultures were then incubated at 37 C for 3–5 hr until the A_{540nm} decreased, and dilutions of the lysate were plated on L-agar at 30 C. The survivors, enriched for cells that had previously lost the Mu-hybrid plasmid, were screened for Km^s (kanamycin sensitivity). Plasmids were extracted from Km^s colonies and examined by agarose gel electrophoresis (AGE).

Pathogenicity tests. Two inoculation methods were used: wounding of the pseudostem (which tests for wilting ability and lesion formation) and whorl inoculation without wounding (which tests only for ability to cause water-soaked lesions). For pseudostem inoculations, 7-day-old Earliking sweet corn seedlings, grown in a controlled environment chamber at 29 C with a 16-hr day length (23,000 lx), were used to assay for virulence of *E. stewartii* strains. Toothpicks dipped into 48-hr L-agar cultures were inserted into young corn seedlings about 1 cm above the soil line. For whorl inoculations, 10-day-old Earliking sweet corn seedlings, grown as described, were used. Bacteria were grown overnight in L-broth at 30 C with agitation. The cultures were diluted to 10^7 cells/ml with 0.01 M potassium phosphate buffer (pH 7.0) containing 0.2% Tween 40, and 0.1–0.2 ml of inoculum was pipetted into the whorls of the young seedlings. Plants were rated for lesions at 4 and 7 days after inoculation.

RESULTS

Transformation of *E. coli* with pDC191 and pDC192. Mu pf7701::pDC190 hybrid plasmids, pDC191 and pDC192, were transformed into an HB101(Mu) recipient by selection for the kanamycin resistance gene of Mu pf7701. Nineteen pDC191

transformants were analyzed by AGE, and five contained pDC191. One transformant, RDF103, contained both pDC191 and pDC170 (43 Mdalton). Similarly, 20 pDC192 transformants were screened by AGE, and three contained pDC192. The sources of the pDC191 and pDC192 DNA used as hybridization probes in the experiments described below were the transformed strains RDF101 and RDF201, respectively.

Occurrence of pDC190 in virulent and avirulent strains. A Southern blot of plasmid DNA from eight virulent and five avirulent strains of *E. stewartii* was hybridized with [^{35}S]-dATP labeled pDC191 DNA (Fig. 1). All eight virulent *E. stewartii* strains contained a plasmid homologous to pDC191. In the six strains containing a 68-Mdalton plasmid, pDC191 hybridized strongly to this plasmid; in the two virulent strains missing the 68-Mdalton plasmid (GC6 and LC), pDC191 hybridized to 47- and 52-Mdalton plasmids, respectively. Two of the five avirulent strains missing a 68-Mdalton plasmid (ES-4 and SS11) also contained smaller plasmids (45 and 41 Mdalton, respectively) with strong homology to pDC191. To determine the extent of homology, a Southern blot of *Bam*H1 restricted plasmid DNA from the same 13 strains was probed with pDC191 (Fig. 2). In strains with a plasmid that hybridized strongly to pDC191, at least eight *Bam*H1 fragments hybridized to pDC191. A common 5.5-Mdalton *Bam*H1 fragment was present in seven of the eight virulent strains and lacking from all five avirulent strains. The single virulent strain that did not have this 5.5-Mdalton *Bam*H1 fragment (GC6) instead had a unique 7.5-Mdalton *Bam*H1 fragment that hybridized to pDC191. In all *E. stewartii* strains examined, there was slight homology with some of the smaller plasmids; the 49-, 43-, 29.5-, and 16.8-Mdalton plasmids hybridized weakly with pDC191 in all cases.

Effect of pDC191 on virulence. In our previous study (3), transfer of pDC191 from DC350-9 into DC336 by conjugation resulted in some transconjugants with reduced virulence. In addition to acquiring pDC191 and losing pDC190, the transconjugants harbored a new 51-Mdalton plasmid. In an effort to repeat these earlier observations, we transferred pDC191 by conjugation from DC350-9 into two virulent recipients, DC336 and DC377, and selected transconjugants for kanamycin resistance. DC377 was mated three times and DC336 was mated once. Transfer occurred at 7×10^{-7} transconjugants per input donor cell with DC377 and at 3×10^{-9} with DC336. Although pDC191 transferred into both of the recipients, none of the transconjugants showed a reduction in virulence when wound-inoculated into the pseudostem of corn seedlings. Several transconjugants from each mating experiment were analyzed for plasmid DNA. In all cases, pDC190 had been displaced by pDC191, and in some transconjugants, new 63-, 51-, 36-, and 34-Mdalton plasmids were observed. A Southern blot of the plasmid DNA from these transconjugants was hybridized with [^{35}S]-dATP labeled pDC191 DNA to determine if these new plasmids had formed by dissociation of the resident 70-Mdalton plasmid in the recipient strains; none, however, hybridized with pDC191.

Characterization of DC336-1. Since we were unable to repeat the isolation of avirulent pDC191 transconjugants, we decided to characterize DC336-1, one of the avirulent mutants obtained in our previous study (3). This strain produced extracellular polysaccharide and caused slight wilting when inoculated into the pseudostem but did not cause any symptoms when inoculated into the whorl. DC336-1 contained pDC191 and a new 51-Mdalton plasmid and was missing pDC190. In contrast to the virulent transconjugants, however, the 51-Mdalton plasmid in DC336-1 did hybridize strongly to pDC191. In order to determine the extent of homology between pDC191 and the 51-Mdalton plasmid in DC336-1, pDC191 first had to be cured from DC336-1; the Km^r marker on pDC191 gave us a way to accomplish this. DC336-1 was grown at 42 C to induce Mu pf7701 replication. This enriched for surviving cells that had previously lost pDC191 because of improper segregation of the plasmid. Among 700 colonies screened, 12 Km^s strains were found, and AGE indicated that pDC191 was apparently lacking in these strains. When *Bam*H1

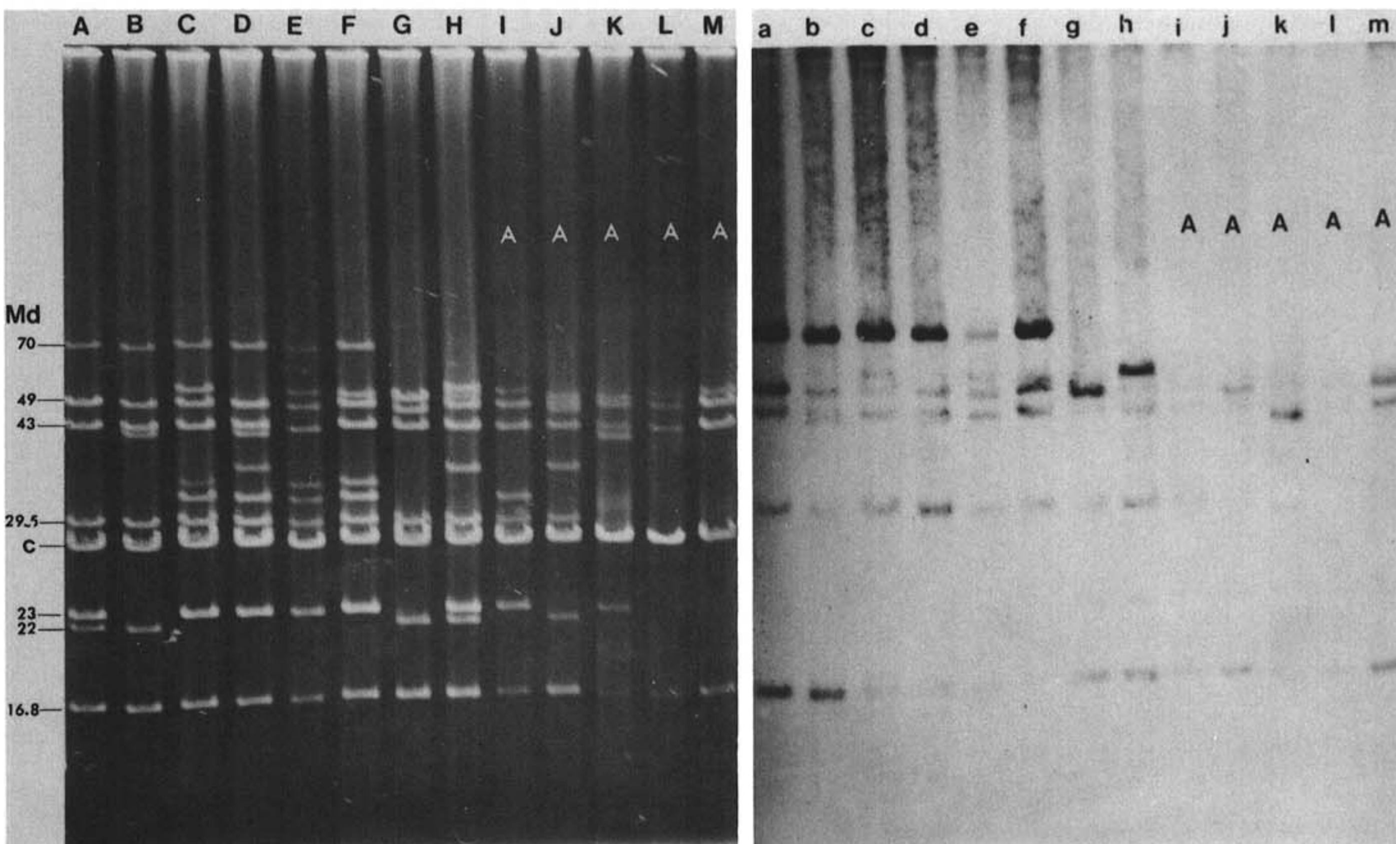

Fig. 1. Hybridization of plasmid DNA from virulent and avirulent strains of *Erwinia stewartii* with [^{35}S]-labeled pDC191 probe. Agarose gel (left) and Southern blot (right) of: **A,** SW18, **B,** SS104, **C,** SW2, **D,** SW3, **E,** SW14, **F,** SW13, **G,** GC6, **H,** LC, **I,** DC150, **J,** ES-4, **K,** SS11, **L,** SS13, and **M,** SS10. A = avirulent strains.

restricted plasmid DNA from one of these cured strains (RDF6011) was probed with pDC191 DNA, seven restriction fragments were found to hybridize (Fig. 3). These corresponded in size to fragments of pDC190 and probably originated from the 51-Mdalton plasmid. The 5.5-Mdalton *Bam*HI fragment that was correlated with virulence was noticeably missing in this strain (Fig. 3). Hybridizations to blots of genomic DNA of RDF6011 did not reveal any additional strongly hybridizing bands that would indicate pDC190 or pDC191 had integrated into the chromosome (data not shown).

Recently, a cosmid library of *E. stewartii* SS104 genomic DNA was constructed (D. L. Coplin et al, *unpublished*) using the cloning vector pVK100 (8). Clones were transferred into RDF6011 via triparental matings using the mobilizing plasmid pRK2013, and a single clone (pES1044) was identified that restored virulence to RDF6011. Restriction analysis followed by hybridization of this clone separately to *E. stewartii* genomic and plasmid DNA showed that the cloned DNA was of chromosomal origin. Although pES1044 did hybridize weakly with the 49-, 43-, and 29.5-Mdalton plasmids, there was no detectable hybridization of pES1044 with pDC190, pDC191, or the 51-Mdalton plasmid of RDF6011 (Fig. 4).

Elimination and transfer of pDC192. McCammon et al (11) obtained another insertion of Mu pf7701 into pDC190 by mutagenesis of DC283 and designated the resulting strain and plasmid SM192 and pDC192, respectively. SM192 remained fully virulent, indicating that any possible virulence genes in pDC190 were still functional in pDC192 but not in pDC191. Comparison of the restriction profiles of pDC191 and pDC192 (Fig. 5) showed that the sites of Mu pf7701 insertion were in fact different in these two plasmids, since different junction fragments were formed. (Further interpretation of this result was not possible without a restriction map of pDC190.) Since SM192 was virulent and contained no plasmids derived from pDC190 other than pDC192, we were able to cure pDC192 and test the effect of its loss on virulence. Using the same heat-shock protocol previously described for isolating RDF6011, we eliminated pDC192 from SM192. Seven hundred colonies were screened for Km^s, and 13 potentially cured strains were found. Blot hybridizations with a pDC191 probe, however, revealed all but one of these contained pDC192 deletion plasmids. In the single cured strain (RDF28303), hybridizations confirmed that pDC190 sequences were absent in both plasmid and chromosomal DNA. SM192 and RDF28303 were fully and equally virulent when inoculated to corn seedlings using both pseudostem wounding and whorl inoculation techniques.

In a further attempt to clarify the role of pDC192 in virulence, we transferred pDC192 from SM192 into RDF6011 by conjugation to see if the acquisition of pDC192 would restore virulence to this strain. A single transconjugant containing pDC192 was obtained, but acquisition of pDC192 did not restore pathogenicity to this strain. Attempts to transfer pDC192 back into RDF28303 by conjugation have been unsuccessful, probably because of zygotic induction of Mu pf7701.

DISCUSSION

Our previous findings that pDC190, a large conjugative plasmid commonly found in *E. stewartii* strains, appeared to be correlated with virulence and that avirulent strains sometimes arose when pDC191 was introduced into a wild-type strain prompted an investigation into the possibility that pDC190 was essential for the virulence of *E. stewartii*. Hybridization of pDC191 DNA to plasmid DNA from various strains of *E. stewartii* confirmed our notion that all of the 68-Mdalton plasmids observed in these strains were in fact related to pDC190. Moreover, the two virulent strains that lacked a 68-Mdalton plasmid (GC6 and LC) and constituted the exceptions to our correlation were shown to contain what appeared to be pDC190 deletion plasmids. Although the other results of the study show that pDC190 is not essential for symptom expression on seedlings, the finding that it is highly conserved in virulent strains suggests that it could have an important role in other aspects of the disease cycle of *E. stewartii*, such as insect transmission or survival.

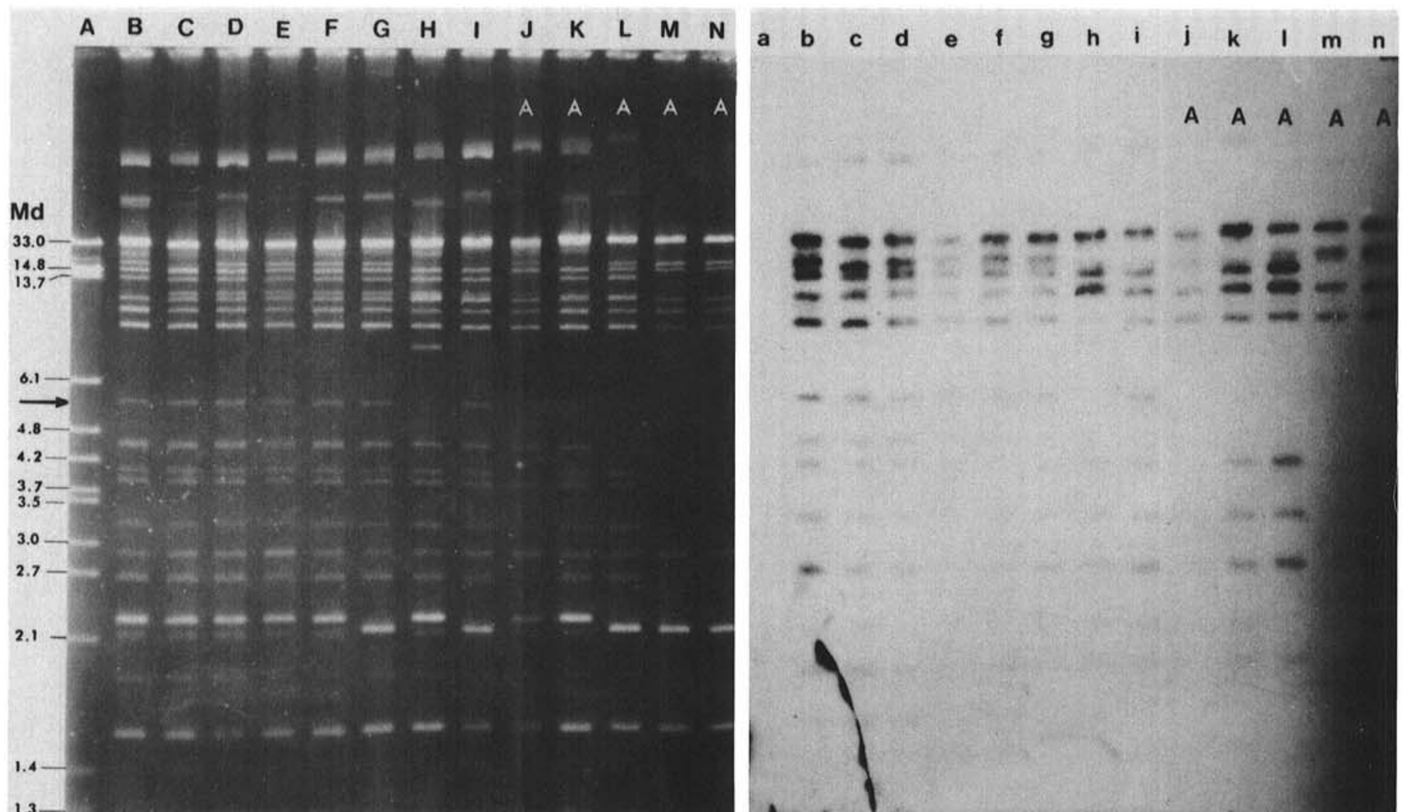

Fig. 2. Hybridization of plasmid DNA from virulent and avirulent strains of *Erwinia stewartii* restricted with *Bam*HI with [^{35}S]-labeled pDC191 probe. Agarose gel (left) and Southern blot (right) of: **A,** λ DNA standards, **B,** SW18, **C,** SS104, **D,** SW2, **E,** SW3, **F,** SW14, **G,** SW13, **H,** GC6, **I,** LC, **J,** DC150, **K,** ES-4, **L,** SS11, **M,** SS13, and **N,** SS10. A = avirulent strains. Arrow indicates 5.5-Mdalton *Bam*HI fragment common to virulent strains.

Isolation of a Mu pf7701 insertion mutant of pDC190 (i.e., pDC192) provided a selectable marker on pDC190 that allowed us to eliminate it from a virulent strain and directly assess its role in virulence. When this experiment was done, the "cured" strain, RDF28303, was still fully virulent. Hybridization of pDC191 to blots of plasmid and chromosomal DNA from RDF28303 confirmed that the plasmid was in fact missing and had not integrated into the chromosome. Thus, pDC190 does not seem to be required for virulence in DC283.

The question remained, however, as to the origin of the avirulent pDC191 transconjugants obtained in our previous study (3). In these strains, pDC191 had apparently displaced the resident pDC190 and a new 51-Mdalton plasmid had been formed. We hypothesized that upon transfer of pDC191 into DC336, the resident pDC190 dissociated in order to resolve incompatibility with pDC191 and in the process formed the 51-Mdalton plasmid. Attempts to repeat the isolation of similar mutants at a later date were unsuccessful. The virulent pDC191 transconjugants from the later experiments were missing pDC190; many had new, smaller plasmids but none of these hybridized to pDC191. In contrast, the 51-Mdalton plasmid of one of the original avirulent transconjugants, RDF6011, hybridized strongly with pDC190 and had a similar restriction profile that supported our supposition it was a deletion mutant of pDC190. Thus, these two sets of transconjugants appeared to differ in the manner in which the incompatibility between pDC190 and pDC191 had been resolved. RDF6011 was missing the 5.5-Mdalton *Bam*HI fragment of pDC190 previously correlated with virulence, and hybridization of pDC191 to chromosomal blots did not indicate that the portion of pDC190 deleted from the 51-Mdalton plasmid had integrated into the chromosome.

In a separate study, we constructed a cosmid library of *E. stewartii* DNA and used it to obtain clones that complement a number of avirulent mutants (D. L. Coplin et al, *unpublished*). One of the mutants used in this study, RDF6011, was complemented with clone pES1044. Hybridization studies showed that pES1044 contained chromosomal DNA and had no homology to pDC190. This finding and the fact that pDC192 did not complement RDF6011 confirmed the chromosomal location of the avirulence mutation in RDF6011.

In Southern blots, pDC191 showed weak homology with some of the other plasmids in *E. stewartii* (i.e., the 49-, 43-, 29.5-, and 16.8-Mdalton plasmids) and also with distinct bands in chromosomal digests. For example, in three avirulent strains lacking pDC190 (DC150, SS10, and SS13; Fig. 2), four *Bam*HI fragments, representing portions of different plasmids, hybridized with pDC191. The hybridizations and subsequent washes were done under very stringent conditions (65 C), suggesting that a short sequence, probably an IS element, was present in multiple copies in the chromosome, pDC190, and other plasmids. Since the pDC191 probe contained Mu pf7701, the possibility of weak homology due to Mu or Tn5 (IS50L) DNA cannot be eliminated.

At present, we do not know how many genes are involved in the pathogenicity of *E. stewartii* or what their products might be. So

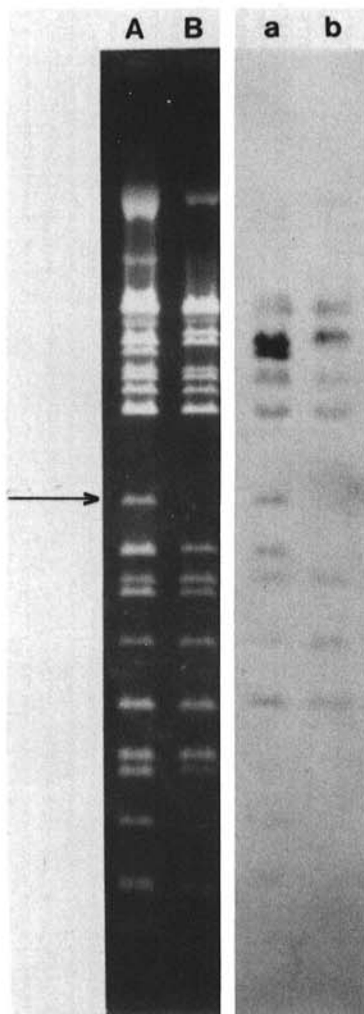

Fig. 3. Hybridization of plasmid DNA from *Erwinia stewartii* strain RDF6011 with [^{35}S]-labeled pDC191 probe. Agarose gel (left) and Southern blot (right) of plasmid DNA restricted with *Bam*HI of **A,** DC336 and **B,** RDF6011. Arrow indicates 5.5-Mdalton *Bam*HI fragment present in DC336 plasmid DNA and missing in RDF6011 plasmid DNA.

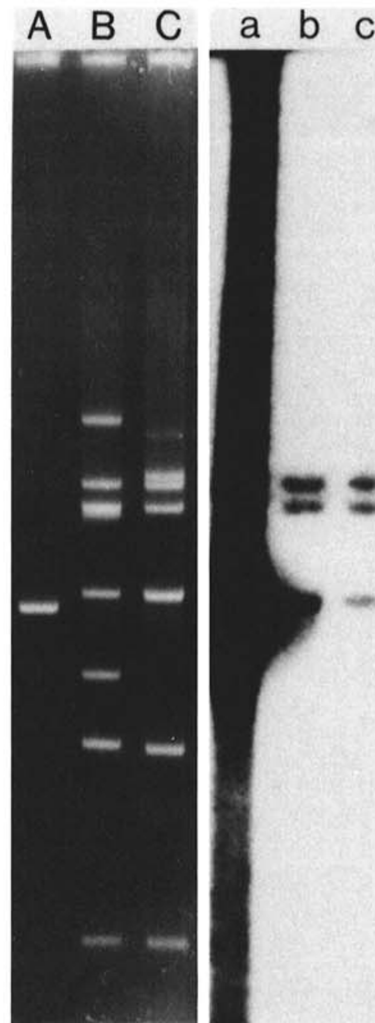

Fig. 4. Hybridization of plasmid DNA from a virulent and an avirulent strain of *Erwinia stewartii* with [^{32}P]-labeled pES1044 probe. Agarose gel (left) and Southern blot (right) of **A,** pES1044, **B,** DC283, and **C,** RDF6011. The slowest migrating plasmids in DC283 and RDF6011 are pDC190 and the 51-Mdalton plasmid, respectively.

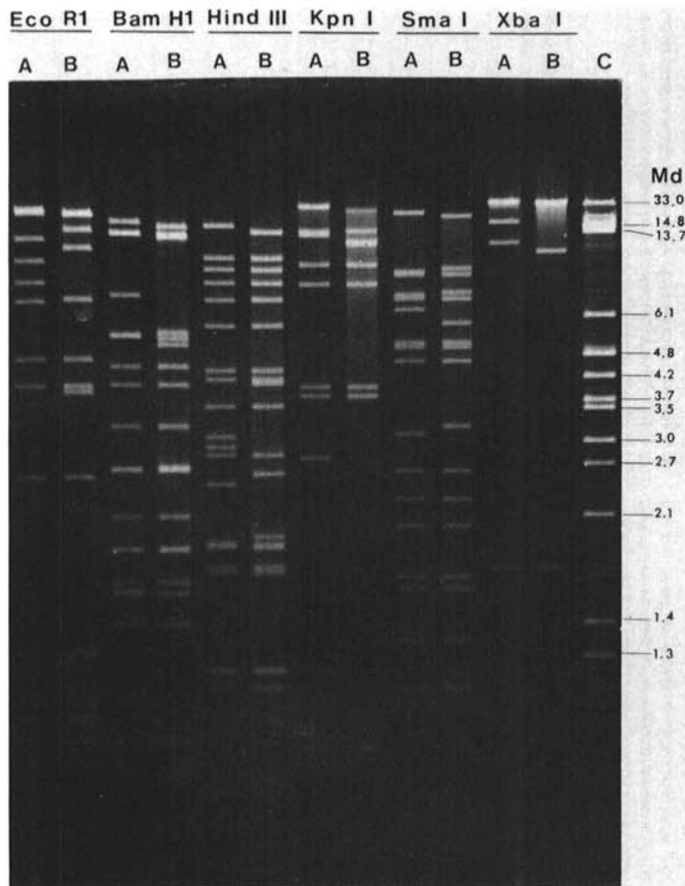

Fig. 5. Restriction analysis of pDC191 and pDC192, two Mu pf7701 labeled derivatives of the *Erwinia stewartii* plasmid pDC190. Agarose gel of **A**, pDC191, **B**, pDC192, and **C**, λ DNA standards.

far, however, the genetics of virulence appears complex. McCammon et al (11) obtained 12 avirulent mutants of *E. stewartii* via Mu pf7701 mutagenesis; none of the mutants contained Mu insertions into any of the cryptic plasmids. Therefore, these preliminary results plus the current evidence that the mutation in RDF6011 is on the chromosome indicate that the majority of virulence genes are chromosomal. It is still possible, however, that plasmids other than pDC190 could be additional determinants of pathogenicity.

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