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Return of *Streptococcus faecalis* DNA Cloned in *Escherichia coli* to Its Original Host via Transformation of *Streptococcus sanguis* Followed by Conjugative Mobilization

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Cloning vectors were introduced into *Streptococcus faecalis* by conjugation. A conjugative plasmid (pVA797) and cloning vector pVA838 recombined in *Streptococcus sanguis* at homologous sequences, forming a cointegrate. The pVA797::pVA838 cointegrate transferred to *S. faecalis* by conjugation. Recombination between homologous sequences resolved the cointegrate in the *S. faecalis* transconjugants, and pVA797 and pVA838 segregated because of incompatibility. *S. faecalis* strains that received pVA838 by this mechanism contained plasmids indistinguishable from authentic pVA838 from *Escherichia coli*. Other plasmids, including pVA736, were introduced into *S. faecalis* by this method. This approach should facilitate the introduction of cloned DNA into *S. faecalis*.

Genetic analysis of *Streptococcus faecalis* can be approached by using techniques that include cloning fragments of DNA in a well-defined *Escherichia coli* system (14). However, since expression of streptococcal (gram-positive) DNA in this background may not always occur, this approach would be more fruitful if there was a way to transfer the cloned DNA back to *S. faecalis*. A return to the original host would also allow for complementation studies. In this report, a method for the transfer of cloned DNA from *E. coli* to *S. faecalis* is described. The transfer process does not require the transformation of *S. faecalis* (a species highly refractile to transformation under conditions whereby *E. coli* or certain species of streptococci are readily transformable [2]), although a protoplast transformation system has recently been developed (M. Smith, E. Ehrenfeld, and D. Clewell, manuscript in preparation). Instead, transfer involves a cloning vehicle (pVA838) able to replicate in *E. coli* and streptococci (14) and a conjugative plasmid (pVA797) that is partially homologous to the cloning vehicle (7). The pVA838 chimera of interest was transformed into *Streptococcus sanguis* (Challis), and pVA797 was used to mobilize the pVA838 derivative to *S. faecalis*. The general approach may be applicable to a variety of streptococci and perhaps other species, although only *S. faecalis* was tested. A similar system for *Yersinia* sp. was recently published (9).

MATERIALS AND METHODS

Bacteria and media. Most of the bacterial strains used are shown in Table 1. Several strains not listed are simple derivatives [e.g., JH2-2(pVA736)]. Bacteria were grown at 37°C in Difco brain heart infusion. Plasmids were transferred by 4-h filter matings in the presence of DNase as described (18) on brain heart infusion agar. When present in selective plates, antibiotics were used at the following concentrations: erythromycin (Em), 10 μg/ml; chloramphenicol (Cm), 5 μg/ml (*S. sanguis*) or 25 μg/ml (*S. faecalis*); rifampin (Rif), 25 μg/ml; fusidic acid (Fus), 25 μg/ml; streptomycin (Str), 1 mg/ml; and spectinomycin (Spc), 0.5 mg/ml. Other reagents and their sources have been described (6, 8). Spectinomycin was a gift from the Upjohn Co.

Isolation of plasmid DNA. Strains were grown to mid-log phase (reading of 30 on a Klett-Summerson colorimeter; no. 54 filter), and nonsterile glycine was added to a final concentration of 5% (15). After an additional 60 min at 37°C, the cells were harvested, washed, incubated for 60 min with 1 mg of lysozyme per ml, and lysed with Sarkosyl, and plasmid DNA was isolated from crude lysates essentially as described elsewhere (5). Briefly, lysates resulting from 100-ml cultures were centrifuged to equilibrium in ethidium bromide-cesium chloride gradients; plasmid bands were visualized by fluoresence under long-wave UV illumination, removed with an 18.5-gauge needle, and recentrifuged in dye-buoyant density gradients. The plasmid DNA was extracted with CsCl-saturated isopropanol, dialyzed, ethanol precipitated, and redissolved in 100 μl of 50 mM Tris (pH 8.0).

Restriction digests and gel electrophoresis. Plasmid DNA was treated with restriction endonucleases from Bethesda Research Laboratories by using conditions previously suggested (16). Electrophoresis was in 0.5% agarose gels at 10 V/cm in TBE (89 mM Tris, 89 mM borate, 2.5 mM EDTA [pH 8.3]) or 0.5 V/cm in Tris-acetate (40 mM Tris, 20 mM sodium acetate [pH 7.8]). Gels were stained with ethidium bromide (1 μg/ml) and transilluminated with short-wave UV light. Lambda DNA was *c*I857 *S*am7 from Bethesda Research Laboratories.

RESULTS

Several streptococcal plasmids which transfer poorly in broth matings transfer efficiently between strains cultured on filter membranes ("filter matings") on agar plates. Most of these plasmids confer erythromycin resistance and transfer among a variety of streptococci (2). One such plasmid, pAMβ1, also transfers to lactobacilli, staphylococci, and *Bacillus subtilis* (2). A similar plasmid, pIP501, confers both erythromycin and chloramphenicol resistance (10). Both pAMβ1 and pIP501 mobilize transfer of nonconjugative plasmids such as pMV163, but there is no evidence that this

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TABLE 1. Bacterial strains

Strain	Description	Construction or reference
S. sanquis		
V288	Transformable	F. Macrina (13)
V797	V288(pVA797)	F. Macrina (7)
V839	Str V288(pVA838)	F. Macrina (14)
V736	V288(pVA736)	F. Macrina (3)
M14	Str V288(pVA797, pVA838)	V797 × V839[a]
S. faecalis		
JH2-2	JH2 Rif Fus	(12)
JH2-SS	JH2 Str Spc	(19)
Y11	JH2-SS *chr*::Tn*916*	(11)
M2	JH2-SS(pVA838)	M14 × JH2-SS[a]
M2C	JH2-SS(pVA797)	JH2-2(pVA797) × M2
FA2-2	JH2 Rif Fus	(3)
FA1001	FA2-2 derivative	F. An (4)[b]
M13	FA2-2(pVA838)	M14 × FA2-2[a]
OG1-RF	OG1 Rif Fus	(6)
39-5	Reference plasmids	(21)[c]
UV202	Recombination deficient	(20)
E. coli		
DB11	HsdR$^-$ Em^s	Julian Davies
DB11(pAM600)		F. Macrina[d]
DB11(pAM601)		F. Macrina[d]
DB11(pVA862)		F. Macrina (14)
V838	DB11(pVA838)	F. Macrina (14)

[a] Transfer by filter mating.
[b] FA2-2 was mutagenized with nitrosoguanidine, and a mutant was isolated which was defective in pheromone production.
[c] A 39-5 derivative lacking the 5-Mdal plasmid was used.
[d] Y. Yagi cloned two *Eco*RI fragments of the Y11 chromosome. These were subcloned by F. Macrina into pVA838, yielding pAM600 and pAM601. These experiments will be described elsewhere.

mobilization involves the formation of a cointegrate (1). Evans and Macrina created a derivative of pIP501 by substituting the multicopy pVA380-1 replicon from *Streptococcus ferus* in place of a segment containing the Em^r determinant in pIP501 (7). The resulting plasmid (pVA797) transfers by filter mating from *S. sanguis* (Challis) donors to both *S. sanguis* and *S. faecalis* recipients and is unique in that it is conjugative and yet confers resistance to chloramphenicol but not erythromycin (7). Macrina and co-workers created the shuttle plasmid pVA838 by joining the pVA380-1 replicon, the Em^r determinant from pAMβ1, and the *E. coli* cloning vector pACYC184 (14). pVA838 replicates and expresses erythromycin resistance in both *E. coli* and *S. sanguis* (14). The Cm^r determinant associated with the pACYC184 moiety of the plasmid does not express significantly in *S. sanguis* (14). Both pVA797 and pVA838 replicate in *S. sanguis* by using the pVA380-1 replicon, and they are incompatible in *S. sanguis* (7, 14).

S. sanguis strains containing both pVA838 and pVA797 were cultured in media containing erythromycin (which selects for pVA838) and chloramphenicol (which selects for pVA797). We found that maintenance of pVA797 and pVA838 in *S. sanguis* resulted in a cointegrate plasmid that transferred to *S. faecalis* by conjugation. The cointegrate resolved in the transconjugants, and the incompatible plasmids pVA797 and pVA838 segregated after resolution. We used this approach to introduce several plasmids to *S. faecalis*.

Evidence for the pVA797::pVA838 cointegrate structure. The restriction maps of pVA797 and pVA838 show the region of DNA which may recombine by homologous recombination (Fig. 1). Note that pVA797 has two *Sph*I sites (spaced by 16.5 and 14.2 kilobases [kb]), and the 9.2-kb pVA838 has one *Sph*I site. Digestion of the pVA797::pVA838 cointegrate should yield two *Sph*I fragments (10.7 and 13.5 kb) not found in either pVA797 or pVA838. Similar predictions can be made about other restriction patterns, and the presence of the pVA797::pVA838 cointegrate can thus be easily verified.

An *S. sanguis* strain (M14) harboring both pVA797 and pVA838 was constructed by transferring pVA797 from V797 to V839 by filter mating. Because pVA797 and pVA838 are incompatible in *S. sanguis* (7), strain M14 was grown on erythromycin (1 μg/ml) and chloramphenicol (1 μg/ml). Undigested plasmid DNA from strain M14 contained a plasmid that migrated like pVA838 and larger plasmid species that were consistent with 30- to 40-kb plasmids (data not shown). The *Sph*I digests of M14 plasmid DNA had the fragments predicted by the structure of the cointegrate in Fig. 1 (Fig. 2, lane 3). The largest band is the 16.5-kb pVA797 fragment, the next two closely spaced bands represent the 14.2-kb pVA797 and 13.5-kb cointegrate fragments, and the small pair of bands represents the 10.7-kb cointegrate fragment and linear 9.2-kb pVA838. It is evident from the relative intensities of the bands that the cointegrate form constituted a significant proportion of the plasmid DNA, but the 10.7-kb *Sph*I fragment of M14 plasmid DNA was less abundant than the 9.2-kb linear pVA838, indicating that there was more pVA838 than cointegrate plasmid in the M14 culture. We performed similar experiments by using the enzymes *Eco*RI (in which a 7.3-kb fragment unique to the

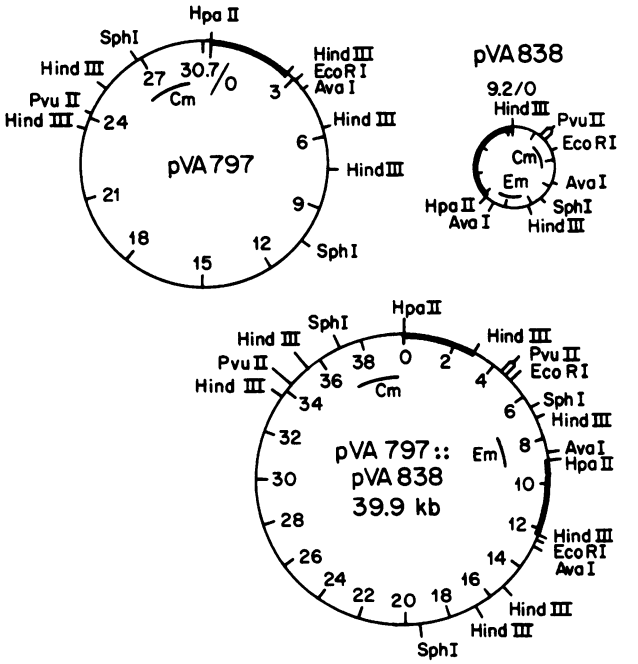

FIG. 1. Restriction maps of pVA797, pVA838, and a cointegrate. The maps are adapted from those of Macrina and co-workers (7, 14). The area of homology between the plasmids is indicated by a heavy line. The cointegrate plasmid pVA797::pVA838 has been drawn assuming that homologous recombination has occurred between pVA797 and pVA838, resulting in a duplication of the homologous segment.

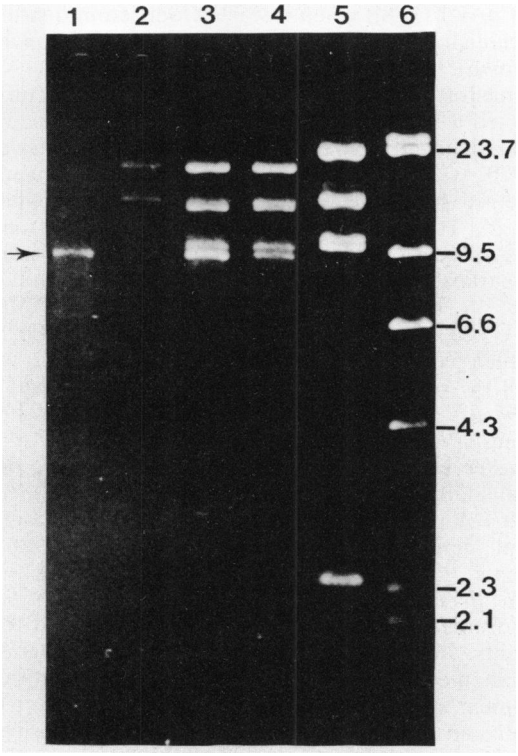

FIG. 2. Agarose gel electrophoresis of restriction endonuclease-treated plasmid or lambda DNA. The sizes (in kb) of lambda *Hin*dIII fragments are indicated on the right. Lane 1, pVA838 from V839 cleaved with *Sph*I. Linear pVA838 is noted by an arrow. Lane 2, pVA797 from V797 cleaved with *Sph*I. Lane 3, M14 plasmid DNA cleaved with *Sph*I. Lane 4, M13 plasmid DNA cleaved with *Sph*I. Lane 5, Lambda DNA cleaved with *Bgl*II (22.8-, 13.6-, 9.8-, and 2.3-kb fragments). Note the 9.8 + 0.46 kb fragment, resulting from annealing of sticky ends. Lane 6, Lambda DNA cleaved with *Hin*dIII.

cointegrate is predicted by the drawing in Fig. 1), *Pvu*II (Fig. 1 predicts a 10.7-kb fragment), and *Ava*I (Fig. 1 predicts a 3.9-kb fragment). In each case the fragment predicted to be unique to pVA797::pVA838 was observed in digests of M14 DNA (data not shown).

TABLE 2. Transfer of pVA797 and pVA838 by filter mating[a]

Donor (resident plasmid) × recipient	Transconjugants per donor selected on:		
	Chloramphenicol	Erythromycin	Chloramphenicol, erythromycin
V797(pVA797) × JH2-SS	1×10^{-3}		
× FA2-2	4×10^{-4}		
V839(pVA838) × FA2-2		$<10^{-9}$	
M14(pVA797, pVA838)			
× JH2-SS	4×10^{-3}	1×10^{-4}	1×10^{-4}
× FA2-2	5×10^{-3}	5×10^{-3}	5×10^{-3}
× UV202	4×10^{-5}	$<10^{-9}$	$<10^{-9}$

[a] Transconjugants were scored as Str^r Spc^r Cm^r and Str^r Spc^r Em^r (for JH2-SS recipients) or Rif^r Fus^r Cm^r and Rif^r Fus^r Em^r (for FA2-2 and UV202 recipients) and stabbed into agar containing erythromycin or chloramphenicol for evidence of cotransfer. This agar also contained either rifampin and fusidic acid or streptomycin and spectinomycin. Donors and recipients, when plated alone, did not grow on agar selective for transconjugants. Transconjugants were identified as *S. faecalis* by colony morphology and growth on bile esculin agar (Difco Laboratories).

TABLE 3. Segregation of Cm and Em markers in pVA797::pVA838 transconjugants

Cells	Selection	No. of transconjugants/total with:	
		Em^r	Cm^r
M14 × FA2-2 mating mixture[a]	Rif Fus Em		60/60
	Rif Fus Cm	8/20	
FA2-2(pVA797::pVA838) transconjugants[b]	Cm	27/56	
	Em		46/48
	No drug	16/58	58/58

[a] A mating mixture resuspended from a filter was plated on selective agar, and transconjugants were stabbed in agar containing chloramphenicol or erythromycin.

[b] Transconjugants selected on rifampin, fusidic acid, erythromycin, and chloramphenicol were grown overnight (ca. 15 generations) without selection and plated on chloramphenicol, erythromycin, or no drug, and colonies were subsequently stabbed into agar containing rifampin, fusidic acid, and chloramphenicol or rifampin, fusidic acid, and erythromycin.

Transfer of pVA797::pVA838 by filter mating. When used as a donor in a filter mating, M14 transferred both Em^r and Cm^r to *S. faecalis*, indicating that the cointegrate was conjugative (Table 2). In one experiment (JH2-SS recipients), transfer of Cm^r from M14 donors was more frequent than transfer of Em^r, suggesting that some of the donors contained pVA797 not as a cointegrate with pVA838. This was consistent with the electrophoresis results (Fig. 2). All Em^r transconjugants from M14 donors were initially also Cm^r (213 of 213), as expected if mobilization of pVA838 required formation of a cointegrate. Plasmid DNA from an FA2-2 transconjugant resistant to erythromycin and chloramphenicol (strain M13) was digested with *Sph*I, and the digest contained the fragments expected from pVA797, pVA838, and the pVA838::pVA797 cointegrate (Fig. 2, lane 4).

Resolution of the pVA797::pVA838 cointegrate in transcon-

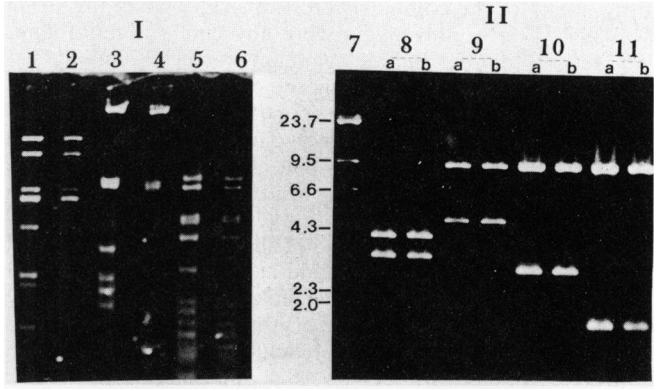

FIG. 3. Gel electrophoresis of restriction endonuclease-digested plasmid DNA. (I) 4% polyacrylamide (10 V/cm in TBE). Shown are pVA838 from M2 (lane 1) and DB11(pVA838) (lane 2), cleaved with *Rsa*I; pVA838 from M2 (lane 3) and DB11(pVA838) (lane 4), cleaved with *Sau*3A; and pVA838 from M2 (lane 5) and DB11(pVA838) (lane 6), cleaved with *Alu*I. (II) 0.7% agarose (0.5 V/cm in Tris-acetate). Lane 7, Lambda DNA cleaved with *Hin*dIII. The sizes (in kb) of the fragments are indicated. Lane 8, pVA736 from V736 (a) and JH2-2(pVA736) (b), cleaved with *Eco*RI and *Hin*dIII. Lane 9, pVA862 from DB11(pVA862) (a) and M2C(pVA862) (b), cleaved with *Eco*RI. Lane 10, pAM600 from DB11(pAM600) (a) and M2C(pAM600) (b), cleaved with *Eco*RI. Lane 11, pAM601 from DB11(pAM601) (a) and M2C(pAM601) (b), cleaved with *Eco*RI.

TABLE 4. Cm^s transconjugants resulting from filter matings

Donor	Recipient	No. of Cm^s transconjugants/ total after 1 pass on erythromycin[a]	Plasmid[b]
V288(pAM600, pAM797)	M2C	14/16	pAM600
	JH2-SS	4/64	pAM603
	JH2-SS(pVA797)	0/57	
	FA1001	0/34	pAM602[c]
	Y11	3/38	pAM604
	Y11 (pVA797)	0/92	
V288(pAM601, pAM797)	M2C	2/6	
	JH2-SS	3/80	
	JH2-SS(pVA797)	0/49	
	FA1001	0/10	
	Y11	1/56	
	Y11(pVA797)	1/30	

[a] Em^r transconjugant colonies from a filter mating were streaked on agar containing erythromycin. Individual colonies were stabbed into agar containing chloramphenicol.

[b] Plasmid obtained from an Em^r Cm^s transconjugant.

[c] For FA1001, such a transconjugant was obtained after repeated passage on erythromycin.

jugants. In the M14 × FA2-2 mating, transconjugants selected on rifampin, fusidic acid, and erythromycin were also Cm^r, whereas 60% of the Rif^r Fus^r Cm^r (selected) transconjugants were sensitive to erythromycin (Em^s) (Table 3). Since Em^r transconjugants were nearly as numerous as Cm^r transconjugants (Table 2), it appears that a significant percentage of the transconjugants selected on chloramphenicol may have been initially also Em^r, but lost the Em^r trait during the growth of the colony (Table 3). An FA2-2(pVA797::pVA838) transconjugant initially selected on erythromycin and chloramphenicol was subcultured overnight in the absence of drug and plated, and individual colonies were tested for the presence of Cm^r or Em^r. When the strain was plated on chloramphenicol or in the absence of drug, many of the colonies were Em^s. Only when the strain was plated on erythromycin were any Cm^s variants found. The reason that the Em^r determinant was preferentially lost from strains initially containing the cointegrate is unknown. (The fact that pAV797 corresponds to two streptococcal replicons may give it an advantage over pVA838.) Isolation of *S. faecalis* transconjugants that contained pVA838 alone was possible, however, if the transconjugant was subcultured in the presence of erythromycin.

In similar experiments (data not shown), variants resistant to only erythromycin were found at a frequency between 1 and 20% of the total isolates tested after one passage of the cointegrate transconjugants on erythromycin. This is consistant with a low frequency of resolution of the cointegrate, followed by segregation of pVA838 and pVA797 due to the known incompatabilities of pVA380-1 derivatives (7). Plasmid DNA from several isolates resistant to erythromycin and sensitive to chloramphenicol comigrated with pVA838 during electrophoresis (data not shown). Plasmid DNA isolated from one such isolate, *S. faecalis* M2, was compared with pVA838 isolated from *E. coli* V838. The *Sau*3A, *Rsa*I, and *Alu*I digestion patterns of the two plasmids were identical (Fig. 3). After daily subcultures of strain M2 in the absence of erythromycin for 3 days, the strain was plated in the absence of drug. All 50 colonies subsequently tested were still resistant to erythromycin. Plasmid pVA838 was not mobilized by the conjugative plasmid pAM211 (a derivative of pAD1 [3, 8]) when it was introduced into strain M2, even though pAM211 transfer was at 0.03 per donor (data not shown).

In an effort to study unresolved cointegrate structures, we attempted to transfer the cointegrate to the isogenic recombination-deficient *S. faecalis* strain UV202. Transfer of Em^r to UV202 was not detectable, even though the same donors transferred Em^r to FA2-2 at easily detectable frequencies (Table 2). Transfer of pVA797 to strain UV202 from M14 donors was easily detected, and UV202(pVA797) transconjugants transferred Cm^r, but not Em^r, to JH2-SS at 0.01 per donor. We transferred pAMβ1 from JH2-SS(pAMβ1) to strain UV202, and UV202(pAMβ1) expressed the Em^r marker, which is identical to that on pVA838 (14), without difficulty (data not shown). It appears that the pVA797::pVA838 cointegrate cannot be stably maintained by strain UV202, but that pVA797 can.

Transfer of other plasmids and chimeras to *S. faecalis*. Several additional plasmids were mobilized to *S. faecalis* by pVA797. A pVA380-1 derivative, pVA736 (13), containing the Em^r determinant of pAMβ1 was mobilized to JH2-2 recipients. pVA838 derivatives containing *Eco*RI inserts from *S. faecalis* plasmid pPD1 (pVA862) and the *S. faecalis* chromosome (pAM600, pAM601) were mobilized to M2C recipients. In each case, resolution of the cointegrate and segregation of the component plasmids occurred at frequencies similar to those shown in Table 3 for pVA838::pVA797 transfer and resulted in *S. faecalis* strains harboring plasmids indistinguishable from the original chimeras from *S. sanguis* or *E. coli* (Fig. 3B).

Aberrant results and deletion formation. For reasons unknown, transfer of cointegrates containing chimeric forms of pVA838 to OG1-RF, FA2-2, FA1001, Y11, and JH2-SS recipients often resulted in transconjugants that did not resolve the cointegrate at a high rate. After repeated passages of transconjugants on erythromycin, only 1 to 10% of the CFU were Cm^s (indicating resolution of the cointegrate). However, these strains contained deletions within the mobilized plasmid. These deletion derivatives conferred Em^r and

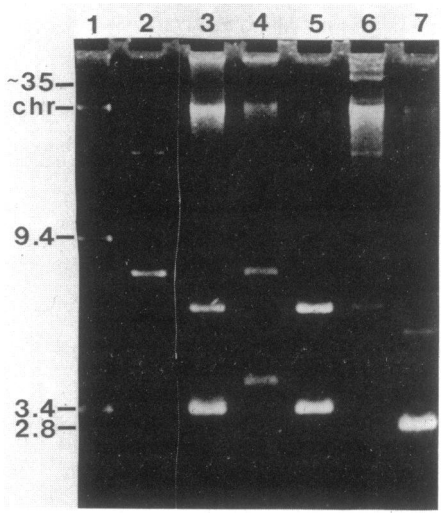

FIG. 4. Agarose gel electrophoresis of plasmid DNA. Chomosomal DNA and the sizes (in Mdal) of 39-5 and pBR322 references plasmids are indicated. Lane 1, 39-5 derivative. Lane 2, pAM600 from DB11(pAM600). Lane 3, pAM603. Lane 4, pAM604. Lane 5, pAM602. Lane 6, Plasmid DNA from FA2-2(pVA797::pAM600). Lane 7, pBR322.

were often (but not always) 3.3 megadaltons (Mdal)—just large enough to contain the Em^r determinant and the pVA380-1 replicon. We have many observations of, but no explanation for, this phenomenon. Again for unknown reasons, these problems were circumvented by making use of an *S. faecalis* strain that had previously harbored pVA838. pVA797 was transferred to strain M2 [*S. faecalis*(pVA838)], and derivatives that had lost pVA838 due to incompatability were identified by their sensitivity to erythromycin. The resulting strain (M2C) was a good recipient for cointegrate transfer (selecting for Em^r transfer), and the cointegrates resolved without deletion formation. An example is shown in Table 4. *S. sanguis* strains containing pVA797 and either pAM600 or pAM601 were used as donors in matings with FA2-2, FA1001, JH2-SS(pVA797), Y11, and Y11(pVA797) recipients. Em^r transconjugants were frequent (10^{-5} per donor), but variants that were Cm^s did not readily appear (Table 4). Those few Em^r Cm^s variants that were found contained a plasmid that was smaller than either pAM600 or pAM601, as Fig. 4 shows for pAM600. Plasmid DNA from a Cm^r Em^r FA2-2 transconjugant contained a large plasmid, consistent with its being the cointegrate; in addition at least two other small plasmids, including one of ca. 3.3 Mdal (5.0 kb), were present (Fig. 4, lane 6). For Y11(pVA797) recipients, a Cm^s Em^r variant was eventually recovered, but it also contained a 3.3-Mdal deleted version of pAM600 (data not shown). These results were repeatedly obtained by using several derivatives of pVA838 that contained inserts from plasmid pPD1, and 3.3-Mdal plasmids were the result in about half the cases (data not shown). In contrast, when the same donors transferred Em^r to M2C recipients, Em^r Cm^s variants of Em^r transconjugants were easily isolated (Table 4). These variants contained authentic pAM600 and pAM601 DNA (Fig. 3). It appears that M2C is a variant in which resolution of the cointegrate and segregation of intact parental plasmids is somehow facilitated.

DISCUSSION

The mobilization approach described here may possibly be used with any organism that can support pVA838 and pVA797, as long as transfer from *S. sanguis* to that organism is reasonably efficient. Since *S. sanguis* and *S. faecalis* can support plasmids based on the *S. ferus*(pVA380-1) replicon, it is reasonable to expect that other streptococci may also be able to support them. Since pIP501 transfers among *S. faecalis*, *Streptococcus agalactiae*, *S. sanguis*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* (2, 10), it is reasonable to expect its derivative, pVA797, to have a broad host range also.

S. faecalis M2C recipients gave much more efficient resolution or segregation, or both, in the case of cointegrates involving pVA838 plasmids containing cloned inserts. This was not because the M2C recipient already contained pVA797, but instead appeared to be due to a difference in strain M2C. There appears to be a correlation between the ability to efficiently resolve cointegrates and the ability to generate undeleted forms of the pVA838 chimeras. The basis of this correlation and the related differences between M2C and other strains remains obscure. Interestingly, very similar results were recently reported for shuttle plasmids in *Bacillus subtilis* which were introduced by transformation (17). Shuttle plasmid pDH5060, constructed from *E. coli*(pBR322) and *B. subtilis*(pOG1196), stably shuttled between the two species, but underwent deletion when derivatives containing inserts were introduced into *B. subtilis*. The problem was alleviated when a *B. subtilis* mutant of unknown character was isolated by introducing a shuttle derivative containing an insert and selecting for a strain that maintained the derivative intact. This mutant, like strain M2C, was then able to accept shuttle chimeras with little difficulty (17).

Despite the pitfalls mentioned above, the mobilization system does offer a means for moving cloned DNA from *E. coli* to *S. faecalis*. Furthermore, the system may be applied to other streptococci since it relies on a broad host range conjugation system. The concept of mobilizing cloning vehicles by partially homologous conjugative plasmids has wider application, as recently shown by Heesman and Laufs (9) in *Yersinia* sp. We designed a second mobilization scheme that does not require recombination and uses a different cloning vehicle able to replicate in *E. coli* and streptococci. Details on this plasmid and its use will be published elsewhere (Smith et al., manuscript in preparation).

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