Properties of F and P Group Plasmids in Erwinia stewartii

D. L. Coplin

Assistant Professor, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, OH 44691.

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ABSTRACT

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The F group plasmids F'*lac*$^+$ and R100*drd*56 and P group plasmids RP1, R68.45, RK212.1, and R751 were transferred into strains of *Erwinia stewartii* from *Escherichia coli*. All antibiotic resistance markers and the *lac*$^+$ genes carried on these plasmids were expressed. The Lac$^+$ transconjugants were generally unstable, insensitive to phage M13, and transferred F'*lac*$^+$ at very low frequency, whereas several RP1 transconjugants were sensitive to phages PRR1 and PRD1 and could donate RP1 at frequencies between 2×10^{-6} and 9×10^{-4}/donor cell. Donor ability correlated with sensitivity to PRR1 and PRD1, and RP1 was stable in all strains. The virulence of RP1-containing strains was essentially the same as that of their wild-type parents. Agarose gel electrophoresis of cleared lysates indicated that RP1 was present as an autonomous plasmid in *E. stewartii*. After transfer back to *E. coli*, RP1 and F'*lac*$^+$ appeared unaltered by passage through *E. stewartii*.

The discovery that plasmids of the F and P1 incompatibility groups can be transferred from *Escherichia coli* and *Pseudomonas aeruginosa* into *Erwinia* spp. has provided the opportunity to study the genetics of these plant pathogens (2, 3, 6, 11). In *E. amylovora* (4, 29) and *E. chrysanthemi* (5), F'*lac*$^+$ has been used to construct Hfr strains and map a number of chromosomal genes. The P1 plasmids originated in *P. aeruginosa* and have uniquely wide host ranges (23) which include many plant pathogenic species (6, 15, 27). Of these plasmids RP1 and RP4 are the best characterized. These plasmids are nearly identical and specify resistance to carbenicillin (Cb^r), kanamycin (Km^r), and tetracycline (Tc^r) and sensitivity to bacteriophages PRR1 and PRD1 (25, 26). A number of P plasmids can mobilize chromosomal genes in *E. coli* (19, 22), *Pseudomonas* spp. (12, 14, 31), *Rhizobium* spp. (1, 18), and *Acinetobacter calcoaceticus* (32). In addition, RP1 has been used to mobilize the virulence plasmid of *Agrobacterium tumefaciens* (8).

The present investigation was undertaken to introduce plasmids with known sex factor activity into *Erwinia stewartii*, the causative agent of Stewart's wilt of corn (28), in order to develop a genetic system for this pathogen. Particular emphasis has been placed on the maintenance and expression of F'*lac*$^+$ and RP1. Part of the information in this paper has been reported previously (7).

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MATERIALS AND METHODS

Media.—Unless otherwise specified, bacteria were grown on L broth and L agar (17). Matings were carried out on Difco Penassay Broth (PAB). When nutritional markers were used, either minimal A medium (21) or minimal DB medium (9) with either glucose or lactose was employed; agar and liquid media contained 0.1% and 1.0% carbohydrate, respectively. Selective media consisted of L or DB agar with appropriate additions of carbenicillin (Pfizer Geopen, 100 μg/ml), kanamycin (Sigma Chemical Company, St. Louis, MO 63178, 20 μg/ml), tetracycline (Sigma, 20 μg/ml), streptomycin (Nutritional Biochemicals Corp., Cleveland, OH 44128, 50 μg/ml), rifampin (Sigma, 100 μg/ml) or nalidixic acid (Sigma, 20 μg/ml). Lactose fermenting (Lac$^+$) colonies were detected on LAC-TZC agar (21) that contained 6 g peptone, 1 g yeast extract, 1 g casamino acids, 1 g NaCl, 10 g lactose, 1 mg 2,3,5-triphenyl-2H-tetrazolium chloride (TZC), and 15 g agar per liter.

Origin of bacterial strains and plasmids.—The bacterial strains used in this study are listed in Table 1. Nineteen additional strains of *E. stewartii*, designated SW4 through SW22, were isolated from corn leaf samples collected from Ohio, Indiana, Illinois, and Kentucky during 1975. All were virulent on sweet corn cultivar Earliking. Cultures taken from L agar were stored in DB containing 40% glycerol at −20 C and transferred to L agar at 28 C as needed.

Spontaneously-occurring nalidixic acid- and rifampin-resistant mutants were obtained by plating ~10^{10} cells on L agar containing either 20 μg/ml nalidixic acid or 100 μg/ml rifampin. Strain DC260, which requires histidine and proline, was derived from DC205 by two successive

cycles of nitrosoguanidine mutagenesis (5 μg/ml for 5 min in 0.1 M citrate buffer, pH 5.5) and penicillin selection (1 mg/ml penicillin G in DB for 4 hr) (21). Strain DC285 was derived from SW2 by nitrosoguanidine mutagenesis followed by overnight growth in L broth containing 100 μg/ml trimethoprim (Sigma). This strain requires 20 μg/ml thymine for maximal growth.

Plasmid transfers.—Cells were grown overnight in PAB and then subcultured until log phase. Donor and recipient cells were mixed in a ratio of 1:10 and collected on Millipore filters (pore size=0.22 μm) according to Chatterjee and Starr (2). Filters were incubated on PAB soft agar overlays at 28 C for P plasmid transfers and 32 C for F′*lac*$^+$ transfers. After mating, cells were washed once in saline (0.15 M), resuspended in 1 ml of saline and 0.1 ml aliquots of appropriate dilutions were plated on selective media. Controls consisted of equivalent numbers of donor and recipient cells plated separately on

TABLE 1. Origin and genetic properties of bacterial strains and plasmids used to study the F and P group plasmids of *Erwinia stewartii*

Strain, plasmid	Relevant characteristics[a]	Source
Pseudomonas aeruginosa		
PA067	His$^-$	R. H. Olsen
PA025	Arg$^-$Leu$^-$	R. V. Miller
Escherichia coli		
C600	Lac$^-$Thr$^-$Leu$^-$Thi$^-$	ICPB2395[b]
DC921	C600 Mod$^-$(K) Res$^-$(K) Nal^r	S. Cohen
DC922	C600 Mod$^-$(K) Res$^-$(K) Str^r	S. Cohen
JC1553	Met$^-$His$^-$Leu$^-$Arg$^-$*rec*A Str^r	C. Gonzalez (22)
J53	Pro$^-$Met$^-$	R. Olsen
UC5006	Ara$^-$Str^r (R100*drd*56)	ICPB3439 (3)
Erwinia herbicola		
Y46 R_{24}	Lac$^-$(F′*lac*$^+$)	ICPB2489 (2)
Erwinia stewartii		
AK239	avirulent, butyrous	A. Kelman
SW2		sweet corn, Wooster, OH
SW3		P. Larsen
SS104		ICPB
SS104R	avirulent	L. N. Gibbins (10)
SS102 (=Z05)		ICPB (7)
104RW18	white, avirulent	L. N. Gibbins (10)
Z09		A. K. Vidaver (9)
Z011		A. K. Vidaver (9)
Z017		A. K. Vidaver (9)
DC205	SW2 Nal^r	spontaneous
DC206	SW2 Rif^r	spontaneous
DC215	SS102 Nal^r	spontaneous
DC221	Z017 Nal^r	spontaneous
DC260	DC205 His$^-$Pro$^-$	nitrosoguanidine
DC285	SW2 Thy$^-$	nitrosoguanidine
DC291	DC285 (RP1)	this paper
Plasmids		
F′*lac*$^+$	Lac$^+$ IncFI	ICPB (2)
R100*drd*56	Tc^r*drd*56 IncFII	ICPB (3)
RP1	Cb^{r}Tc^r Km/Nm^r IncP1	R. Olsen (23)
R68.45	Cb^{r}Tc^r Km/Nm^r IncP1	R. V. Miller (12)
RK212.1	RK2 Cb^{r}Tc^{r}Km^s IncP1	N. Panopoulos
R751	Tp^r IncP1	R. Olsen

[a]Plasmid markers designate resistances to carbenicillin (Cb^r), kanamycin (Km^r), neomycin (Nm^r), tetracycline (Tc^r), and trimethoprim (Tp^r). Abbreviations: Inc denotes the plasmid incompatibility group and *drd* is derepressed for conjugal transfer; Str^r, Nal^r, and Rif^r denote chromosomal resistance to streptomycin, nalidixic acid, and rifampin, respectively; His$^-$, Arg$^-$, Leu$^-$, Thr$^-$, Met$^-$, Pro$^-$, Thy$^-$, and Thi$^-$ denote the requirements for histidine, arginine, leucine, threonine, methionine, proline, thymine, and thiamine, respectively; Lac$^-$ and Ara$^-$ indicate the inability to utilize lactose and arabinose, respectively; and Rec$^-$, Mod$^-$, and Res$^-$ indicate deficiencies in recombination, modification, and restriction, respectively.

[b]The abbreviation ICPB refers to the International Collection of Phytopathogenic Bacteria, University of California, Davis. M. P. Starr, Curator.

selective media. Either drug-resistant recipients or auxotrophic donors were used for counterselection.

Measurement of minimal inhibitory concentrations.—Transconjugants and parental strains were replica-plated onto L agar containing stepped concentrations of kanamycin (100 to 250 μg/ml), tetracycline (75 to 125 μg/ml) or carbenicillin (500 to 1,250 μg/ml). The minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic upon which no growth was observed after 3 days.

Sensitivity to donor-specific phages.—The F-dependent phage M13 was obtained from D. Pratt and the RP1-dependent phages, PRD1 and PRR1, from R. Olsen. Phage M13 was propagated on *E. coli* C600 (F'*lac*$^+$) and PRR1 and PRD1 were propagated on *P. aeruginosa* PA067 (RP1). All experiments were done with L top agar, base agar, and broth. For PRD1 infection, 5mM $CaCl_2$ was added (24). *Escherichia coli* and *P. aeruginosa* were incubated at 37 C and *E. stewartii* at 28 C. Spot tests were conducted with phage stocks at titers of 2×10^{10} to 4×10^{10} plaque-forming units (PFU) per ml.

Isolation and electrophoresis of plasmid DNA.—Bacteria were grown overnight in 30 ml of L broth and DNA was prepared and electrophoresed according to the method of Meyers et al (20) in 0.5% or 0.7% agarose (Seakem ME) disc gels. Gels were 6 × 90 mm and run at 60V for 4 hr. Plasmid bands were stained with ethidium bromide, visualized under shortwave UV light and photographed on Polaroid PN55 film through a Wratten G filter.

TABLE 2. Frequency of transfer of F'*lac*$^+$ from *Escherichia coli* C600 (F'*lac*$^+$) to *Erwinia stewartii*

Recipient	Lac$^+$ transconjugants per donor[a]
SW2	8×10^{-8}
SS102	2×10^{-4}
SS104	2×10^{-5}
ZO9	2×10^{-6}
ZO11	1×10^{-6}
ZO17	7×10^{-5}
AK239	5×10^{-5}
C600 Str^r	1×10^{-2}[b]

[a]Matings on filters for 2-3 hr at 32-35 C. Selection on DB lactose agar. Donor cells were titered at end of mating.
[b]Selection on DB lactose streptomycin agar.

RESULTS

Transfer of plasmids to Erwinia stewartii.—Plasmids F'*lac*$^+$ and RP1 were transferred to a number of *E. stewartii* strains from *E. coli*. Frequencies of F'*lac*$^+$ transfer (Table 2) ranged from 8×10^{-8} to 2×10^{-4} per donor cell. It was not possible to demonstrate transfer of F'*lac*$^+$ from *E. herbicola* Y46R$_{24}$ to *E. stewartii* although transfer from this donor to *E. coli* was normal. Transfer of F'*lac*$^+$ was higher when filters were incubated at 32 than at 28 or 30 C. In nonquantitative matings the drug resistance plasmid R100*drd*56 was transferred to SS102 and Z017 from UC5006 and Tc^r transconjugants were obtained.

Erwinia stewartii readily accepted RP1 with transfer frequencies as high as 0.2/donor cell (Table 3). Occasionally transfer of Cb^r appeared somewhat higher than Tc^r. This discrepancy was probably due to the release of β-lactamase into the medium which allowed the growth of Cb-sensitive colonies in the vicinity of resistant colonies. To avoid this problem, Tc^r was selected in most

TABLE 3. Transfer of IncP plasmids to *Erwinia stewartii* from *Escherichia coli* JC1553 and J53 and retransfer of them among *E. stewartii* strains

Donor	*E. stewartii* recipient	Selection[a]	Duration of mating (hr)	Transconjugants per donor
E. coli				
JC1553 (RP1)	DC205	Cb^r	3	9×10^{-2}
		Tc^r	3	2×10^{-2}
	DC215	Cb^r	3	1×10^{-2}
		Tc^r	4	2×10^{-1}
	DC221	Cb^r	3	8×10^{-2}
	DC285	Tc^r	3	6×10^{-3}
J53 (R751)	DC206	Tp^r	7	2×10^{-2}
E. stewartii				
SW2 (RP1)	DC260	Tc^r	4	nd[d]
SS104 (RP1)	DC260	Tc^r	6	nd
SW3 (RP1)	DC260	Tc^r	6	2×10^{-5}
SS104R (RP1)	DC260	Tc^r	4	8×10^{-6}
104RW18 (RP1)	DC260	Tc^r	4	9×10^{-4}
DC291 (RP1)	DC260	Tc^r	6	6×10^{-5}
DC206 (R751)	DC260	Tp^r	18	nd
DC260 (R68.45)	SW3	Tc^r[b]	18	4×10^{-6}
SW2 (RK212.1)	DC206	Tc^r[c]	3	2×10^{-5}

[a]Counterselection on LB agar with nalidixic acid unless otherwise indicated.
[b]Nutritional counterselection on DB agar.
[c]Counterselection on LB agar with rifampin.
[d]The abbreviation nd = not detected under experimental conditions; $< 1 \times 10^{-8}$/donor.

experiments. Controls consisting of donor or recipient cells by themselves rarely yielded spontaneous mutants resistant to nalidixic acid or rifampin. *Erwinia stewartii* transconjugants were distinguished easily from *E. coli* donors by their yellow pigmentation. The frequency of transfer for RP1 was tenfold greater on filters than in broth matings (D. L. Coplin, *unpublished*).

Several other P group plasmids also were transferred to *E. stewartii* in nonquantitative matings; R68.45 was transferred from PA025 to Z017 and SS102 with selection for Tc^r, R751 from J53 to DC206 with selection for trimethoprim resistance (Tp^r, 50 μg/ml) and RK212.1 from J53 into SW2 with selection for Tc^r.

Characteristics of transconjugants.—*Erwinia stewartii* displays delayed fermentation of lactose and for the purpose of these experiments can be considered Lac^-. Transconjugants receiving F′*lac*$^+$ grew as well on lactose as they did on glucose; colonies were easily counted after 4 days on DB lactose agar. In contrast, several weeks were required for wild-type strains to form colonies on the same medium. Most F′*lac*$^+$ transconjugants were highly unstable and purified colonies taken from DB lactose agar typically contained a majority of Lac^- cells when dilution-plated on LAC-TZC agar. In one such experiment, a colony of SW2 (F′*lac*$^+$) was transferred from DB lactose agar to L broth and the frequency of Lac^- cells in the population increased from 58 to 75% after ten doublings. It was possible, however, to find stable strains. When F′*lac*$^+$ was transferred into strains SW3 through SW22, AK239, Z09, Z011, and Z017 in spot-plate matings, stable clones were obtained from AK239, SW11, SW14, SW18, SW19, SW20, and Z011 after several single-colony transfers on LAC-TZC agar.

Transconjugants of *E. stewartii* containing plasmids RP1, R68.45, and RK212.1 expressed the antibiotic resistance patterns characteristic of each plasmid. For example, 250 clones each of SW2 (RP1) and SS102 (RP1) were selected for Tc^r and each of them also acquired Cb^r and Km^r. Minimal inhibitory concentrations for several strains containing RP1 are given in Table 4. In the cases of SS104R and 104RW18 a number of transconjugants were tested and the expression of Tc^r and Cb^r was found to vary among them.

In the absence of selection, RP1 was quite stable in all *E. stewartii* strains. Loss of the plasmid could not be detected following growth of SW2 (RP1) in L broth for 24 hr (250 clones tested) or after storage of SW3 (RP1), SS104R (RP1) or SW2 (RP1) on L agar slants at 4 C for 1 to 2 mo (100 colonies of each tested). Attempts to eliminate RP1 by growth for several days in L broth containing up to 10% sodium dodecyl sulfate were unsuccessful. Likewise RP1 was not eliminated from DC285 (RP1) by thymine starvation (2 to 10 μg/ml thymine in DB broth for 48 hr). However, RP1 was eliminated when another IncP1 plasmid was transferred into the same host. Transfer of R751 into SW2 (RP1) from J53 occurred at 1×10^{-8}/donor cell, whereas the frequency of transfer of this plasmid into DC206 was 2×10^{-2}/donor cell. The reduced frequency of transfer into SW2 (RP1) is indicative of entry exclusion of R751 by RP1 (24). Several Tp^r clones from the cross J53 (R751) × SW2 (RP1) were obtained and all had lost drug resistances associated with RP1 and sensitivity to phage PRD1.

Transconjugants harboring RP1 were similar to wild-type strains with respect to colony morphology and growth rate in vitro. The virulence of DC206 (RP1) and SS104 (RP1) was compared with that of their R^- parents with a quantal response infectivity titration assay (30) based on the inoculation technique of Lockwood and Williams (16). Sweet corn seedlings of cultivar Earliking were grown in a controlled environment chamber (16 hr photoperiod, 30,000 lux, 26 C days and 17 C nights) and inoculated 10 days after planting. Seedlings were cut off just below the first leaf and a 5 μliter drop of inoculum was placed on the cut end. Twenty-four seedlings were inoculated at each cell concentration and the experiment was repeated once. The ED_{50} for RP1 exconjugants averaged 6 ± 4 cells/plant and that for the R^- parents was 13 ± 4 cells/plant. These values were not significantly different. Although growth rates for the four cultures were the same in DB broth plus 0.1% casamino acids, the time required for all responses to appear in infectivity tests was 3 wk for RP1 transconjugants as compared to 2 wk for their R^- parents. There were no differences in disease severity after 4 wk. Bacteria isolated from infected plants retained RP1 markers.

Sensitivity to donor-specific bacteriophages.—Twenty-seven strains of *E. stewartii* containing F′*lac*$^+$ were tested for sensitivity to the F-specific phage M13 in spot tests; none was sensitive. Log phase cultures of six Lac^+ strains and their corresponding F^- parents then

TABLE 4. Minimal inhibitory concentrations (MIC) of carbenicillin, tetracycline, and kanamycin to RP1-containing strains of *Erwinia stewartii*

Strain	MIC[a] (μg/ml)			Clones tested (no.)
	Carbenicillin	Tetracycline	Kanamycin	
SW2[b]	5	1	1	1
DC260 (RP1)	250	>125[c]	250	2
SW3 (RP1)	1,000	>125	>250	1
SS104R (RP1)	500–>1,250	>125	>250	9
104RW18 (RP1)	500–>1,250	125	150–>250	9
JC1553 (RP1)	1,250	100	>250	1

[a]The lowest concentration at which all growth was completely inhibited. When variability among clones was observed, a range is given.

[b]Similar results were obtained for strains DC260, SW3, SS104R, and 104RW18.

[c]The symbol > denotes growth at highest concentration tested.

were infected with M13 at a multiplicity of infection of 0.005 PFU/cell for 5 hr at 33 C. The titer of M13 was measured at the beginning and end of the incubation period C600 (F'*lac*$^+$) as an indicator strain. Although increases between two- and eightfold were observed in some cultures, these increases were not statistically significant.

In a similar experiment, 21 *E. stewartii* strains containing RP1 were tested for sensitivity to the P-dependent phages PRR1 and PRD1 (25, 26). In spot tests most strains showed slight clearing with PRD1 and none with PRR1. Phage PRD1 produced clear lysis on SS102 (RP1) in spot tests but formed indistinct plaques when diluted. Only strains SW3 (RP1), SS104 (RP1) and 104RW18 (RP1) were sensitive to both phages; they gave clear plaques with PRD1 and hazy plaques with PRR1. Wild-type strains were not sensitive to either phage. The efficiency of plating (EOP) of PRD1 on SW3 (RP1), JC1553 (RP1) and PA067 (RP1) was similar regardless which of these hosts was used to produce the phage stocks, which indicated that restriction was not a problem in these tests. *Erwinia stewartii* SW3 (RP1) plated PRD1 more efficiently (EOP = 72%) than *E. coli* JC1533 (RP1) (EOP = 36%) but less efficiently than *P. aeruginosa* PA067 (RP1) (EOP = 100%).

Donor ability of Erwinia stewartii transconjugants.—Transconjugants of strains SW2, SS102, Z011, and AK239 harboring F'*lac*$^+$ were mated for 3 hr with SS102 and JC1553; SW18 (F'*lac*$^+$), SW19 (F'*lac*$^+$) and SW20 (F'*lac*$^+$) were mated overnight with their F$^-$Nal^r parents. Transfer of F'*lac*$^+$ could not be detected in any of these crosses. However, F'*lac*$^+$ was transferred from unstable Lac$^+$ clones of SW2, SW20, and SS102 to DC922 at low frequencies in spot plate matings on supplemented minimal A agar containing streptomycin and lactose. The Lac$^+$ DC922 transconjugants from these crosses were sensitive to M13 and donated F'*lac*$^+$ at normal frequencies in subsequent matings. In similar experiments it was not possible to demonstrate transfer of R100*drd*56 from DC215 to DC921.

Initial crosses in which wild-type RP1 donors such as SW2 (RP1) and SS102 (RP1) were used did not yield any transconjugants ($<1 \times 10^{-8}$/donor cell). However, the PRR1- and PRD1-sensitive strains SS104R (RP1), 104RW18 (RP1) and SW3 (RP1), described in the preceding section could donate RP1 at frequencies ranging from 8×10^{-6} to 9 to 10^{-4}/donor cell (Table 3). Transconjugants of SW2 containing R68.45 and RK212.1 also donated these plasmids at low frequencies (Table 3). Transfer of R751 from DC206 to DC260 could not be detected.

Since differences in donor ability could be due to the presence of cryptic plasmids in *E. stewartii* that inhibit fertility of RP1, it was decided to attempt to eliminate such plasmids by thymine starvation and then examine the survivors for increased sensitivity to PRR1 and PRD1. Forty clones of DC285 (RP1) were isolated following growth for 48 hr in DB liquid medium containing 10 μg/ml thymine; survival was 1.6%. One of these clones, designated DC291, was sensitive to both PRR1 and PRD1. This strain donated RP1 to DC260 at 2×10^{-5}/donor cell, whereas the other clones could not transfer RP1.

Isolation of plasmid DNA.—Salt-cleared lysates were prepared from unstable Lac$^+$ transconjugants of SW2, SW11, and SW20 and stable transconjugants of SW14 and SW20 and then were subjected to electrophoresis on 0.5% agarose gels. Only the unstable SW20 transconjugant contained a very faint band which migrated with an F'*lac*$^+$ DNA standard (D. L. Coplin, *unpublished*). This suggests that F'*lac*$^+$ either does not exist as an autonomous plasmid in *E. stewartii* or has an extremely low copy number since this technique gave good recovery of the cryptic plasmids from these strains and F'*lac*$^+$ from C600 (F'*lac*$^+$).

Attempts to demonstrate RP1 DNA in SW3 (RP1) were complicated by the presence of many cryptic plasmids. The molecular weight of RP1 is 36×10^6 daltons and most strains of *E. stewartii* contain a 39×10^6 dalton resident plasmid. Extended electrophoresis of plasmid DNA on 0.5% agarose gels just barely separated these two plasmids (Fig. 1, gel B).

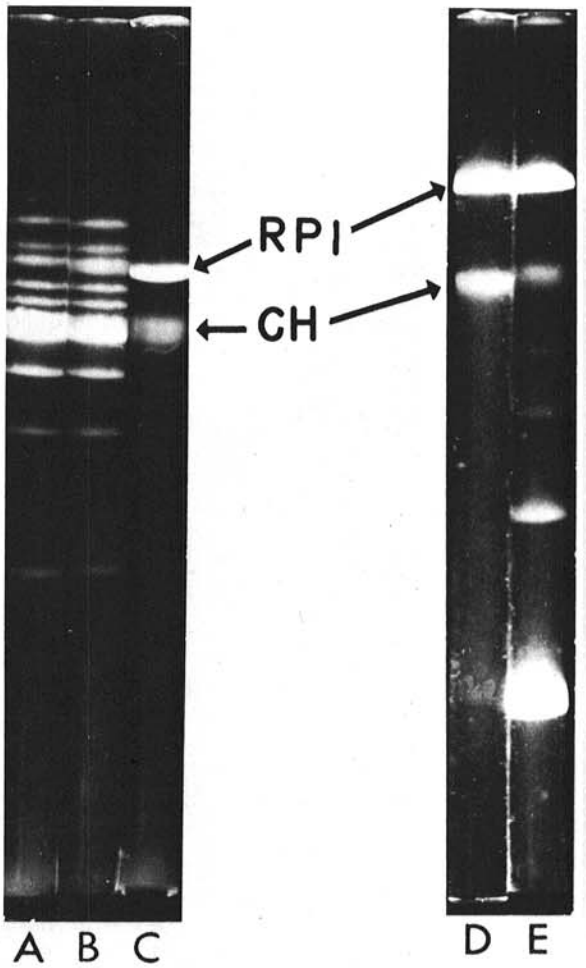

Fig. 1 (A to E). Agarose gel electrophoresis of cleared lysates containing cryptic and RP1 plasmid DNA: (A-C) 0.5% agarose; (D and E) 0.7% agarose. **A)** *Erwinia stewartii* SW3. **B)** SW3 (RP1). **C)** *Escherichia coli* JC1553 (RP1). **D)** JC1553 (RP1). **E)** Transconjugant from SW3 (RP1) × *E. coli* DC921. The abbreviation CH indicates chromosomal DNA.

As additional evidence that RP1 had not recombined with other replicons in *E. stewartii,* SW3 (RP1) was mated overnight with *E. coli* DC921 and DNA was extracted from four Tc^r transconjugants. A 36×10^6 dalton plasmid which coelectrophoresed with RP1 isolated from the original *E. coli* donor strain, JC1553 (RP1) (Fig. 1, gels D and E), as well as a 3×10^6 dalton cryptic plasmid, was present in all four DC921 transconjugants. The cryptic plasmid also was present in gels of cleared lysates from SW3 (D. L. Coplin, *unpublished*) but not from DC921, indicating that this plasmid had been mobilized by RP1 during conjugation. Additional bands seen in gel E may represent the monomeric open circular form of this plasmid and the open and closed circular forms of its dimer.

DISCUSSION

Results of the present study demonstrated that most strains of *E. stewartii* will accept IncF and IncP plasmids from *E. coli* and the resulting transconjugants express the expected drug resistant or Lac^+ phenotypes. Fertility of the IncF plasmids F′*lac*$^+$ and R100*drd*56 was almost completely inhibited in *E. stewartii,* whereas fertility of IncP plasmids was partially expressed in some strains. Both F′*lac*$^+$ and RP1 were capable of normal conjugation frequencies after transfer back to *E. coli* indicating that a difference in the host rather than a change in the plasmids themselves is responsible for their inhibition in *E. stewartii.* Closed circular RP1 DNA was detected in *E. stewartii* transconjugants, which demonstrated that this plasmid was maintained in an autonomous state without detectable changes in its physical characteristics. Further evidence for the maintenance of RP1 markers on a plasmid in *E. stewartii* was their displacement by an incompatible plasmid, R751. In contrast, the maintenance of F′*lac*$^+$ in *E. stewartii* is unclear since this plasmid could not be detected in cleared lysates from most strains. We are currently investigating the possibility that this plasmid integrates into the chromosome to form unstable Hfrs.

The behavior of RP1 in *E. stewartii* was similar to that observed in other erwinias. Lacy and Kelman (13) reported difficulty in transferring RP1 into corn isolates of *E. chrysanthemi* and low frequencies of intraspecific transfer. Gibbins et al (11) likewise reported that RP1 is expressed in *E. herbicola* but that transconjugants are poor donors. They also included *E. stewartii* strain SS104R in their study and obtained essentially the same results as presented in this paper, except that they did not attempt intraspecific matings.

The sensitivity of RP1 transconjugants to phages PRR1 and PRD1 correlated with their ability to donate RP1. Because plaques of PRR1 (which attaches to pili) were initially hazy and became increasingly clear after several days, it is probable that all cells are capable of producing pili at some point in their growth cycle but at a given time only a small percentage are piliated. Since *E. stewartii* is a good recipient in matings with *E. coli,* the low frequencies of retransfer from the transconjugants are probably due to its capacity to act as a donor. The inability of *E. stewartii* to produce effective pili and form mating pairs and/or fertility inhibition by resident plasmids are probably responsible for this. With regard to the first explanation, Lai et al (15) suggested that capsular slime may interfere with pair formation in *Xanthomonas vesicatoria* because RP1 was transferred at higher frequencies in a minimal medium in which slime was not produced. This is likely because strains containing RP1 form weak mating pairs (12). Unfortunately, such an increase could not be demonstrated for *E. stewartii.* This may be due to a low level of slime production by *E. stewartii* in L broth or PAB which lack sugars.

Olsen and Shipley (24) have demonstrated that fertility of RP1 is severely repressed by IncN plasmids. Two lines of evidence support the concept that fertility inhibition by cryptic plasmids may likewise be responsible for lowered conjugation frequencies in *E. stewartii.* Most strains of *E. stewartii* contain at least eight species of plasmid DNA (D. L. Coplin and M. Rudinski, *unpublished*) which are evident in Fig. 1-A. Secondly, recent studies have shown that three strains capable of donating RP1, SW3, SS104R, and 104RW18, lack one or two plasmids present in nondonor strains SW2 and SS104. Because even the best RP1 donors found in this study did not transfer RP1 as well as *E. coli,* either a combination of host factors or fertility inhibition is involved. In the case of F′*lac*$^+$ plasmids, the finding that the stable Lac^+ derivative of SW11 is missing the 44 and 48×10^6 dalton plasmids originally present in SW11 (D. L. Coplin and R. Rowan, *unpublished*) suggests that a resident IncF plasmid could be responsible for the low frequency of transfer from *E. coli* into *E. stewartii* and the subsequent instability of the transconjugants.

Recent attempts to demonstrate mobilization of auxotrophic markers using RP1, R68.45, and F′*lac*$^+$ in intrastrain matings have not been successful. Since the frequency at which RP1, R68.45, and F′ plasmids mobilize chromosomal markers is usually several orders of magnitude less than the frequency of transfer for the plasmid itself, it is unlikely that F or P plasmids can be used for chromosome mapping at present. However, RP1 mobilized a 3×10^6 dalton cryptic plasmid from SW3 into *E. coli* (Fig. 1) and may prove valuable in transferring other nonconjugative plasmids into *E. coli* where they may be studied in a known genetic background. As more information is obtained, it should be possible to identify certain plasmids responsible for fertility inhibition of F′*lac*$^+$ and R68.45, eliminate them, and develop better donor strains.

In view of recent concern that acquisition of nonindigenous plasmids by plant pathogens, such as those used as molecular vehicles, might increase their virulence or survival potential, it was interesting to find that RP1 had very little effect on the pathogenicity of *E. stewartii.* In fact, the observed differences in response time suggest that strains harboring RP1 may grow more slowly in vivo.

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