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Plasmids and Transposable Elements in *Salmonella wien*

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The plasmids from six clinical strains of *Salmonella wien* have been characterized. All the *S. wien* strains were found to carry three types of plasmids: an IncFI R-Tc Cm Km Ap (resistance to tetracycline, chloramphenicol, kanamycin, and ampicillin) plasmid, either conjugative or nonconjugative, of large size (90 to 100 megadaltons); an R-Ap Su Sm (resistance to ampicillin, sulfonamide, and streptomycin) plasmid of 9 megadaltons; and a very small (1.4 megadaltons) cryptic plasmid. The characteristics of conjugative R plasmids, recombinant between F′*lac pro* and the FI nonconjugative plasmid, indicated that regions coding for the donor phenotype were present on this plasmid. The molecular and genetic features of the R plasmids were very close to those described for the R plasmids isolated from *S. wien* strains of different origin. This fact supported the hypothesis of a clonal distribution of this serotype in Algeria and Europe. The analysis used to identify transposable elements showed the presence of only Tn*A* elements, which were located on both the R-Tc Cm Km Ap and R-Ap Su Sm plasmids. They contained the structural gene for a TEM-type β-lactamase and had translocation properties analogous to those reported for other Tn*A*'s.

The serotype *Salmonella wien* occurred at low frequency in human pathology until 1969. The first epidemic outburst occurred in Algiers in 1969 (18). Between 1970 and 1972 *S. wien* reached a high incidence among salmonellosis cases in France (20%) (18). In Italy this serotype caused at least 2,399 cases in 1975 and 2,535 in 1976 (M. Fantasia Mazzotti, personal communication); in some Italian regions about 60% of all *Salmonella* infections were due to *S. wien* (1). Sporadic cases of *S. wien* infection also occurred in Britain from 1973 onwards (2).

The strains showed multiple drug resistance, and the most common type (over 80%) was characterized as resistant to tetracycline, chloramphenicol, kanamycin, ampicillin, sulfonamide, and streptomycin. An interesting feature of *S. wien* is that, although the majority of isolates are able to transfer their drug resistance, some isolates showed no such ability in spite of the fact that they displayed the same resistance pattern (1, 2). In Italy, these nontransferring multiply antibiotic-resistant strains are represented among clinical isolates at high frequency (10 to 30%) (1; Maimone, Nicoletti, and Casalino, unpublished data).

In this paper the plasmids from six *S. wien* strains isolated in Italy are characterized and the results are compared with those described for R plasmids from *S. wien* strains of different origins (2, 3, 26).

Additional information about the genetic structure of naturally occurring R plasmids may be obtained by identifying the types of possible transposable (Tn) elements situated on such plasmids. For this purpose, some genetic and structural properties of Tn*A* elements associated with three classes of R plasmids from *S. wien* are also examined.

MATERIALS AND METHODS

Bacterial strains and plasmids. *S. wien* strains WZM1, WZM2, WZM3, WZM4, WZM5, and WZM6 are independent clinical isolates, collected during 1974 and 1976 in the provinces of Latina and Rome. All the strains had the same pattern of antibiotic resistance, namely to tetracycline (Tc), chloramphenicol (Cm), kanamycin (Km), ampicillin (Ap), sulfonamide (Su), and streptomycin (Sm). They were sensitive to gentamicin (Gm), rifampin (Rif), and nalidixic acid (Nal).

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Escherichia coli K-12 CSH26, CSH27, and CSH28 were gifts from J. H. Miller (21). ZM46 was a nalidixic acid-resistant mutant of strain CSH26. *E. coli* 803 r^- m^- (27) and EB93 (RP4) were provided by E. Calef. ZM41 was a nalidixic acid- and rifampin-resistant two-step mutant of strain 803. HfrC D7011, W3110 (4), and JG184 (*rha lacY14 rps L recA56*) were obtained from G. P. Tocchini Valentini. *E. coli* C600 (*thr leu thi lacY*) and the plasmids R1*drd-19* (IncFII), R124 (IncFIV), F*olac* (IncFV), R144 (IncIα), and R27 (IncH1) were kindly provided by E. M. Lederberg, Plasmid Reference Center. pZM40 was a tetracycline-sensitive mutant of the plasmid RP4. The other very frequently used plasmids are listed in Table 1. Phenotypic symbols are according to Novick et al. (22). Tn numbers are those assigned by the Plasmid Reference Center.

Matings and plasmid interaction. *S. wien* donor cells were mated in a ratio of 1:4 with *E. coli* K-12 cells in 2× Nutrient Broth (2NB) for 18 h at 37°C. In *E. coli* × *E. coli* interrupted crosses, exponential-phase broth cultures of donor and recipient strains were mixed in a ratio of 1:4. Matings were generally interrupted after 30 min. Triparental crosses were performed as described by Guerry et al. (10); when the intermediates were *S. wien* strains, the mating mixture of donors and intermediates was incubated for 10 h instead of 2 h. One hundred colonies able to grow on the selection plates separately with each drug were tested by replica plating for their resistance patterns; antibiotics used were: Tc, 5 and 10 μg/ml; Cm, 25 μg/ml; Km, 25 μg/ml; Ap, 30 μg/ml; Sm, 10 μg/ml; Su, 800 μg/ml. Drug concentrations used as counterselection were: Nal, 50 μg/ml; Sm, 80 μg/ml; Rif, 100 μg/ml. The frequency of transfer was always expressed as the number of resistant recipient cells per donor cell in the mating mixture at the time of plating.

Compatibility experiments were performed by introducing one plasmid into a strain carrying another and selecting for characters of the donor plasmid. Transconjugants were purified on nonselective medium and tested, by replica plating, for the presence of both the incoming and resident plasmids.

Demonstration of Fi^+ (F) character was obtained by measuring sensitivity of HfrC(R^+) cells to phage MS2 in the presence of chloramphenicol. Derepressed mutants of conjugative plasmids were isolated after treatment with ethyl methane sulfonate as described by Edwards and Meynell (7). Mutant plasmids, used to perform incompatibility or mobilization tests, were isolated after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as described by J. H. Miller (21).

DNA isolation. Plasmid DNA was isolated by ethanol precipitation of DNA from sodium dodecyl sulfate-salt-cleared lysates as described by Meyers et al. (20).

Agarose gel electrophoresis of DNA. Plasmid DNA was subjected to electrophoresis in 0.7% agarose gels as described by Meyers et al. (20).

Electron microscopy. Spreading and staining of plasmid DNA for electron microscopy were performed as described by Gandini Attardi et al. (9). DNA molecules with clearly defined contours were photographed, and relative contour lengths were calculated with a Hewlett-Packard calculator digitizer (9864A-9810). Simian virus 40 DNA was used as an internal marker. The size assigned to the simian virus 40 DNA was 5,224 base pairs (8), and the molecular masses were calculated on the assumption that 1,000 base pairs correspond to 0.665 megadaltons (Mdal) (15).

β-Lactamases. β-Lactamases were characterized by their substrate-inhibitor profiles according to Matthew and Sykes (Table 1 of ref. 19). Substrates used were: penicillin (Pc), ampicillin (Ap), oxacillin (Ox), and cephaloridine (Cr). Cloxacillin (Cx) was used as an inhibitor.

Restriction endonuclease digestion. *Eco*RI endonuclease digestions of plasmid DNA, eluted from the agarose gels, were performed by the method described by Thompson et al. (25). The fragments were analyzed by electrophoresis in 0.7% agarose gels.

Transformation. Plasmid DNA was used to transform *E. coli* strains as described by Cohen et al. (5). The transformation mixtures were plated after a 120-

TABLE 1. *Bacterial plasmids*

Plasmid	Relevant characters	Source of derivation and reference
Conjugative		
F'*lac pro*	Lac Pro IncFI	CSH28, J. H. Miller (21)
pZM33	Tc Cm Km Ap IncFI, containing Tn*1931*(Ap)	*S. wien* WZM3, clinical isolate, Italy 1976 (this paper)
pZM64	Tc Cm Km Ap IncFI	Triple cross CSH28 × WZM6 × ZM41, recombinant pZM61 × F'*lac pro* (this paper)
RP4	Tc Km Ap IncP	EB93, E. Calef (6)
Nonconjugative		
pZM61	Tc Cm Km Ap IncFI, 96 Mdal, containing Tn*1932*(Ap)	*S. wien* WZM6, clinical isolate, Italy 1974 (this paper)
pZM62	Ap Su Sm, 9 Mdal, containing Tn*1933*(Ap)	*S. wien* WZM6
pZM63	Cryptic, 1.4 Mdal	*S. wien* WZM6
pSC101	Tc, 6 Mdal	E. M. Lederberg, Plasmid Reference Center (15)
RSF1010	Su Sm, 5.5 Mdal	E. M. Lederberg, Plasmid Reference Center (23)
ColE1	Cel, 4.2 Mdal	E. M. Lederberg, Plasmid Reference Center (13)

min incubation period in 2NB to allow phenotypic expression of resistance determinants.

Method for estimation of translocation frequency. The donor plasmids pZM33, pZM61, and pZM62 were introduced into JG184(pSC101), 803(pSC101), and C600(ColE1) strains by transformation (pZM62) or by conjugation at 42°C (pZM33 and pZM61). Strains carrying the donor and recipient plasmids were isolated and purified on the same selection medium at 42°C (to maintain inhibition of translocation). Single colonies were incubated in 2NB for 3 h at 42°C. The cultures were diluted into fresh prewarmed 2NB at a density of 200 to 400 cells per ml and incubated with shaking at 32 and 42°C. After the appropriate number of generations, plasmid DNA was extracted from both the cultures at 32 and 42°C and used to transform strain 803 cells. When the pSC101 plasmid was the recipient plasmid, transformants were selected on Tc and Tc-Ap plates separately. From 300 to 400 independent isolates from each group were analyzed for their resistance pattern by replica plating. The Tn*A* translocation frequency was expressed as the number of pSC101::Tn*A* transformants (resistant only to Tc and Ap) relative to the total number of pSC101 transformants (Tc^r).

Tests of the ColE1 or ColE1::Tn*A* transformants for production of active colicin and colicin immunity were performed as described by Inselburg (12).

The *recA* mutation in strain JG184 was monitored by sensitivity to UV.

RESULTS

Preliminary analysis of *S. wien* strains WZM1-6. *S. wien* strains WZM1-5 were able to transfer all the resistance markers after overnight matings with *E. coli* K-12 F^- CSH27 (Nal^r). The resistance patterns in the transconjugants, the frequencies of transfer, and the results of successive transfer to another K-12 strain revealed, in all the strains of *S. wien* tested, an R-Tc Cm Km Ap (resistance to Tc, Cm, Km, and Ap) conjugative plasmid and an independent R-Ap Su Sm nonconjugative plasmid.

Strain WZM6 was unable to transfer its resistance determinants. In triparental crosses the mobilization of the resistance genes of WZM6 by an FII conjugative plasmid (a derivative of R1*drd-19*) showed that in WZM6, as in the other strains, the genes for resistance to Tc, Cm, Km, and Ap formed a linkage group which was, however, on a plasmid that lacked the capacity for conjugation. The R-Ap Su Sm replicon was present also in this strain.

DNA gel electrophoresis and electron microscopy revealed in all the *S. wien* strains a third very small plasmid that was silent with regard to all the phenotypic characters investigated.

IncFI plasmids. The R-Tc Cm Km Ap conjugative plasmids transferred their own markers at a frequency of 1×10^{-5} to 4×10^{-5} in *E. coli* × *E. coli* matings of 30 min. They were Fi^+ (F) and a derepressed mutant of pZM33 (the R-Tc Cm Km Ap plasmid from *S. wien* WZM3) isolated after treatment with ethyl methane sulfonate conferred sensitivity to phage MS2.

The molecular weight of the R-Tc Cm Km Ap nonconjugative plasmid (designated pZM61) from *S. wien* WZM6 and *E. coli* transconjugants (two examined) was found by electron microscopy to be 96×10^6 (144.4 ± 5.4 kilobase pairs; 18 molecules measured). All the plasmids were examined by gel electrophoresis; their covalently closed circular DNA migrated to the same position characteristic of pZM61 DNA.

The R-Tc Cm Km Ap plasmids were tested for compatibility with plasmids of incompatibility groups FI, FII, FIV, FV, Iα, H1, and P; they belonged to incompatibility group FI, but they had a stability of about 99% in HfrC D7011.

To demonstrate the presence of regions coding for the donor phenotype on the pZM61 nonconjugative plasmid from WZM6, the interaction between pZM61 and F′*lac pro* was investigated. F′*lac pro* × pZM61 recombinant plasmids were isolated from triparental matings CSH28 × WZM6 × ZM41 and CSH28 × ZM41(pZM61) × CSH26. The frequency of pZM61 mobilization by F′*lac pro* was on the average 2×10^{-7} to 3×10^{-7}, and the two major classes of conjugative recombinant R plasmids were R-Tc Cm Km plasmids and R-Tc Cm Km Ap plasmids. The R-Tc Cm Km recombinant plasmids were of very large size (they did not enter the gel under our conditions), and they were transmitted by conjugation at a frequency of 10^{-1} in 30-min *E. coli* × *E. coli* crosses.

Eight independently isolated R-Tc Cm Km Ap recombinant plasmids were examined by gel electrophoresis. No difference was detectable between their size and the size of pZM61 (96 Mdal). When the frequency of transfer (6×10^{-4} to 10×10^{-4} in 30-min *E. coli* × *E. coli* crosses) and the ability to mobilize a small resistance replicon (pZM62, see below) were measured, these R-Tc Cm Km Ap conjugative recombinant plasmids displayed phenotypic properties intermediate between those of F′*lac pro* and those of the naturally occurring conjugative plasmids from *S. wien* strains WZM1-5 (an example is shown in Table 2, matings 1, 2, and 3). The existence of these recombinant plasmids suggested that DNA sequences on pZM61 shared homology with the regions of the F DNA where transfer functions are located.

In accordance with the results of Anderson et al. (2), no mobilization of pZM61 was detected with the RP4 conjugative plasmid.

R-Ap Su Sm plasmids. All the *S. wien* tested

TABLE 2. *Transfer and mobilization of conjugative and nonconjugative plasmids*

Mating[a]	Time	(I) Frequency of conjugative plasmid transfer	(II) Frequency of nonconjugative plasmid mobilization	(II)/(I)	Transconjugants[b] receiving only nonconjugative plasmid (%)
1. CSH26 F′*lac pro*(pZM62) × ZM46	30 min	2×10^{-1}	8.5×10^{-6}	4.3×10^{-5}	21
2. CSH26(pZM64)(pZM62) × ZM46	30 min	1×10^{-3}	8.7×10^{-7}	8.7×10^{-4}	5
3. CSH26(pZM33)(pZM62) × ZM46	30 min	2.8×10^{-5}	1.3×10^{-7}	4.6×10^{-3}	ND[c]
4. CSH26 F′*lac pro*(pZM62) × ZM46	18 h	1.2	5.6×10^{-5}	4.7×10^{-5}	20
5. JG184 F′*lac pro*(pSC101) × ZM46	18 h	1.5	2.9×10^{-4}	1.9×10^{-4}	ND
6. CSH26(RP4)(pZM62) × ZM46	18 h	0.6	0.6	1	1
7. JG184(pZM40)(pSC101) × ZM46	18 h	0.4	0.4	1	ND
8. CSH26(RP4) × ZM46	30 min	6×10^{-5}			
9. CSH26(RP4)(RSF1010) × ZM46	30 min	7.2×10^{-5}	4×10^{-4}	5.6	82
10. CSH26(RP4)(pZM62) × ZM46[d]	20 min	4×10^{-5}	6×10^{-4}	15	93.5
	30 min	6.5×10^{-5}	7.3×10^{-4}	10.8	91[e]
	3 h	4.7×10^{-4}	8×10^{-4}	1.7	ND

[a] The spontaneous loss of resistance markers in the donor strains was less than 0.7%.

[b] Two hundred colonies were purified by streaking on the same selection medium and tested by replica plating.

[c] ND, Not done.

[d] If samples of this mating were plated at the same time without mechanical interruption, under our conditions of selection and counterselection (50 μg of Nal per ml), no consistent difference in frequencies was observed.

[e] Each of 10 Ap^r Su^r Sm^r Tc^s Km^s transconjugants analyzed by gel electrophoresis contained only one plasmid, corresponding to pZM62.

in this investigation carried a nonconjugative R-Ap Su Sm plasmid whose molecular weight, determined by gel electrophoresis, was about 9 × 10^6. These plasmids are similar to the R-Ap Su Sm nonconjugative plasmids found in other enterobacterial strains (11, 24). They were mobilized by the FI conjugative plasmids at a frequency of 10^{-6} in the mating *S. wien* × *E. coli* K-12. Plasmid pZM62 (isolated from *S. wien* WZM6) was used as the prototype of this class of resistance plasmids; its molecular weight was 9 × 10^6 (13.5 ± 0.4 kilobase pairs; 40 molecules measured). The mobilization of pZM62 by F′*lac pro* and RP4 in *E. coli* K-12 strains was similar to those of RSF1010 and pSC101 plasmids (Table 2, matings 4 to 10). The RP4 transfer factor mobilized pZM62 and pSC101 at the same frequency as its own conjugative transfer in crosses of 18 h (Table 2, matings 6 and 7). In the early stages of the cross (20 to 30 min) the mobilization experiments with pZM62 and RSF1010 revealed a number of conjugative events, efficient in DNA transfer, 5 to 10 times higher than the value determined by the transfer of the RP4 DNA alone (Table 2, matings 8 to 10). This unexpected finding must result from specific characteristics of the conjugative system of RP4 and indirectly stresses the similarity of behavior that pZM62, RSF1010, and pSC101 showed in their interaction with this conjugative system.

Tn elements. Analysis of transposable antibiotic resistance elements on the R plasmids from the *S. wien* strains was performed in K-12 strains JG184 *rec*$^-$, 803 *rec*$^+$, and C600 *rec*$^+$. The donor plasmids were pZM33, pZM61, and pZM62. The recipient plasmids were pSC101 and ColE1. pSC101::Ap^r (ampicillin-resistant) or ColE1::Ap^r recombinant plasmids were isolated using each of the three donor plasmids. Even though the transposition experiments with pZM33 and pZM61 plasmids were carried out with C600 or JG184 cultures grown at 32°C for 90 generations, no Cm-resistant, Km-resistant, or Tc-resistant ColE1 or pSC101 recombinant plasmid was isolated (translocation frequency $<2 \times 10^{-5}$ to 6×10^{-5}).

We have examined some properties of the transposable elements carrying Ap resistance. They were similar to those of the Tn*A* elements described by other authors (14). The recombinant plasmids pSC101::Tn*1931*, pSC101::Tn*1932*, pSC101::Tn*1933*, ColE1::Tn*1931*, and ColE1::Tn*1932* (five examined by gel electrophoresis from each experiment) had molecular weights corresponding to the molecular weight of pSC101 or ColE1 plus an approximately 3.1 × 10^6-molecular-weight DNA segment which did not contain any *Eco*RI restriction sites. In all three cases the Ap resistance of the Tn*A* elements was associated with a structural gene encoding for a TEM-type β-lactamase (Table 3). The translocation process of Tn*1931*, Tn*1932*, and Tn*1933* was temperature sensitive and independent of the normal *recA* function of *E. coli* (Tables 4 and 5). In the experiments with C600, the insertion of Tn*1931* and Tn*1932* in ColE1 DNA affected the expression of colicin production in 15 to 23% of the isolates. Tn*3* insertions

in ColE1 DNA cause this phenotype at an analogous frequency (13). Table 5 shows the dependence of the translocation frequency of Tn*1933* on the number of generations of cultures of cells carrying the donor plasmid pZM62 and the recipient plasmid pSC101. Dependence on the number of generations has been reported by Kretschmer and Cohen (17) for the translocation of Tn*3* from chromosome to plasmid.

Since we obtained a very high translocation frequency in the experiments performed after 90 generations of growth at 32°C, we attempted to see if Tn*1933* insertions into the pSC101 recipient plasmid corresponded to Tn*1933* excisions from the R-Ap Su Sm pZM62 donor plasmid. We examined, by replica plating, 9,900 streptomycin-resistant transformants isolated after transformation by plasmid DNA from strain 803(pZM62)(pSC101). No Sm^r transformant was Ap^s. Thus, our experiments showed no correspondence between the insertion and excision frequencies. This finding is in agreement with what has been found for Tn*5* and Tn*10* (14).

Cryptic plasmids. All the six *S. wien* strains carried a third very small plasmid which was silent with respect to the phenotypic traits we have studied. Its molecular weight was calculated by gel electrophoresis and electron microscopy to be 1.4×10^6 (2.1 ± 0.2 kilobase pairs; 52 molecules measured). On the microscope grids prepared with DNA from WZM6 we could count between five and eight times more molecules of this cryptic plasmid (designated pZM63) than molecules of pZM62. This plasmid was not found in any of the drug-resistant transformants or transconjugants of *E. coli* K-12 that we examined by gel electrophoresis or by electron microscopy. Although the other plasmids present in *S. wien* "accepted" the transposable Ap re-

TABLE 3. *Biochemical properties of the β-lactamases specified by pSC101::Tn1931, pSC101::Tn1932, and pSC101::Tn1933*

β-lactamase specified by	Relative rate of hydrolysis[a]				Cx[b]
	Pc[c]	Ap	Ox	Cr	
Tn*1931*	100	170	0	135	S
Tn*1932*	100	150	0	120	S
Tn*1933*	100	180	0	140	S

[a] The rate of hydrolysis of β-lactam substrates was determined by the iodometric method (19).

[b] Inhibition of β-lactamase activity was measured spectrophotometrically using cephaloridine as the substrate (19). S, Sensitive.

[c] Pc, Penicillin; Ap, ampicillin; Ox, oxacillin; Cr, cephaloridine; Cx, cloxacillin.

TABLE 4. *Effect of temperature and recA genotype on translocation frequency of Tn1931 and Tn1932*

Donor plasmid	Temp (°C)	Host strain[a]	Translocation frequency,[b] Tc^r Ap^r/Tc^r (%)
pZM33	32	803 rec^+	0.65
	32	JG184 *rec*	0.5
	42	JG184 *rec*	<0.01
pZM61	32	803 rec^+	0.6
	32	JG184 *rec*	0.75
	42	JG184 *rec*	<0.01

[a] Cultures of cells carrying the donor plasmid (pZM33 or pZM61) and the recipient plasmid pSC101 were grown at 32 and 42°C for 90 generations.

[b] In each of the experiments no Cm-resistant or Km-resistant transformant was isolated (transformation frequency less than 5×10^{-10}).

TABLE 5. *Effect of temperature, recA genotype, and number of generations on translocation frequency of Tn1933 from pZM62 to pSC101*

No. of generations[a]	Temp (°C)	Host strain	Tc^r Ap^r/Tc^r [b] (%)	Tc^r Ap^r Sm^s/Tc^r Ap^r [b] (%)	Translocation frequency,[c] Tc^r Ap^r Sm^s/Tc^r (%)
90	32	803 rec^+	14	18	2.5
	32	JG184 *rec*	8	31.6	2.5
	32	JG184 *rec*	7.5	26.6	2
	42	JG184 *rec*	7	<0.3	<0.02
18 to 20	32	JG184 *rec*	1	43	0.43
	32	JG184 *rec*	6.6	5.2	0.34
	32	JG184 *rec*	4.5	7.1	0.32

[a] Cultures of cells carrying the donor plasmid pZM62 and the recipient plasmid pSC101 were grown for 20 and 90 generations. In each of the experiments, including those at 42°C, the spontaneous loss of Tc or Ap resistance markers was less than 1%.

[b] Transformants were selected and analyzed as described in Materials and Methods.

[c] The translocation frequency was estimated by dividing the number of pSC101::Tn*1933* transformants (Tc^r Ap^r Sm^s) by the total number of pSC101 transformants (Tc^r).

sistance element, no Tn*A* insertion into the pZM63 cryptic plasmid was detected. We were not able to isolate any Ap^r Su^s Sm^s transformant (3,200 Ap^r examined) from extrachromosomal DNA from *S. wien* WZM6 cultures grown at 32°C for 90 generations.

DISCUSSION

The six *S. wien* strains exhibited uniform plasmid content: an IncFI conjugative or nonconjugative R-Tc Cm Km Ap plasmid of large size (about 90 to 100 Mdal); an independent, 9-Mdal, probably multicopy R-Ap Su Sm plasmid; and a very small (1.4 Mdal) multicopy cryptic plasmid. The genetic and molecular features of conjugative recombinant R plasmids such as pZM64, obtained by the interaction of F′*lac pro* with the IncFI nonconjugative plasmid pZM61, give more precise information as to the nature of the large FI plasmid present in *S. wien* strains and support the hypothesis that plasmids such as pZM61 are defective conjugative mutants of the IncFI conjugative plasmid, which is present in the other *S. wien* strains. The IncFI defective conjugative R plasmids are found in clinical *S. wien* strains at high frequency (10 to 30%); the reasons for this phenomenon still remain obscure, and further investigation of their origin is required.

All the genetic and molecular characteristics of the FI plasmids we have described are very similar to those reported for the FI plasmids from *S. wien* strains isolated in Algeria, France, Britain, and Austria, including the defective conjugative members (2, 26). Furthermore, all these *S. wien* strains carried an independent Ap Su Sm determinant. The identity of the plasmid content strongly supports the view that the distribution of this serotype in Europe is due to only one strain of *S. wien* which was probably imported from Algeria (2, 18).

The analysis used to identify Tn elements capable of translocation to the pSC101 and ColE1 plasmids showed the presence of only Tn*A* elements, which were located on both the IncFI and R-Ap Su Sm plasmids. They contained the structural gene for a TEM-type β-lactamase and had translocation properties analogous to those described for other Tn*A*'s (13-17, 23).

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