

## Characterization of Plasmids in *Erwinia stewartii*†

D. L. COPLIN,\* R. G. ROWAN, D. A. CHISHOLM, AND R. E. WHITMOYER

Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

Received 16 March 1981/Accepted 29 June 81

Plasmids in 39 strains of *Erwinia stewartii* were examined by agarose gel electrophoresis. Most virulent strains had from 11 to 13 plasmids ranging in molecular mass from 2.8 to 210 megadaltons and contained plasmids of 210, 70, 49, 43, 29.5, 16.8, 8.8, and 2.8 megadaltons. Plasmids in strains SW2 and SS104 were characterized by both electron microscopy and agarose gel electrophoresis and may be useful as convenient references for sizing plasmids by electrophoresis. Specific size classes of plasmids could not be associated with antibiotic and heavy metal resistance, carbohydrate utilization, bacteriocin production, or pathogenicity to corn. However, avirulent strains tended to have fewer plasmids than virulent strains.

*Erwinia stewartii* (E. F. Smith) Dye can cause a devastating disease of both sweet and field corn. The bacterium grows in the intercellular spaces of leaves, causing localized lesions, and in the xylem vessels, causing systemic wilting. During the summer, *E. stewartii* is transmitted by the corn flea beetle (*Chaetocnema pulicaria* Melch) and can overwinter in the beetle's alimentary tract. We have been using *E. stewartii* as a model system for studying mechanisms of virulence and host resistance and have previously demonstrated that it readily exchanges R-factors with *Escherichia coli* (5) and contains several derepressed conjugative plasmids which can mobilize pCR1 between *E. stewartii* and *E. coli* (6). The purpose of this study was to verify that the DNA species previously observed in strains SW2 and SS104 by agarose gel electrophoresis (AGE) (5, 6) are plasmid DNAs, to show that multiple plasmids are characteristic of this species, and to identify, for future study, size classes of plasmids common in virulent strains.

### MATERIALS AND METHODS

**Bacterial strains.** *E. stewartii* strains that were analyzed for plasmids are given in Table 1. All *E. coli* R-plasmids and strain V517 were obtained from E. M. Lederberg, Plasmid Reference Center, Stanford University, Palo Alto, Calif. *Pseudomonas aeruginosa* plasmids pMG1 and pMG5 (12) were obtained from R. Olsen, University of Michigan Medical School, Ann Arbor.

Culture media, growth conditions, and storage of strains have been previously described (5). Antibiotic sensitivity tests were done with Difco antibiotic sensitivity disks (Difco Laboratories, Detroit, Mich.) on

† Journal Article no. 40-81 of the Ohio Agricultural Research and Development Center.

L. agar. Minimal inhibitory concentrations of AgCl, Na<sub>2</sub>SeO<sub>3</sub>, K<sub>2</sub>TeO<sub>3</sub>, HgCl<sub>2</sub>, and Na<sub>2</sub>HAsO<sub>4</sub> were determined in DB minimal agar at 10<sup>-3</sup>, 10<sup>-4</sup>, and 10<sup>-5</sup> M. Strains were also tested for utilization of carbohydrates (9) and bacteriocin production (21).

**Isolation of plasmid DNA.** Plasmid DNA for molecular mass determinations was prepared by the method of Currier and Nester (7). For gel electrophoresis, CsCl density gradient purification was sometimes omitted to give better recovery of large plasmids. The rapid alkaline lysis procedure of Birnboim and Doly (1) was used for plasmid screening. For detection of small plasmids, samples were treated with 15 μg of RNase A per ml at 37°C for 15 min before electrophoresis.

In general, most methods of plasmid isolation that use sodium dodecyl sulfate or sodium dodecyl sarcosine worked for *E. stewartii* without modification (1, 3, 7, 12, 16). However, this species is resistant to lysozyme, so pretreatment with penicillin G to produce spheroplasts was necessary to obtain lysis with gentler detergents, such as Triton X-100. The recovery of all plasmids was optimal with techniques that use alkaline denaturation (1, 3, 7).

To determine the amount of plasmid DNA in strain SW2, exponentially growing cells (8 × 10<sup>8</sup> cells per ml) were labeled for 4 h with 5 μCi of [*methyl*-<sup>3</sup>H]thymidine (specific activity, 50.8 Ci/mmol) per ml in minimal glucose medium containing 0.1% Casamino Acids and 250 μg of 2'-deoxyadenosine per ml. Cells were washed and suspended in 1.0 ml of 16% sucrose; 0.2 ml of 0.25 M tris(hydroxymethyl)aminomethane (Tris) chloride buffer (pH 8.0), 0.4 ml of 0.25 M ethylenediaminetetraacetic acid (EDTA) (pH 8.0), and 1.6 ml of 1.6% sodium dodecyl sarcosine-0.05 M Tris-0.05 M EDTA (pH 8.0) were then added. The viscosity of the lysate was reduced by mixing for 30 s on a Vortex mixer. Plasmid DNA was separated by isopycnic centrifugation in ethidium bromide-cesium chloride density gradients (7). Fractions were collected, and radioactivity in the two DNA bands was determined (20).

**Gel electrophoresis.** Molecular masses of plas-

TABLE 1. *Origins of E. stewartii* strains

Strain <sup>a</sup>	Origin	Source
SW1, SW2	Ohio, 1974	Coplin
SW3	Ohio, 1970	Coplin
SW11	Illinois, 1975	Coplin
SW13, SW14	Indiana, 1975	Coplin
SW18, SW63	Ohio, 1975	Coplin
SW19, SW20	Kentucky, 1975	Coplin
SW36	Tennessee, 1975	Coplin
SW39	N. Carolina, 1975	Coplin
SW45	Missouri, 1975	Coplin
SW51	Virginia, 1975	Coplin
SW65	Ohio, 1977	Coplin
SW69	Ohio, 1976	Coplin
SW70, SW71	Ohio, 1979	Coplin
E141 through E144	Connecticut, 1975	D. Sands
SS104	Illinois, 1967	ICPB <sup>b</sup>
Z05, Z017	New York (8)	A. Vidaver
22A, GC6	Missouri, 1975	A. Karr
ES-1, ES-2, ES-4	New York, 1975	T. Woods
DC150, DC155	Illinois, 1976	M. Turner
SS10, SS12, SS13	Lindstrom, Iowa, 1940	ICPB
SS11	Lindstrom, Iowa, 1940	ATCC8199
104W18, 104W13	Nonpigmented variants of SS104 by growth at 37°C (10).	L. N. Gibbins

<sup>a</sup> Strains SW2 and SS104 have been deposited with the Plasmid Reference Center, Stanford University, (E. M. Lederberg, curator). All isolates are virulent and from corn except E141 through E144, which are from flea beetles, and SS10 through SS13, DC150, ES2, ES4, and 104W18, which are avirulent.

<sup>b</sup> ICPB, International Collection of Phytopathogenic Bacteria, Davis, Calif., (M. P. Starr, curator).

mids were determined by horizontal AGE in 0.7% Seakem ME agarose (Marine Colloids, Rockland, Maine) gels in either TA buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, pH 8.0) at 5.0 to 5.5 V/cm or TB buffer (44.5 mM Tris, 44.5 mM boric acid, 2.5 mM EDTA, pH 8.0) at 8.0 to 9.0 V/cm. Gels were stained with 0.5  $\mu$ g of ethidium bromide per ml and photographed under ultraviolet light. Plasmids RA1 (86 megadaltons [Mdal]), R1 (62 Mdal), RP1 (39.5 Mdal), pRO161 (25.5 Mdal), and pML2 (8.9 Mdal) were used as size references for unknown plasmids between 16 and 70 Mdal, and plasmid pML2 and strain V517 were used for unknown plasmids smaller than 16 Mdal. Masses were calculated by linear regression of log relative mobility versus log molecular mass (12, 16); pRO161 DNA was added to *E. stewartii* samples as an internal reference for calculating relative mobility. Plasmids pMG1 (312 Mdal), pMG5 (280 Mdal), R478 (166 Mdal), and Rts1 (126 Mdal) were used as standards for the largest unknown plasmid, and molecular masses were determined graphically.

To screen *E. stewartii* strains for plasmid content, DNA preparations were electrophoresed on both 0.7 and 0.5% TA gels. The use of two gel concentrations allowed the identification of chromosomal and open circular plasmid bands and so detected plasmids that might otherwise have been obscured by the chromosomal bands. Plasmids of strains SW2 and SS104 were used as molecular size references.

**Electron microscopy.** CsCl density gradient-purified plasmid DNA in STE buffer (50 mM Tris, 50 mM NaCl, 5 mM EDTA, pH 8.0) containing 20  $\mu$ g of ethidium bromide per ml was nicked by exposure to a

40-W cool white fluorescent lamp at a distance of 10 cm for 2 h. Ethidium bromide was removed by dialysis, and the DNA was spread for electron microscopy (EM) according to Kleinschmidt (13). Pure carbon support films on 200-mesh copper grids were used. All material was viewed and photographed with a Phillips EM201 electron microscope. DNA molecules were measured from photographic enlargements (final magnification,  $\times$  160,000) with a Numonics model 250-113 graphics calculator. ColE1 DNA (4.2 Mdal) was used as an internal standard.

## RESULTS

**Plasmid DNA in SW2 and SS104.** AGE of gradient-purified DNA revealed 13 plasmids in SW2 and 11 plasmids in SS104. Their molecular masses were determined by AGE and EM (Fig. 1 and Table 2) and ranged from 2.8 to 210 Mdal. In each strain, AGE resolved three more plasmids than did EM. With the exception of the 210-Mdal plasmid, the following observations indicate that all of the bands identified as plasmids in our gels were covalently closed circular DNA: (i) all circular DNA species seen by EM corresponded to gel bands; (ii) the relative order of migration in gels was not affected by changes in voltage, buffer composition, or agarose concentration; (iii) heating and quick cooling of the samples before electrophoresis (19) did not remove bands; and (iv) nicking (as described for

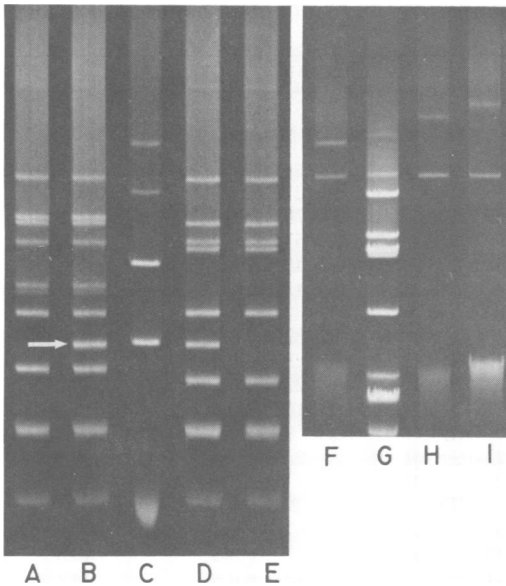


FIG. 1. AGE of plasmid DNA from *E. stewartii* strains SW2 and SS104 and R-plasmids used as molecular mass standards. (A) SW2; (B) SW2 + pRO161 (arrow); (C) pML2 (8.9 Mdal) + pRO161 (25.5 Mdal) + RP1 (39.5 Mdal) + R1 (62 Mdal) + RA1 (86 Mdal); (D) SS104 + pRO161; (E) SS104; (F) RA1 + R478 (166 Mdal); (G) SS104 + RA1; (H) RA1 + pMG5 (280 Mdal); (I) RA1 + pMG1 (312 Mdal).

EM) prevented most of the DNA from entering the gel and produced several new bands that corresponded to open circular forms of the smaller plasmids. Evidence for the 210-Mdal plasmid is not as convincing because it was not observed by EM, it was not efficiently recovered from CsCl density gradients, and it was removed by heating. However, the fact that its relative mobility was unaffected by changing electrophoretic conditions and it was susceptible to nicking suggest that it is a covalently closed circular species. In addition, this plasmid was only isolated by procedures designed for extraction of large plasmids.

The plasmid DNA of strain SW2 comprised  $14.6 \pm 1.6\%$  of the total DNA. Given the molecular masses in Table 2 and assuming shearing of the 210-Mdal plasmid during extraction, 10 copies per genome each of the 2.7- and 2.8-Mdal plasmids and 1 copy each of the remaining plasmids, and an *E. stewartii* genome size equivalent to that of *E. coli* ( $2.5 \times 10^9$  dal), we would expect to recover 17% plasmid DNA. Thus, our results suggest that most of the plasmids in SW2 are present in low copy number. Furthermore, if these assumptions are true and we did not get complete recovery of large plasmids, then SW2 could contain as much as 25% plasmid DNA.

**Stability of plasmids in culture.** Two cultures of SW2 were alternately grown in L broth and L agar to stationary phase for a total of 28 transfers. Plasmids were then isolated from five clones from each culture. In one culture the plasmids were unchanged, whereas in the other the 49-Mdal plasmid had apparently formed a dimer.

**Characterization of strains.** To select strains for plasmid screening which represented a range of variability in *E. stewartii*, 94 strains were tested for utilization of 26 carbohydrates, and 69 strains were selected for resistance to five heavy metal salts (Ag, Se, Te, Hg, and As) and to 14 antibiotics (penicillin, ampicillin, carbenicillin, kanamycin, neomycin, chloramphenicol, sulfathiazole, sulfadiazine, gentamicin, colistimethate sodium, streptomycin, polymyxin, tetracycline, and trimethoprim). With only four exceptions, all of our strains conformed to the description of *E. stewartii* in *Bergey's Manual of Determinative Bacteriology* (14) and its antibiotic sensitivity pattern reported by Garibaldi and Gibbins (10). No differences in the minimal inhibitory concentrations of heavy metals were observed between strains. Minimal inhibitory concentrations were  $10^{-3}$  M for Ag and Se,  $10^{-3}$  M for As and Hg, and  $10^{-4}$  M for Te. The high minimal inhibitory concentrations for Hg indicate that *E. stewartii* is more resistant to this metal than are most bacteria. Four strains from Connecticut (ES141 through ES144) utilized maltose and rhamnose, but not raffinose and melibiose.

All combinations of 20 strains of *E. stewartii* and several strains of *Erwinia herbicola* and *E. coli* were tested, both as bacteriocin producers and as indicators. Although lysogenic phage were present in about half of the strains, bacteriocin production was not detected.

**Distribution of plasmids.** To identify those plasmids most frequently found in virulent strains, we used a rapid alkaline lysis technique (1) to screen 31 virulent and 8 avirulent strains for plasmids (Fig. 2, Tables 3 and 4). These strains represented different geographic areas and times of isolation. All of the strains contained multiple plasmids. Twenty-nine of 31 virulent strains had from 11 to 13 plasmids; the smallest number was 8 in 1 strain. In each case, the plasmid profiles were very similar (Fig. 2). Eight size classes of plasmids (2.8, 8.8, 16.8, 29.5, 43, 49, 70, and 210 Mdal) were found in 87% or more of the virulent strains (Table 3). In cases where one of these "common" plasmids was missing, a unique plasmid, which could have been derived from the missing plasmid, was often present. For example, three of four strains missing the 8.8-Mdal plasmid had a 10.2-Mdal

TABLE 2. Molecular masses of plasmids in *E. stewartii* strains SW2 and SS104 as determined by EM and AGE

Strain	EM <sup>a</sup>		AGE <sup>b</sup> mol. mass (Mdal ± SD) of plasmids			
	Mol. mass (Mdal ± SD) of plasmids	No. of molecules measured	TB buffer	TA buffer	Avg	
SW2			ND <sup>c</sup>	210 ± 5	210	
	70.7 ± 1.3	11	69.2 ± 0.5	70.3 ± 1.8	69.8	
	49.9 ± 0.9	10	52.0 ± 0.7	51.3 ± 0.3	51.6	
	46.1 ± 0.7	18	50.3 ± 0.8	48.1 ± 0.3	49.2	
	41.8 ± 0.9	30	44.2 ± 0.7	42.4 ± 0.1	43.3	
	34.8 ± 0.6	36	35.2 ± 0.4	33.7 ± 0.2	34.5	
			33.8 ± 0.6	32.1 ± 0.2	33.0	
	30.8 ± 0.6	31	30.1 ± 0.4	28.8 ± 0.2	29.5	
	23.6 ± 0.5	47	23.4 ± 0.6	22.9 ± 0.1	23.2	
	16.8 ± 0.5	34	17.1 ± 0.3	16.7 ± 0.1	16.9	
	8.9 ± 0.2	21	ND	8.8 ± 0.1	8.8	
	2.8 ± 0.1	159	2.80 ± 0.01	2.78 ± .02	2.8	
			2.70 ± 0.01	2.70 ± .01	2.7	
	SS104			ND	210 ± 5	210
		69.0 ± 1.6	13	67.7 ± 0.8	67.7 ± 0.4	67.7
46.0 ± 0.8		12	49.8 ± 0.6	47.1 ± 0.8	48.5	
			44.5 ± 0.7	42.0 ± 0.6	43.2	
41.6 ± 1.0		32	42.5 ± 0.3	40.5 ± 0.6	41.5	
30.6 ± 0.5		13	30.2 ± 0.3	28.4 ± 0.3	29.3	
22.4 ± 0.6		12	22.2 ± 0.8	21.0 ± 0.1	21.6	
16.7 ± 0.4		21	17.0 ± 0.2	16.4 ± 0.2	16.7	
8.6 ± 0.4		13	ND	8.55 ± 0.14	8.8	
2.8 ± 0.1		39	2.80 ± 0.01	2.80 ± 0.01	2.8	
			2.70 ± 0.01	2.70 ± 0.01	2.7	

<sup>a</sup> ColE1 DNA (4.2 Mdal) was used as an internal standard.

<sup>b</sup> DNA was electrophoresed in 0.7% agarose gels at 8 to 9 V/cm in TB buffer and 5.0 to 5.5 V/cm in TA buffer.

<sup>c</sup> ND, not done.

plasmid, SW11 was missing the 43-Mdal plasmid and had a 28-Mdal plasmid (Fig. 2), and SW3 was missing the 210-Mdal plasmid and had a 130-Mdal plasmid (Fig. 2). Other size classes of plasmids were also quite frequent: 23-, 33-, 34.5-, and 51-Mdal plasmids were found in 29 to 65% of the strains.

Avirulent strains (SS10 through SS13), which had been in culture over 40 years, had fewer plasmids, but those present were typical of the size classes found in contemporary strains. In regard to the common plasmids, six of eight avirulent strains were lacking the 70-Mdal plasmid and five of eight strains were either missing the 29.5-Mdal plasmid or appeared to have a small deletion. Compared with their parent (SS104), strains 104W13 and 104W18 were missing the 210-, 49-, 41.5-, 21.5-, and 2.7-Mdal plasmids and had a 1-Mdal deletion in the 29.5-Mdal plasmid.

The four strains from Connecticut which were Mal<sup>+</sup> Rha<sup>+</sup> did not contain any unique size classes of plasmids, nor did they share any plasmids other than those previously designated as common. Compared with SW2, E141 lacked the 51.5-, 34.5-, and 23-Mdal plasmids and had a

36.5-Mdal plasmid; E142 and E143 lacked the 51.5-, 23-, and 8.8-Mdal plasmids and had 40- and 10.2-Mdal plasmids; and E144 lacked the 33- and 34.5-Mdal plasmids.

## DISCUSSION

The presence of an unusually large number of plasmids was characteristic of all the *E. stewartii* strains used. In the case of a typical strain (SW2), plasmid DNA could theoretically comprise as much as 25% of the genome. This is not characteristic of erwinias, since plasmids have been isolated from *Erwinia chrysanthemi* (18), *Erwinia amylovora* (17), and *E. herbicola* (4, 11), and none contains as many plasmids as *E. stewartii*. Plasmid profiles of *E. stewartii* showed remarkable similarities among isolates from diverse geographic and temporal origins. Eight size classes of plasmids (2.8, 8.8, 16.8, 29.5, 43, 49, 70, and 210 Mdal) were found in over 87% of our virulent isolates. In fact, we have found the rapid plasmid screening procedure (1) to be a useful aid in identifying this species in epidemiological studies. It will be interesting to see if molecular hybridization studies confirm the identity of similar-sized plasmids in different *E.*

*stewartii* strains and can detect sequences from a given common plasmid in virulent strains which appear to be missing that plasmid.

Once the plasmids in strains SW2 and SS104 were characterized, we found them to be convenient single sources of molecular mass standards for AGE. Since *E. stewartii* plasmids are large and cover a wide range of molecular mass, they complement the smaller plasmids found in *E. coli* V517 (15). Also, we are not aware of very many good plasmid standards in the 10- to 40-Mdal range. The agreement between the molecular masses determined by EM and AGE, however, was dependent on electrophoretic conditions. At low voltages, small plasmids (8 to 23 Mdal) migrated 10 to 20% slower and larger

plasmids (30 to 50 Mdal) migrated 2 to 5% faster than predicted on the basis of their EM molecular masses and the standard curve obtained by using R-factors with pRO161 as an internal reference (D. L. Coplin and D. A. Chisholm, manuscript in preparation). We therefore recommend that molecular mass determinations with SW2 or SS104 plasmids as standards be electrophoresed at 5.0 V/cm in 0.7% Tris-acetic acid gels or 8.0 V/cm in 0.7% Tris-boric acid gels.

Recent studies have shown that the 33- and 34.5-Mdal plasmids in SW2 are responsible for the conjugative mobilization of pCR1 previously reported (6). The 33-Mdal plasmid pDC250 has been labeled with Tn10 and Mu *cts62* pf7701 (S. L. McCammon, R. G. Rowan, and D. L. Coplin. Annu. Meet. Am. Phytopathol. Soc., abstr. no. 194, 1980) and is currently the basis for studies on gene exchange and transposon mutagenesis in *E. stewartii*. Other than this, plasmids in *E.*

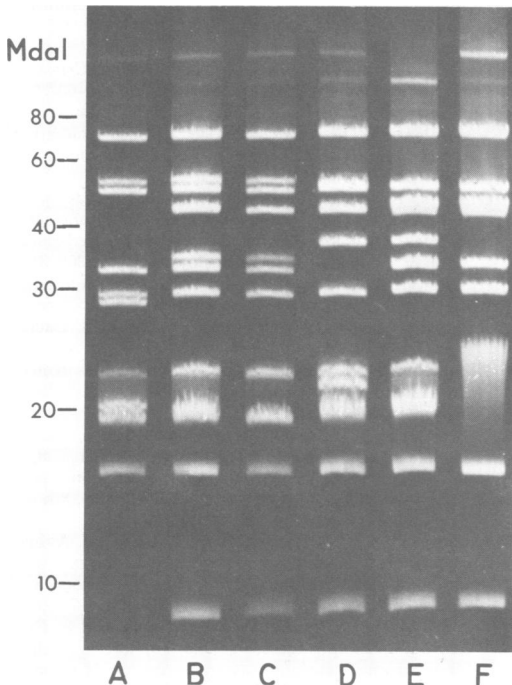


FIG. 2. AGE of plasmid DNA from different strains of *E. stewartii*. (A) SW11; (B) SW14; (C) SW2; (D) SW20; (E) SW3; (F) 22A. The band at the 19-Mdal position is linear chromosomal DNA.

TABLE 3. Distribution of plasmids in virulent strains of *E. stewartii*<sup>a</sup>

Size class (Mdal)	Presence of plasmid	
	No. of strains	%
205-215	30	97
130	1	3
77	1	3
68-70	30	97
51-52	9	29
50-51	7	23
48-50	27	87
46	1	3
43-44	30	97
41-43	8	26
38-40	5	16
36-37	3	10
34-35	20	65
33-34	20	65
31	1	3
29-30	29	94
28	1	3
23-24	15	48
21-22	5	16
20	1	3
16-17	31	100
10-11	4	13
8-9	27	87
2.5-3.0	31	100

<sup>a</sup> Thirty-one strains were examined by AGE (1).

TABLE 4. Plasmids in avirulent strains of *E. stewartii*

Strains	Molecular mass (Mdal) <sup>a</sup> by size class												
SS10	210	51	49	43	28	16.8	2.8	2.7					
SS11		50	49	43	41	23	16.8	8.8	2.8	2.7			
SS12	210	51	49	43			16.8		2.8	2.7			
SS13		51	49	43		28	16.8		2.8	2.7			
DC150	200	51	49	43	33	29.5	23	16.8	8.8	2.8	ND <sup>b</sup>		
ES-2	210	70	52	49	43	34.5	33	29.5	23	16.8	8.8	2.8	ND <sup>b</sup>
ES-4	210		49	45	43	36.5		29.5	21.5	16.8	8.8	2.8	ND <sup>b</sup>
104W18, 104W13		68			43			28.5		16.8	8.8	2.8	

<sup>a</sup> Molecular mass determined by AGE in 0.5% TA gels at 5.0 V/cm with SW2 plasmids as standards.

<sup>b</sup> ND, Not determined.

*stewartii* remain genetically cryptic. Because we found a large number of common plasmids, no unusual antibiotic resistance, and very little variability in nutritional capabilities or heavy metal resistance in our strains, it is difficult to assign a phenotype to any plasmid. Strains from Connecticut which fermented maltose and rhamnose did not contain plasmids obviously related to these properties, nor could they donate the Mal<sup>+</sup>Rha<sup>+</sup> traits to SW2 and SS104 by conjugation. Bacteriocin production was not detected in any of our strains. However, if this property is determined by plasmids, such as the 2.8-, 8.8-, or 16.8-Mdal plasmids, then all of the strains tested would have been producers and, hence, bacteriocin resistant.

To date, experiments aimed at curing putative virulence plasmids by treatment with acridine orange, ethidium bromide, mitomycin C, sodium dodecyl sulfate, and high temperature have not yielded any avirulent strains that are missing plasmids. Spontaneous non-encapsulated mutants that appear in storage likewise are not missing plasmids (2). However, the finding of fewer plasmids in many avirulent strains and the loss of plasmids after heat treatment in Garibaldi and Gibbins' variants (10) suggest that some of the larger common plasmids, such as the 29.5-, 49-, 70-, and 210-Mdal plasmids, may be related to virulence. It is also possible that some of these plasmids may enhance the growth and survival of this bacterium in the flea beetle gut and may be important for transmission by and overwintering in this vector.

It is puzzling that *E. stewartii* normally maintains such a large number of plasmids, especially when it shows little nutritional diversity. Perhaps some of the plasmids carry vital genes which are usually located on the chromosome in other bacteria. This notion would account for the stability of these plasmids in nature and in culture. Furthermore, such stability would be advantageous. Since *E. stewartii* alternatively lives for extended periods of time in two very different niches, i.e., a plant and an insect, it must maintain its plasmids in the periodic absence of phenotypic selection.

#### ACKNOWLEDGMENTS

We thank M. Rudinski, W. Emch, and E. Kretzschmar for technical assistance.

This research was supported by the Science and Education Administration of the U.S. Department of Agriculture under grant no. 78-00625 from the Competitive Research Grants Office.

#### LITERATURE CITED

- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl. Acids Res.* 7:1513-1523.
- Bradshaw-Rouse, J. J., M. H. Whatley, D. L. Coplin, A. Woods, L. Sequeira, and A. Kelman. 1981. Agglutination of strains of *Erwinia stewartii* with a corn agglutinin: correlation with extracellular polysaccharide production and pathogenicity. *Appl. Environ. Microbiol.* 42:344-350.
- Casse, F., C. Boucher, J. S. Julliot, M. Michel, and J. Dénarié. 1979. Identification and characterization of large plasmids in *Rhizobium meliloti* using agarose gel electrophoresis. *J. Gen. Microbiol.* 113:229-242.
- Chatterjee, A. K., M. K. Behrens, and M. P. Starr. 1979. Genetic and molecular properties of *E'lac*<sup>+</sup>, a transmissible plasmid of *Erwinia herbicola*. *Proc. Int. Conf. Plant Pathog. Bact.* 4:75-79.
- Coplin, D. L. 1978. Properties of F and P group plasmids in *Erwinia stewartii*. *Phytopathology* 68:1637-1643.
- Coplin, D. L., and R. G. Rowan. 1979. Conjugative plasmids in *Erwinia stewartii*. *Proc. Int. Conf. Plant Pathog. Bact.* 4:67-73.
- Currier, T. C., and E. W. Nester. 1976. Isolation of covalently closed circular DNA of high molecular weight from bacteria. *Anal. Biochem.* 76:431-441.
- Dye, D. W. 1963. The taxonomic position of *Xanthomonas stewartii* (Erw. Smith 1914) Dowson 1939. *N. Z. J. Sci.* 6:495-506.
- Dye, D. W. 1968. A taxonomic study of the genus *Erwinia*. I. The "amylovora" group. *N. Z. J. Sci.* 11:590-607.
- Garibaldi, A., and L. N. Gibbins. 1975. Induction of avirulent variants in *Erwinia stewartii* by incubation at supraoptimal temperatures. *Can. J. Microbiol.* 21:1282-1287.
- Gibbins, L. N., P. M. Bennett, J. R. Saunders, J. Grinstead, and J. C. Connolly. 1976. Acceptance and transfer of R-factor RP1 by members of the "herbicola" group of the genus *Erwinia*. *J. Bacteriol.* 128:309-316.
- Hansen, J. B., and R. H. Olsen. 1978. Isolation of large bacterial plasmids and characterization of the P2 incompatibility group plasmids pMG1 and pMG5. *J. Bacteriol.* 135:227-238.
- Kleinschmidt, A. K. 1968. Monolayer techniques in electron microscopy of nucleic acid molecules. *Methods Enzymol.* 21B:361-377.
- Lelliott, R. A. 1974. Genus *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers, and Smith 1920, 209, p. 332-339. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Macrina, F. L., D. Kopecko, K. R. Jones, D. J. Ayers, and S. M. McCowen. 1978. A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. *Plasmid* 1:417-420.
- Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow. 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. *J. Bacteriol.* 127:1529-1537.
- Panopoulos, N. J., W. V. Guimaraes, S-S. Hua, C. Sabersky-Lehman, S. Resnik, M. Lai, and S. Schaffer. 1978. Plasmids in phytopathogenic bacteria, p. 238-241. In D. Schlessinger (ed.), *Microbiology—1978*. American Society for Microbiology, Washington, D.C.
- Sparks, R. B., and G. H. Lacy. 1980. Purification and characterization of cryptic plasmids pLS1 and pLS2 from *Erwinia chrysanthemi*. *Phytopathology* 70:369-373.
- Vanden Hondel, C. A. M. J. J., W. Keegstra, W. E. Borrias, and G. A. Van Arkel. 1979. Homology of plasmids in strains of unicellular cyanobacteria. *Plasmid* 2:323-333.
- Van Etten, J. L., A. K. Vidaver, R. K. Koski, and J. S. Semancik. 1973. RNA polymerase activity associated with bacteriophage  $\phi 6$ . *J. Virol.* 12:464-471.
- Vidaver, A. K., M. L. Mathys, M. E. Thomas, and M. L. Schuster. 1972. Bacteriocins of the phytopathogens *Pseudomonas syringae*, *P. glycinea*, and *P. phaseolicola*. *Can. J. Microbiol.* 18:705-713.