JOURNAL OF BACTERIOLOGY, June 1983, p. 1347–1355
0021-9193/83/061347-09$02.00/0

Vol. 154, No. 3

Streptococcal R Plasmid pIP501: Endonuclease Site Map, Resistance Determinant Location, and Construction of Novel Derivatives

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Received 12 November 1982/Accepted 14 March 1983

The streptococcal resistance plasmid pIP501 (30 kilobase pairs [kb]) encodes resistance to chloramphenicol (Cm^r) and erythromycin (Em^r) and is capable of conjugative transfer among numerous streptococcal species. By using a streptococcal host-vector recombinant DNA system, the Cm^r and Em^r determinants of pIP501 were localized to 6.3-kb *Hin*dIII and 2.1-kb *Hin*dIII-*Ava*I fragments, respectively. pIP501 was lost at a frequency of 22% in *Streptococcus sanguis* cells grown at 42°C but was stable in cells grown at 37°C (<1% frequency of loss). Sequences from a cryptic multicopy plasmid, pVA380-1, were substituted for the pIP501 Em^r determinant in vitro, and the resulting recombinant plasmid, designated pVA797, was recoverd in transformed *S. sanguis* cells. The replication of pVA797 was governed by the pVA380-1 sequences based on temperature-stable replication and incompatibility with pVA380-1-derived replicons. The self-ligation of partially cleaved *Hin*dIII pIP501 DNA fragments allowed the localization of a pIP501 region involved in autonomous plasmid replication. A small pIP501 derivative (pVA798) obtained from this experiment had a greatly increased copy number but was unstably inherited. Our data indicate that the sequences encoding the resistance determinants and some of the plasmid replication machinery are relatively clustered on the pIP501 molecule. The properties of pVA797 and pVA798 indicate that these molecules will enhance current streptococcal genetic systems from the standpoint of conjugative mobilization (pVA797) and gene amplification (pVA798).

Evidence for conjugative transfer of streptococcal resistance (R) plasmids was demonstrated first by Jacob and Hobbs (15) in Lancefield group D streptococci. More recently, conjugative R plasmids in Lancefield groups A (25), B (13), C (3), G (3), and N (26) have been reported. In contrast to some Lancefield group D conjugal plasmids which are transmissible in mixed broth cultures (5), certain streptococcal conjugative R plasmids require cell-to-cell contact of donors and recipients on filter membranes for transfer. Several plasmids of this latter group can transfer to a wide range of hosts and confer resistance to erythromycin (Em^r). An example of one such plasmid is pAMβ1 (4), which is transmissible to at least 10 streptococcal species (6, 8, 13, 18, 25), *Staphylococcus aureus* (6), *Bacillus subtilis* (16), and *Lactobacillus casei* (10).

Horodniceanu et al. (14) isolated an Em^r-conferring conjugative plasmid, pIP501, from *Streptococcus agalactiae* (Lancefield group B) that was 30 kilobase pairs (kb) in size. pIP501 also conferred resistance to chloramphenicol (Cm^r). Intergeneric transfer of pIP501 to Lancefield groups A (25), C (3), D (25), F (13), G (3), and H (25) and to *Streptococcus pneumoniae* (32) has been reported. In addition to self-transmissibility, pIP501 is capable of mobilizing smaller non-selftransmissible R plasmids (32). Deletion-bearing derivatives of pIP501 have been utilized as streptococcal cloning vehicles (1, 2). Based on host range and restriction endonuclease fragment similarities, Hershfield (13) suggested that pIP501 is a member of a family of streptococcal conjugative plasmids which share related Em^r determinants, a general size range of 23 to 37 kb, and a similar broad host range.

Streptococcal broad-host-range resistance plasmids warrant further attention because of both their clinical importance and their use as genetic tools. In this report we detail our studies of pIP501. A restriction endonuclease site map of pIP501 has been constructed and, using recombinant DNA methodologies, we have localized the resistance determinants and some of the plasmid replication machinery. A high-copy-number mini-pIP501 derivative also was isolated and characterized. In addition, a genetically

TABLE 1. Bacterial strains[a]

Strain	Laboratory no.	Chromosomal phenotype	Plasmid			Remarks/reference
			Designation	Phenotype	Size (kb)	
S. sanguis	V288					(20)
S. faecalis	V348	Rif^r Fus^r Str^r				JH2-2 derivative from D. B. Clewell
S. sanguis	V481	Rif^r				Rif^r mutant of V288
S. sanguis	V683		pIP501	Em^r Cm^r Tra$^+$	30	(13, 14)
S. sanguis	V685		pVA380-1	Cryptic	4.2	(20)

[a] Rif^r, growth on 50 μg of rifampin per ml; Fus^r, growth on 50 μg of fusidate per ml; Str^r, growth on 1,000 μg of streptomycin per ml; Em^r, growth on 10 μg of erythromycin per ml; Cm^r, growth on 5 μg of chloramphenicol per ml; Tra$^+$, exhibits genetic transfer by a conjugation-like mechanism.

stable, elevated-copy-number, conjugative derivative of pIP501 bearing only the Cm^r determinant was constructed and characterized.

MATERIALS AND METHODS

Bacterial strains and media. The strains used in this work are listed in Table 1. Strains were grown routinely in brain heart infusion broth or Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) at 37°C under anaerobic conditions. Solid medium was made by the addition of 1.5% agar (Difco Laboratories) to broth. Chloramphenicol, erythromycin, and rifampin were employed in selective media at concentrations of 5, 10, and 50 μg/ml, respectively. Bacterial stocks were stored at −70°C in brain heart infusion supplemented with 30% glycerol. The sources of all chemicals and enzymes used in these studies were as previously described (33).

Isolation of plasmid DNA. Covalently closed circular DNA was isolated by a modified method of Hansen and Olsen (12) as reported by Tobian and Macrina (33). Rapid screening of transformants for plasmid DNA was as previously described (24).

DNA enzymology. For digestions involving single restriction endonucleases, standard buffers specified by Bethesda Research Laboratories (Rockville, Md.) were used. For simultaneous digestions with multiple restriction endonucleases, the buffers used were as previously reported (20). Ligation of restricted plasmid DNA fragments with T4 DNA ligase was performed as described by the supplier (Bethesda Research Laboratories). Ligation mixtures were used without modification to transform competent *Streptococcus sanguis* cells (17).

Conjugal mating procedure. Filter matings were performed by the Hershfield (13) method modified as follows. Overnight cultures of donor and recipient cells were diluted 1/10 and mixed in a ratio of 1:10 (donor/recipient) to give a final volume of 1.0 ml in a 1.5-ml conical polypropylene centrifuge tube. After centrifugation for 2 min in an Eppendorf centrifuge, the supernatant was decanted and the cells were suspended in 50 μl of brain heart infusion broth by mechanical agitation. The cells then were pipetted onto 0.45-μm-pore size nitrocellulose (Millipore Corp., Bedford, Mass.) filters, and the filters were placed on nonselective media and allowed to incubate anaerobically for 6 h at 37°C. The cells then were washed from the filter by mechanical agitation in 1 ml of brain heart infusion broth, and 0.1-ml samples were plated on selective media.

Plasmid curing and copy number. Plasmid copy numbers were determined as previously described (22). Copy number determinations were always performed using selectively grown cells (chloramphenicol, 4 μg/ml). Plasmid loss in cells grown at 37 or 42°C was monitored as earlier reported (21).

Containment. The recombinant DNA experiments with streptococcal plasmids described in this report fall into exemption category III-D-3 of the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (21 April 1982).

RESULTS

Restriction map of pIP501. Covalently closed circular pIP501 DNA from *S. sanguis* V683 was subjected to single and double digestion with restriction endonucleases, and the resultant fragments were sized on vertical agarose gels (Table 2). The positions of the restriction sites on pIP501 (Fig. 1) were deduced from the sizes of fragments liberated by appropriate combinations of enzymes (11). No sites for restriction endonucleases *Bam*HI, *Eco*RI, *Sal*I, *Xba*I, and *Xho*I were found on the pIP501 molecule. Restriction enzymes *Ava*I, *Bcl*I, *Hae*III, *Hpa*II, and *Pvu*II each cleaved the pIP501 molecule at a single site. Multiple fragments were liberated from pIP501 by digestion with *Sph*I, *Ava*II, *Hpa*I, *Kpn*I, *Bst*EII, and *Hin*dIII (Table 2). The location of the resistance determinants and some of the replication region (Fig. 1) followed from data presented below.

Cloning of the pIP501 Em^r and Cm^r determinants. Using recombinant DNA methodology, we localized the Em^r and Cm^r determinants to specific pIP501 restriction fragments. pIP501 and pVA380-1 digested simultaneously with *Hin*dIII and *Ava*I (Fig. 2) were ligated and used to transform *S. sanguis*. Resultant Em^r transformants recovered at a frequency of 9×10^{-4} per recipient contained a single plasmid class, 6.0 kb in size; a representative plasmid was designated pVA794. Restriction endonuclease site analysis showed that pVA794 resulted from

TABLE 2. Restriction endonuclease fragments of pIP501

Restriction endonuclease or sum	Fragment designation	Fragment size[a] (kb)
*Hin*dIII	A	6.3 ± 0.05
	B	4.8 ± 0.13
	C and C'	3.5 ± 0.05
	D	2.1 ± 0.11
	E	1.9 ± 0.13
	F	1.5 ± 0.10
	G	1.4 ± 0.08
	H	1.2 ± 0.51
	I	1.1 ± 0.25
	J	1.0 ± 0.14
	K	0.75 ± 0.08
	L	0.60[b]
	M	0.15[b]
*Hin*dIII fragment sum		29.80
*Bst*EII	A	12.6 ± 0.40
	B	10.0 ± 0.26
	C	5.8 ± 0.55
	D	1.6 ± 0.10
*Bst*EII fragment sum		30.00
*Hpa*I	A	23.11 ± 2.1
	B	6.6 ± 0.30
	C	1.7 ± 0.02
*Hpa*I fragment sum		31.4
*Kpn*I	A	21.0 ± 1.1
	B	4.5 ± 0.60
	C	4.3 ± 0.56
*Kpn*I fragment sum		29.8
*Ava*II	A	14.5 ± 0.21
	B	8.0 ± 0.10
	C	7.5 ± 0.16
*Ava*II fragment sum		30.0
*Sph*I	A	16.0 ± 0.21
	B	13.9 ± 0.21
*Sph*I fragment sum		29.9
*Ava*I, *Bcl*I, *Hae*III, *Hpa*II, and *Pvu*II	A	30.0 ± 1.6

[a] Size determination (in kb ± standard deviation) based on at least three independently performed digestions.

[b] Based on one determination.

the ligation of the 2.1-kb *Hin*dIII-*Ava*I fragment of pIP501 with the large *Hin*dIII-*Ava*I fragment of pVA380-1. Minimum inhibitory concentrations (1,000 μg of erythromycin per ml) and inducibility of Em^r conferred by the pIP501 Em^r determinant were identical (data not shown) in *S. sanguis* containing pVA794 (V794) and *S. sanguis* containing pIP501 (V683).

The Cm^r determinant of pIP501 previously was shown to reside on the *Hin*dIII A fragment (1, 20). *Hin*dIII-cleaved pIP501 was ligated with *Hin*dIII-linearized pVA380-1 (Fig. 2), and the ligation mixture was used to transform *S. sanguis* cells, with selection for Cm^r. Cm^r transformants were recovered at a frequency of 10^{-4} per recipient. Plasmids pVA795 and pVA796 (10.5 kb) each contained the *Hin*dIII A fragment of pIP501 but in opposite orientation with respect to pVA380-1. Expression of Cm^r was identical in V795(pVA795), V796(pVA796), and V683 (growth on 5 μg of chloramphenicol per ml; inducible expression [data not shown]).

Construction of pVA797. With a knowledge of the pIP501 restriction site map, a pIP501 derivative was constructed which exhibited temperature-stable replication (pIP501 displays thermosensitive replication in *S. sanguis* [see below]), a unique *Eco*RI cleavage site, and erythromycin sensitivity due to deletion of the Em^r determinant. pIP501 was cleaved with *Hpa*II-*Ava*I and ligated with similarly treated pVA380-1 (Fig. 3). These ligation products were used to transform *S. sanguis* V288 to Cm^r. Cm^r transformants were recovered at a frequency of 3.6×10^{-5} per recipient, and 82/100 of such transformants were Em^s. Strains that were Em^r Cm^r harbored plasmids identical to pIP501. A representative Cm^r Em^s plasmid, designated pVA797, was examined by restriction endonuclease analysis. It was found to consist of the 3.4-kb pVA380-1 *Hpa*II-*Ava*I A fragment inserted at the unique *Hpa*II and *Ava*I sites of pIP501. The large *Hpa*II-*Ava*I fragment of pVA380-1 is known to be able to support plasmid replication in *S. sanguis* (19). pVA797 was 30.7 kb in size and could be readily distinguished from pIP501 by failure to confer Em^r and by the possession of a single *Eco*RI cleavage site of pVA380-1 origin (pIP501 has no *Eco*RI cleavage sites; Fig. 3).

Plasmid pVA797 was tested for self-transmissibility to *S. sanguis* and *S. faecalis* recipients bearing a chromosomal rifampin resistance (Rif^r) marker. pVA797 was able to transfer Cm^r during filter matings to both recipients (Table 3). Differences between pIP501 transfer frequencies and pVA797 transfer frequencies always were within one order of magnitude.

Miniplasmid derivative of pIP501 containing a replication region. Behnke and Gilmore (1) have reported the isolation of two deletion derivatives of pGB301, itself a deletion derivative of pIP501, which define a region involved in autonomous replication and copy number control. The identification of regions required for autonomous plasmid replication has been approached in other systems by self-ligation of limit-digested plasmid DNA and, after transformation, screening for plasmids of reduced size in transformants (7). pIP501 DNA partially digested with *Hin*dIII was self-ligated and transformed, with selection

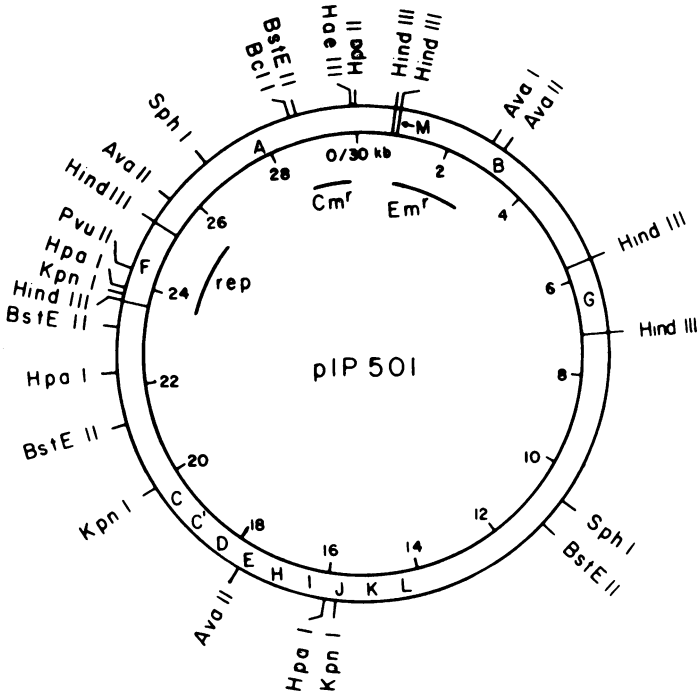

FIG. 1. Restriction site map of pIP501. The inside circle shows the location of pIP501 *Hin*dIII fragments designated with capital letters A through M. Unmapped fragments (C, C', D, E, H through L) are grouped together. The positions of the Cm^r and Em^r determinants and the replication region were assigned as described in the text. The numbers on the inside of the circular map refer to kb coordinates. The rep region refers to a putative region governing autonomous replication, copy number control, and plasmid stability.

for Cm^r and Em^r transformants. No Em^r transformants were detected; however, Cm^r transformants were obtained at a frequency of 1.05×10^{-6} per recipient (10 total colonies). All of the Cm^r transformants were also Em^s and harbored a plasmid of identical size (7.8 kb), designated pVA798. *Hin*dIII-digested pVA798 liberated the 6.3-kb *Hin*dIII A (contains Cm^r determinant; see above) and 1.5-kb *Hin*dIII F fragments of pIP501 (Fig. 1). Repeated attempts to remove the *Hin*dIII F fragment by further subcloning of pVA798 were unsuccessful. The *Hin*dIII A and F fragments of pIP501 were contiguous on the pIP501 molecule as shown by map distances of several restriction endonuclease sites and comparison of *Hin*fI-generated fragments of pVA798 and pIP501 (Fig. 1; data not shown).

Plasmid stability. Plasmid loss from strains V683(pIP501), V797(pVA797), and V798(pVA798) was determined (Table 4). pIP501 was stably maintained at 37°C but was lost during growth at 42°C with a frequency of 22%. In contrast, pVA797 was stable at both 37 and 42°C, whereas pVA798 was unstable during growth at both temperatures (Table 4).

Estimation of number of plasmid copies per chromosome. Crude lysates of strains V683, V797, and V798, grown in the presence of 4 μg

TABLE 3. Inter- and intraspecies transfer frequencies of pIP501 and pVA797

Donor	Plasmid	Recipient	Transconjugants/donor cell[a]	
			Rif^r Cm^r [b]	Rif^r Em^r [b]
S. sanguis V683	pIP501	*S. faecalis* V348	5.5×10^{-2}	1.1×10^{-2}
S. sanguis V797	pVA797	*S. faecalis* V348	4.3×10^{-3}	
S. sanguis V683	pIP501	*S. sanguis* V481	7.4×10^{-4}	2.1×10^{-4}
S. sanguis V797	pVA797	*S. sanguis* V481	6.0×10^{-3}	

[a] Time of mating contact was 6 h. All matings were performed in the presence of 100 μg of DNase I per ml.
[b] Drugs were used in selective media at the following concentrations: rifampin, 50 μg/ml; erythromycin, 10μg/ml; and chloramphenicol, 5 μg/ml.

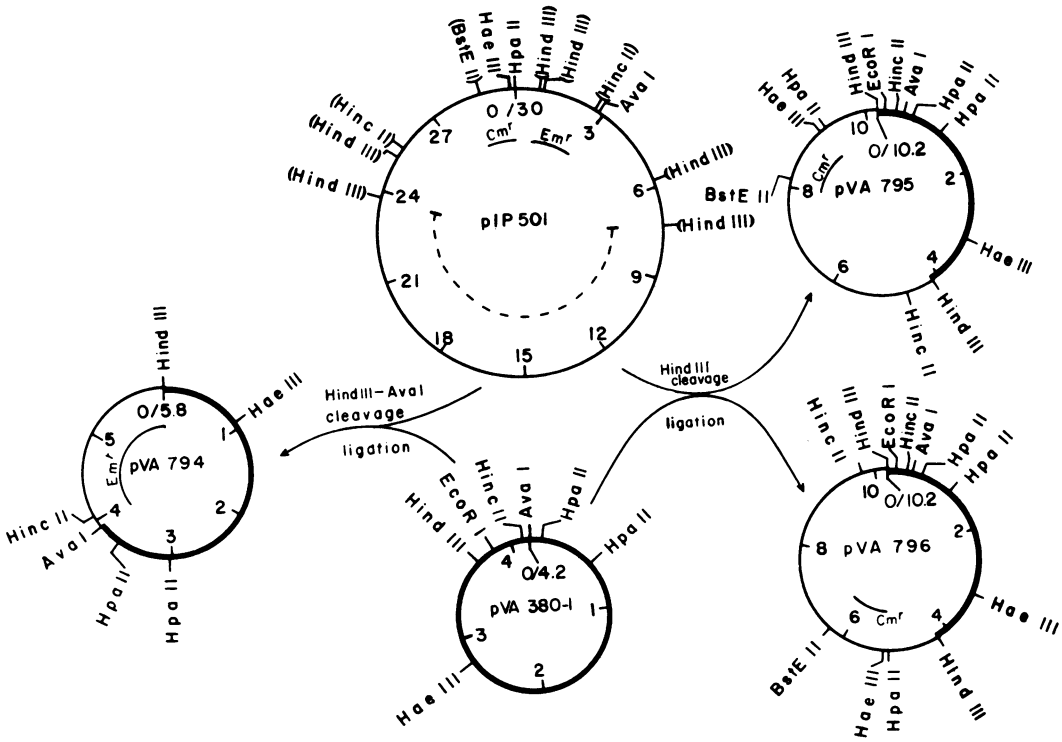

FIG. 2. Cloning of the pIP501 Em^r and Cm^r determinants. DNA fragments from pIP501 (narrow line) containing the Em^r and Cm^r determinants were cloned into pVA380-1 (heavy line), a streptococcal vector useful for cloning directly selectable markers (20). Restriction endonuclease cleavage sites and genetic markers relevant to the construction of these plasmids are indicated. The *Hin*dIII, *Bst*EII, and *Hin*cII sites (in parentheses) are shown; however additional sites exist in the region denoted by the dashed line. In the case of *Hin*cII, at least six other sites exist. A more extensive map of pIP501 is shown in Fig. 1. The molecules are not drawn to scale with respect to one another. Exact Cm^r placement is known by comparison with pGB301, a deletion plasmid of pIP501 described by Behnke and Gilmore (1).

of chloramphenicol per ml, were analyzed on cesium chloride-ethidium bromide gradients. The amount of plasmid DNA was compared with that of chromosomal DNA by summation of radioactivity under the respective peaks. The data from two experiments are compiled in Table 5. pIP501 represented approximately 6 to 7% of the chromosomal DNA in V683, reflecting some six copies per genome equivalent. pVA797, which contains replication regions from both pIP501 and pVA380-1, was maintained at 10 copies per genome equivalent in V797. The miniplasmid derivative of pIP501, pVA798, was present as 28% of the chromosomal DNA, representing some 95 copies per genome equivalent.

DISCUSSION

In this paper we have detailed the molecular organization of the pIP501 plasmid. Information in this regard had emerged from the work of Behnke et al. (1, 2), who have used deletion derivatives of pIP501 as molecular cloning vehicles. Our work represents the first report of a systematic dissection of a broad-host-range streptococcal plasmid.

The positioning of the resistance determinants of pIP501 (Fig. 1) was accomplished by using the results of our cloning work and the deletion and cloning analyses of Behnke and Gilmore (1). The work of these latter authors (1) has established that the *Bst*EII site at the 28.8-kb coordinate on the pIP501 map (Fig. 1) resides within the Cm^r gene, because insertions into that site eliminate the Cm^r phenotype. The Cm^r gene itself appears to reside within the *Bcl*I site at kb coordinate 28.5 and the *Hpa*II site at kb coordinate 30. The *Bcl*I site is unique on pVA798 (see above), and this site can accomodate inserted DNA without loss of Cm^r or replicative ability (manuscript in preparation). The successful construction of pVA797 (see above; Fig. 2) indicates that insertion into the *Hpa*II site (30-kb coordinate) does

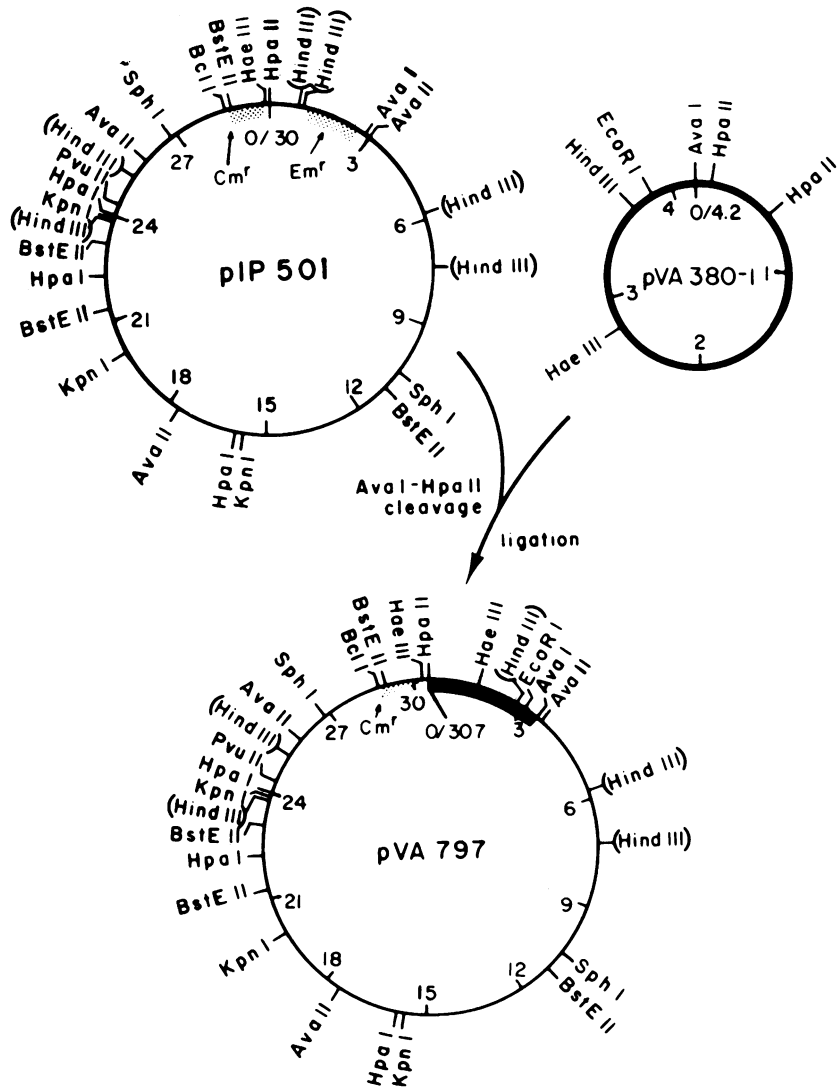

FIG. 3. Construction of pVA797. *Hpa*II-*Ava*I-cleaved molecules of pVA380-1 and pIP501 were ligated and transformed into *S. sanguis*, with selection for Cm^r. The *Hin*dIII sites are shown in parentheses to indicate that other such sites exist on pVA797 (Fig. 1).

not affect Cm^r. The fact that the *Hin*dIII A fragment (Cm^r) of pIP501 was recovered in both orientations in pVA380-1 (pVA795 and pVA796; Fig. 2) suggests that Cm^r expression is under the control of a pIP501 promoter sequence carried by the *Hin*dIII A fragment.

The pVA798 plasmid constructed by *Hin*dIII limit digest product ligation consists of the contiguous *Hin*dIII A and *Hin*dIII F fragments of pIP501. Self-ligation of the Cm^r determinant-containing *Hin*dIII A fragment was not possible on repeated attempts, implying that there are required replication functions on the *Hin*dIII F fragment. Behnke and Gilmore (1) have reported that the replication region of pGB301 is bounded by *Ava*II sites; however, the replication region of pIP501 is bounded by a single *Ava*II site. The deletion event(s) which yielded pGB301 from pIP501 resulted in the placement of one of the two additional *Ava*II sites near the pIP501 *Hin*dIII site at kb coordinate 23.6 (Fig. 1). A deletion event(s) which resulted in loss of regions between kb coordinates 3.2 and 23.3 including the *Bst*EII site at kb coordinate 23.2 is the simplest description of the origin of pGB301 (1, 2).

TABLE 4. Plasmid stability

Strain	Plasmid	Growth conditions[a]	Expt	No. of colonies scored	% Cm^s [b]
S. sanguis V683	pIP501	37	1	50	<1
			2	132	<1
			3	111	<1
		42	1	89	29
			2	130	16
			3	107	20
S. sanguis V797	pVA797	37	1	29	<1
			2	150	<1
			3	87	<1
		42	1	92	<1
			2	130	<1
			3	207	<1
S. sanguis V798	pVA798	37	1	ND[c]	
			2	157	34
			3	200	30
		42	1	ND	
			2	144	28
			3	208	41

[a] An overnight broth culture was diluted 10^5, grown for 18 h at the temperature indicated, and then plated on nonselective media. Individual colonies then were screened for the Cm^r phenotype. Cm^s colonies of V683 were also Em^s.

[b] No growth on 5 μg of chloramphenicol per ml.

[c] ND, Not done.

Several results indicate that pVA798 does not contain all of the pIP501 replication machinery. Comparison of loss of pVA798 and pIP501 from isogenic strains after serial passage revealed significant differences (Table 4), most notably, the extensive loss of pVA798 from cells grown at 37°C. pVA798 also exhibits a dramatic increase in copy number over pIP501 (95 copies per chromosomal equivalent versus 6 copies per chromosomal equivalent for pIP501). Our data, taken with that of Behnke and Gilmore (1), suggest that the region between the *Bst*EII cleavage site at kb coordinate 23.2 and the adjacent *Hin*dIII cleavage site at kb coordinate 23.6 is required for copy number control and plasmid stability (Fig. 1). Behnke and Gilmore (1) first reported the involvement of this region in copy number control but did not obtain as dramatic an elevation in copy number. pVA798 segregation in cells grown at 37°C can be attributed to the deletion of a locus involved in partitioning (*par*) of plasmid copies to daughter cells (27–29). The high segregation frequency of pVA798 cannot be explained, however, as the loss of plasmid copies due to random segregation alone. Clearly, additional factors involved in the maintenance of pIP501 await elucidation.

The high copy number of pVA798 and its relatively small size (7.8 kb) suggest its use as a production vehicle in the streptococci. pVA798 can be maintained stably in cells grown under selective pressure (chloramphenicol, 5 μg/ml), and a number of unique restriction endonuclease sites can be used to insert DNA into this plasmid (manuscript in preparation).

Conjugal plasmid pVA797 is a hybrid consisting of the pIP501 and pVA380-1 replicons (Fig. 3). This plasmid was formed by the ligation of *Hpa*II-*Ava*I-cleaved pIP501 and pVA380-1 DNAs with the resultant loss of pIP501 regions between kb coordinates 0 and 2.7 (Fig. 3). Thus, pVA797 is slightly larger than pIP501 (30.7 and 30 kb, respectively) and has lost the Em^r determinant, but still retains the Cm^r determinant and the ability to transfer by conjugation (Table 3). Because many of the available, nonconjugative plasmid cloning vehicles of the streptococci bear Em^r genes, pVA797 will be useful as a mobilizing plasmid for chimeras because of its Cm^r Em^s phenotype.

Hybrid plasmids which contain two or more replicons are generally under the replication control of the replicon with the least amount of requirements for replication initiation (30, 31, 34). Three lines of evidence support the hypothesis that pVA797 replication is driven by the pVA380-1 replicon rather than the pIP501 replicative machinery. First, a minimum estimation of plasmid copy number showed that pVA797 is maintained at 10 copies per chromosomal equiv-

TABLE 5. Plasmid copy number in *S. sanguis*

Strain	Plasmid	Plasmid size (kb)	Expt no.	% Chromosome	Copies/chromosomal equivalent[a]
S. sanguis V683	pIP501	30	1	6.1	6
			2	7.6	7
S. sanguis V797	pVA797	30.7	1	11.0	10
			2	13.0	12
S. sanguis V798	pVA798	7.8	1	28.0	95
			2	28.0	95

[a] The size of the *S. sanguis* genome was assumed to be 1.8×10^9 daltons (22).

alent, whereas pIP501 exists at 6 copies per chromosomal equivalent (Table 5). Although this elevation in copy number of pVA797 relative to that of pIP501 is reproducible, its significance is difficult to evaluate. Although it is suggestive of some replication initiation at the pVA380-1 locus, proof of this awaits further investigation. Second, pVA797 is stably maintained at 42°C in contrast to the 22% frequency loss of pIP501 in strains grown at 42°C (Table 3). We have previously demonstrated the stability of pVA380-1 and cloning vectors derived from pVA380-1 in *S. sanguis* cells grown at 42°C (20). Finally, pVA797 is unable to coexist stably in *S. sanguis* harboring pVA380-1 derivatives (e.g., pVA736 [Em^r, see reference 20], pVA838 [Em^r, see reference 23]), indicating its incompatibility with pVA380-1 (unpublished data).

Conjugal transfer-deficient deletion derivatives of pIP501 missing nearly one-half of the pIP501 molecule tend to suffer deletions predominately between kb coordinates 3 and 23 (unpublished data). Attempts to clone a fragment(s) that encodes conjugal transfer ability have been unsuccessful to date. pVA797 will prove useful in the elucidation of the conjugal transfer genes of streptococcal plasmid pIP501 and in the development of genetic exchange systems in the streptococci. pVA797 has been used to mobilize an Em^r *Escherichia coli-S. sanguis* shuttle plasmid, pVA838 (23), from an *S. sanguis* host to a *S. faecalis* recipient (M. Smith and D. B. Clewell, personal communication). In addition, the entire pVA797 molecule recently has been cloned on an *E. coli* plasmid vector and introduced into *E. coli* via transformation. This chimera containing pVA797 segregates into minicells and directs the synthesis of several polypeptides (unpublished data). We are currently using this system to begin identification of pVA797-specific polypeptides that are involved in conjugal transfer.

ACKNOWLEDGMENTS

R.P.E. is the recipient of the Virginia Commonwealth University School of Graduate Studies Fellowship Award. F.L.M. is the recipient of Public Health Service Research Career Development Award DE00081 from the National Institute of Dental Research. This work was supported by Public Health Service grant DE04224 from the National Institute of Dental Research and by the Consumer Products Group of the Warner Lambert Co.

ADDENDUM IN PROOF

pIP501 DNA prepared from host *S. faecalis* or *S. agalactiae* contains a second *Pvu*II site at kb coordinate 15.4 on the pIP501 restriction endonuclease site map. This additional *Pvu*II site remains refractory to cleavage on pIP501 molecules prepared from *S. sanguis* V288.

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