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Genetic Transformation of *Streptococcus sanguis* (Challis) with Cryptic Plasmids from *Streptococcus ferus*

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By using the basic methodology initially published by Kretschmer et al. (J. Bacteriol. **124**:225-231, 1975), we have been able to introduce phenotypically cryptic plasmids from *Streptococcus ferus* (formerly *Streptococcus mutans* subsp. *ferus*) into *Streptococcus sanguis* by genetic transformation. In this system, the entry of the cryptic plasmids is selected indirectly. This is effected with transforming deoxyribonucleic acid mixtures in which the cryptic plasmid deoxyribonucleic acid is present in an approximate 10-fold molar excess with respect to a plasmid (pVA1) known to confer erythromycin resistance. Under such conditions, 5 to 10% of the pVA1-containing erythromycin-resistant transformants were cotransformed with cryptic plasmid deoxyribonucleic acid. pVA1 may be selectively eliminated by growth of its *S. sanguis* host strain at 42°C, enabling the construction of isogenic strains with and without *S. ferus* cryptic plasmids. Comparative physiological studies of such strains have failed to reveal any plasmid-conferred phenotypes in *S. sanguis*. With this procedure, we have been able to physically separate two small cryptic plasmids (2.4×10^6 and 2.8×10^6 daltons) of *S. ferus*. Although these plasmids were found naturally to exist in a single *S. ferus* host, they were able to replicate independently of one another in *S. sanguis*. Restriction enzyme fingerprinting indicated that these plasmids did not share a common ancestry.

The search for naturally occurring extrachromosomal genetic elements in the human oral streptococci has led to the discovery of only a few plasmid-containing strains (1, 16, 18, 20). In general, these plasmids are small in size, and evidence supporting the dissemination of one such plasmid among different *Streptococcus mutans* strains has been presented (17). With regard to the oral streptococci, the only definitive report of a naturally occurring plasmid bearing a phenotypic marker was published by Yagi et al. (23). Using both curing studies and genetic transformation, these authors demonstrated the existence of a 4.6×10^6 dalton (4.6 megadalton [Mdal]) plasmid which conferred inducibly expressed macrolide-lincosamide-streptogramin B antibiotic resistance.

Two groups (7, 8, 10) have suggested that a plasmid (or plasmids) confers sucrose-utilizing exopolysaccharide synthesis on cariogenic *S. mutans*. However, such studies relied only on phenotypic curing as a means of identifying plasmid-conferred phenotype. The establishment of plasmid-encoded exopolysaccharide production in *S. mutans* would be significant since such polymers are believed to play a key role in the oral colonizing ability and cariogenicity of this species (6).

We have sought to introduce phenotypically cryptic plasmids from *Streptococcus ferus* (formerly *S. mutans* subsp. *ferus* [4, 5]) into *Streptococcus sanguis* as a means of exploring the possible phenotypic expression of such extrachromosomal elements. In doing so, we hope further to use such small plasmids to develop a molecular cloning system in *S. sanguis*. Using the *S. sanguis* transformation system, we have adapted the technique of Kretschmer et al. (11) for introducing cryptic plasmids into a cell by indirect selection. We report here the successful introduction of two different-sized cryptic plasmids from *S. ferus* into *S. sanguis* (strain Challis). Isogenic strain pairs with and without these plasmids have been physiologically studied, but no plasmid-encoded, phenotypically expressed traits have been identified.

MATERIALS AND METHODS

Bacterial strains and media. The strains used or developed in this work are described in Table 1. The maintenance of strains was as previously published (20). Todd-Hewitt (TH) broth (Difco Laboratories, Detroit, Mich.) was used as the complex liquid medium. Brain heart infusion (Difco Laboratories) was used for transformation experiments. Agar (Difco Laboratories) was added to a final concentration of 1.5% when a solid medium was desired. Carbohydrate fermentation medium and testing were as described previously (16). Erythromycin was added to the medium

TABLE 1. *Principal bacterial strains*

Strain	Lab designation	Relevant phenotype[a]	Plasmid content and size[b]	Remarks/references
S. sanguis	V288	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^s	None	Strain Challis
S. ferus	V380	Lac$^-$ Sor$^+$ Mtl$^+$ Ery^s	pVA380 (2.4) pVA380 (2.8)	Designated *S. ferus* 4S1 by Coykendall (4, 5)
S. sanguis	V486	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^r	pVA1 (7.3)	pVA1 plasmid confers Ery^r; Macrina et al. (Plasmid, in press)
S. sanguis	V676	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^r	pVA1 (7.3) pVA380 (2.4)	This paper
S. sanguis	V678	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^r	pVA1 (7.3) pVA380-1 (2.8)	This paper
S. sanguis	V719	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^r	pVA1 (7.3) pVA380 (2.4) pVA380-1 (2.8)	This paper
S. sanguis	V679	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^s	pVA380 (2.4)	This paper
S. sanguis	V685	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^s	pVA380-1 (2.8)	This paper
S. sanguis	V721	Lac$^+$ Sor$^-$ Mtl$^-$ Ery^s	pVA380 (2.4) pVA380-1 (2.8)	This paper

[a] Lac$^+$, Acid produced from lactose; Lac$^-$, no acid produced from lactose; Sor$^+$, acid produced from sorbitol; Sor$^-$, no acid produced from sorbitol; Mtl$^+$, acid produced from mannitol; Mtl$^-$, no acid produced from mannitol; Ery^s, inhibited by 10 μg of erythromycin per ml; Ery^r, not inhibited by 10 μg of erythromycin per ml.

[b] Numbers given within parentheses represent size in Mdal.

after autoclaving to a final concentration of 10 μg/ml unless otherwise stated. All cultures were incubated anaerobically at 37°C.

Chemicals and enzymes. Cesium chloride (technical grade) was obtained from Kawecki Berylco Industries, New York, N.Y. Agarose (type II), lysozyme (grade I), protease (grade I), deoxyribonuclease I, ribonuclease T_1, and ethidium bromide were purchased from the Sigma Chemical Co., St. Louis, Mo. Sarkosyl NL 97 (sodium dodecyl sarcosinate) was a gift of the Ciba-Geigy Corp., Greensboro, N.C. Erythromycin was a gift of The Upjohn Co., Kalamazoo, Mich. Restriction endonucleases and bacteriophage lambda deoxyribonucleic acid (DNA) were purchased from Bethesda Research Laboratories, Rockville, Md. Restriction endonuclease digestions of streptococcal plasmid DNA were for 16 h in a reaction volume of 50 μl containing 0.5 to 1.0 μg of DNA. Digestions were carried out at 37°C for *Hin*fI or 65°C for *Taq*I with 10 U of enzyme per μg of DNA. Reactions were stopped by the addition of one-fourth volume of an aqueous solution containing 0.07% bromophenol blue, 33% glycerol, and 7% sodium dodecyl sulfate.

Isolation and characterization of plasmid DNA. The preparation of cleared cell lysates of *S. sanguis* was performed as described previously (16, 17). Covalently closed circular DNA was isolated from such lysates by cesium chloride-ethidium bromide centrifugation. Purified plasmid DNA was stored in TE buffer [0.01 M tris(hydroxymethyl)aminomethane, 0.001 M ethylenediaminetetraacetic acid, pH 8]. Plasmid DNA and plasmid restriction digest products were examined by agarose gel electrophoresis by the method of Meyers et al. (19). Agarose gel size reference markers consisted of plasmids from *Escherichia coli* V517 (15) as covalently closed circular reference molecules or of *Hin*dIII digest products of phage lambda DNA as linear reference molecules.

Transformant clones were screened for plasmid DNA by a rapid lysis method (18a). In short, logarithmic-phase cells (from 1.5-ml broth cultures) grown in the presence of 5% glycine were harvested by centrifugation, washed, and suspended in hypertonic glucose. These cells then were treated sequentially with lysozyme, ribonuclease, and protease; sodium dodecyl sulfate was used to effect lysis. Such lysates were hydrodynamically sheared, and small samples were analyzed directly on agarose gels by low-voltage electrophoresis (2 V/cm of gel length). Under these conditions, reliable detection of plasmids of 10 Mdal or less could be made.

Genetic transformation. The transformation protocol used in this work was as described by Lawson and Gooder (12).

Plasmid-curing protocol. Selective elimination of pVA1 from *S. sanguis* was accomplished by growth at 42°C. Several colonies of a pure culture were picked and grown to stationary phase in TH broth. This culture was diluted in fresh TH broth and used to inoculate a 10-ml TH broth culture such that the starting cell density was 10^4 cells per ml. This culture then was incubated at 42°C. After 24 h of incubation, cells were plated on solid TH medium. Single colonies which grew on such plates then were transferred with a toothpick or replica plated onto erythromycin-containing medium to score for the loss of erythromycin resistance. Erythromycin-sensitive clones then were examined for plasmid DNA with the minilysis protocol (see above).

RESULTS

Genetic transformation of *S. sanguis* V288 with plasmid DNA. Kretschmer et al. (11) reported that an indicator plasmid (conferring drug resistance) can be used to identify *E. coli* cells which have been cotransformed with a

second plasmid bearing no selectable marker. Such cotransformation is effected when the indicator plasmid is present in low concentration relative to the plasmid being selected indirectly. We adapted their method to the *S. sanguis* genetic transformation system with pVA1 as an indicator plasmid. pVA1 is a 7.3-Mdal plasmid conferring erythromycin (macrolide-lincosamide-streptogramin B) resistance on the streptococci (Macrina et al., Plasmid, in press). It is a deletion derivative of the 17-Mdal pAMβ1 plasmid (2). pVA1 DNA can be introduced into *S. sanguis* via genetic transformation, and it is relatively stably maintained in this host (<5% spontaneous loss frequency at 37°C). At 42°C, however, this plasmid is rapidly segregated from growing cells (>90% frequency of loss [Macrina el al., Plasmid, in press]).

Various mixtures of pVA1 and the two phenotypically cryptic plasmids of *S. ferus* V380 were used to transform *S. sanguis* V288. The plasmid DNA was mixed with competent cells, and the incubation of DNA and cells at 37°C was allowed to proceed for 3 h. Samples (0.1 ml each) of this mixture then were spread onto TH agar plates containing 10 μg of erythromycin per ml. Data from a typical set of experiments are shown in Table 2. pVA1 alone was able to effect the transformation of V288 at a frequency of about 10^{-2} transformants per recipient. The addition of cryptic plasmid DNA in a 2.7- to 9-fold molar excess of pVA1 resulted in a significant depression of pVA1-mediated transformation to erythromycin resistance. As the molar ratio of indicator plasmid to cryptic plasmid DNA approached 10^{-2}, transformants were rarely detected.

In the experiment described in Table 2, cotransformants were detected at a frequency of ~8% when the indicator/cryptic plasmid molar ratio was 1.1×10^{-1}, whereas only 2.5% of the examined clones were cotransformed when the plasmid DNA molar ratio was 1.7×10^{-1}. As shown in Table 2, cotransformed cells bearing either pVA380 or pVA380-1 were detected. The physical analysis of parental and transformed strains is shown in Fig. 1. Cesium chloride-ethidium bromide-purified plasmid DNA from *S. ferus* V380 is shown in lane B, and pVA1 from *S. sanguis* V486 is shown in lane C.

Typical cotransformants bearing pVA1 and pVA380 (V676) or pVA1 and pVA380-1 (V678) are shown in lanes D and E, respectively, of Fig. 1. When these strains were grown at 42°C as described above, erythromycin-sensitive isolates bearing only the respective cryptic plasmids were obtained (Fig. 1, lanes G and H). Both pVA380 and pVA380-1 appeared to be unaffected by the growth of their host strain at 42°C. Of the 50 (25 for V676 and 25 for V678) erythromycin-sensitive clones examined, none was found to be cured of its cryptic plasmid, despite the loss of pVA1.

To construct an *S. sanguis* strain bearing both pVA380 and pVA380-1, *S. sanguis* V679 (carrying pVA380) was transformed with a mixture of pVA1 (0.9 μg) and pVA380/pVA380-1 (5 μg). Erythromycin-resistant transformants were found at a frequency of $\sim 5 \times 10^{-7}$, and 1 out of 20 clones screened was found to contain both pVA380 and pVA380-1. The plasmid profiles of this strain, designated V719, and its pVA1 temperature-cured derivative, V721, are shown in lanes F and I of Fig. 1.

Physical analysis of pVA380 and pVA380-1. The construction of *S. sanguis* V679 and V685 clearly established that pVA380 and pVA380-1 were capable of replicating independ-

TABLE 2. *Transformation of S. sanguis V288 with streptococcal plasmids*

Plasmid DNA (μg per 6.6×10^6 cells[a])		Molar ratio[b] (pVA1/pVA380 and pVA380-1)	Ery^r transformants per recipient[c]	pVA1 transformants bearing:	
pVA1	pVA380 and pVA380-1[d]			pVA380	pVA380-1
0.9	0		2×10^{-2}[e]		
0.9	5.4	1.1×10^{-1}	5.6×10^{-6}	2/38	1/38
0.9	3.6	1.7×10^{-1}	3.0×10^{-4}	1/38	0/38
0.9	1.8	3.6×10^{-1}	1.7×10^{-4}	0/38	0/38

[a] Plasmid DNA concentration estimated by absorbance at 260 nm; 1 U of absorbance at 260 nm is equal to a concentration of 50 μg/ml; the final volume of DNA plus competent cell culture was always 0.43 ml.

[b] Approximate molar ratios were calculated by using the following size values: pVA1 = 7.3 Mdal (Macrina et al., Plasmid, in press), pVA380 = 2.4 Mdal (18), and pVA380- 1 = 2.8 Mdal (18).

[c] Recipient cell count determined at time DNA was added to culture.

[d] Plasmid mixture isolated from *S. ferus* V380; each plasmid has been shown to be present to the extent of ~30 copies per chromosome (18).

[e] This frequency is ~100-fold higher than that obtained with pAMβ1 DNA from *Streptococcus faecalis*. Since the pVA1 DNA was purified from *S. sanguis*, this high transformation frequency is likely due to host-controlled restriction mechanisms (13).

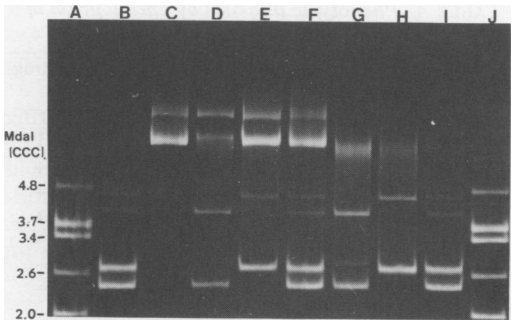

FIG. 1. *Agarose gel electrophoretic analysis of parental and transformed streptococcal strains. All plasmid DNAs analyzed were purified by one cycle of cesium chloride-ethidium bromide centrifugation. (A and J) Size reference covalently closed circular (CCC) molecules; sizes (Mdal) are given to the left of the photograph. (B) Plasmids from S. ferus V380: pVA380 (2.4 Mdal) and pVA380-1 (2.8 Mdal); corresponding open circular molecules are seen as slower-migrating faint components. (C) pVA1 plasmid DNA (7.3 Mdal); the slower-migrating, faint component is the open circular form of pVA1. (D) Plasmid DNA from V676; the faint component seen in the 5-Mdal range is consistent with the migration of linear pVA1 DNA. (E) Plasmid DNA from V678. (F) Plasmid DNA from V719. (G) Plasmid DNA from V679; the faint component seen migrating above covalently closed circular pVA380 is consistent with linear pVA380 DNA. (H) Plasmid DNA from V685. (I) Plasmid DNA from V721. The diffuse components seen in the 5- to 7-Mdal range in (G) and (H) represent contaminating host chromosomal DNA. The agarose concentration was 0.7%, and migration was from top to bottom. The gel was stained for 15 min in an aqueous solution of 5 μg of ethidium bromide per ml.*

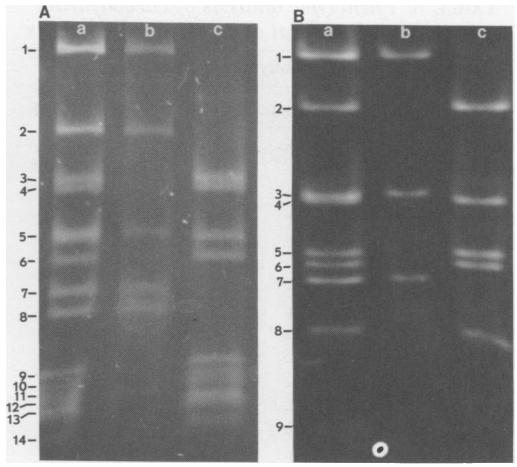

FIG. 2. *Restriction enzyme analysis of pVA380 and pVA380-1. Cesium chloride-ethidium bromide-purified plasmid DNA from S. ferus V380, S. sanguis V679, and S. sanguis V685 was digested with TaqI (A) and HinfI (B). For (A) and (B), the lanes represent the following: (a) enzyme digest products of pVA380 and pVA380-1 from strain V380; (b) enzyme digest products of pVA380 from strain V679; (c) enzyme digest products of pVA380-1 from strain V685. The agarose concentration was 1.6%, and migration was top to bottom.*

ently of one another. To probe the possibility that these two plasmids shared a common molecular organization, pVA380 and pVA380-1 were purified separately by using the V679 and V685 host strains. Plasmid DNA then was digested with the restriction enzyme *Taq*I (Fig. 2A) or *Hinf*I (Fig. 2B).The *Taq*I digest of the two plasmids isolated from their original host (*S. ferus* V380) is shown in lane a of Fig. 2A and reveals 14 components, some of which correspond to doublets. Lane b of Fig. 2A shows that components 1, 2, 5 (one member of doublet), 7, 8, and 12 correspond to the pVA380 plasmid. Component 12 is not clearly resolved in lane a of Fig. 2A and shows up as only a faint band in lane b of Fig. 2A. Components 3, 4, 5 (one member of doublet), 6, 9, 10, 11, 13, and 14 correspond to pVA380-1 (Fig. 2A, lane c).

Nine *Hinf*I components are revealed after digestion of pVA380 and pVA380-1 isolated from *S. ferus* V380 (Fig. 2B, lane a). Components 1, 3, 7, and 9 (barely visible) are seen to correspond to pVA380 (Fig. 2B, lane b), whereas components 2, 4, 5, 6, and 8 correspond to pVA380-1. As expected from these data, the *Taq*I and *Hinf*I digest products of pVA380 and pVA380-1 isolated from *S. sanquis* V721 did not differ from the digest fingerprints of the plasmid mixture isolated from *S. ferus* V380 shown in lane a of Fig. 2A and B.

Physiological studies of isogenic strains. *S. sanguis* V288, V679, V685, and V721 (Table 1) represent an isogenic set of strains. As such, these strains were used in an attempt to identify traits conferred by pVA380 or pVA380-1. Also included in all such comparative physiological studies was *S. ferus* V380. Tables 3 through 5 summarize data from these experiments and reveal no differences between the plasmidless V288 and its plasmid-containing derivatives (V679, V685, and V721). In the case of the bacteriocin assays (Table 5), neither bacteriocin production nor sensitivity was altered by the presence of the pVA380 or the pVA380-1 plasmid. All *S. sanguis* strains (V288, V679, V685, and V721) displayed alpha-hemolysis on 5% sheep blood agar, whereas *S. ferus* V380 was only weakly alpha-hemolytic.

Figure 3 is a composite of photographs of the above strains grown on mitis salivarius (high-sucrose) agar (Difco Laboratories). There were no differences in the colonial morphology of any of the isogenic *S. sanguis* strains. In general, *S.*

TABLE 3. *Phenotypic analysis by carbohydrate fermentation of parental and transformed strains*

Carbohydrate	Carbohydrate fermentation[a]				
	V288	V380	V679	V685	V721
Mannitol	−	+	−	−	−
Sorbitol	−	+	−	−	−
Lactose	+	−	+	+	+
Salicin	+	+	+	+	+
Levulose	+	+	+	+	+
Inositol	−	−	−	−	−
Rhamnose	−	−	−	−	−
Trehalose	+	+	+	+	+
Dulcitol	−	−	−	−	−
Melibiose	−	−	−	−	−
Mannose	−	−	−	−	−
Galactose	+	−	+	+	+
Raffinose	−	+	−	−	−
Fructose	+	+	+	+	+
Glucose	+	+	+	+	+

[a] Acid production determined by growth on phenol red agar (Difco Laboratories) containing 0.5% carbohydrate; +, acid produced; −, no acid produced.

ferus V380 (Fig. 3E) produced a smaller colony than that produced by *S. sanguis* on mitis salivarius agar. All of the *S. sanguis* strains (V288, V679, V685, and V721) produced colonies which were hard (granular) in consistency and tended to be attached to the mitis salivarius agar surface. This was also the case for *S. ferus* V380.

DISCUSSION

Kretschmer et al. (11) were the first to report that phenotypically cryptic plasmids may be introduced into a cell by indirect selection. They used an indicator plasmid conferring tetracycline and kanamycin resistance (pSC201) which was a thermosensitive replication-defective mutant. When pSC201 DNA was mixed with a 1.5-Mdal cryptic plasmid at a molar ratio of 4×10^{-4}, ~66% of the *E. coli* drug-resistant transformants were found to carry the cryptic plasmid. By using the basic approach of Kretschmer et al. (11), we have been able to introduce cryptic plasmids from *S. ferus* into *S. sanguis*. Our work established that cryptic plasmids may be indirectly selected in the streptococcal transformation assay. Within this context, it is important to note that genetic transformation in *S. sanguis* is a naturally occurring process in that, unlike the *E. coli* system, the cells need not be pretreated with $CaCl_2$ or other agents to develop a competent (transformable) state.

Two noteworthy differences exist between the results obtained in the streptococcal system and those found with *E. coli*. First, Kretschmer et al. (11) were able to use mixtures of indicator plasmid to cryptic plasmid in the 10^{-4} to 10^{-5} molar ratio range. When we attempted to use molar ratios which were 10^{-2} or less, erythromycin-resistant transformants usually were not detected. This is most likely due to competition effects of the large amount of cryptic plasmid DNA as compared with the indicator plasmid (pVA1). A similar effect was not noted in the *E. coli* system described by Kretschmer et al. (11). *E. coli* cells are made competent by $CaCl_2$ shock, a treatment which facilitates the entry of the DNA into the cell by a means which is not clear (3, 9). This is in contrast to the *S. sanguis* transformation system in which the competent state is achieved during early logarithmic growth and appears to involve the producton of a competence protein (or proteins) which presumably plays a role in the specific transport of DNA into the streptococcal cell (21). The lack of presumed competition effects in the *E. coli* system (11) as compared with the streptococcal system could be due to these fundamental differences in the two transformation processes.

Second, the frequencies of double transform-

TABLE 4. *Phenotypic analysis by antibiogram of parental and transformed strains*

Antibiotic	Antibiogram (growth on 5 μg of drug per ml[a])				
	V288	V380	V679	V685	V721
Streptomycin	+	+	+	+	+
Gentamicin	+	+	+	+	+
Kanamycin	+	+	+	+	+
Penicillin	−	−	−	−	−
Erythromycin	−	−	−	−	−
Chloramphenicol	−	−	−	−	−
Tetracycline	−	−	−	−	−
Cephalothin	−	−	−	−	−

[a] A loopful of saturated broth culture was streaked onto TH agar containing 5 μg of antibiotic per ml; growth was scored after 24 h of incubation; +, growth; −, no growth.

TABLE 5. *Bacteriocin testing of streptococcal strains*

Indicator strain	Zone size (mm)[a] of producing strain					
	V288	V380	V403[b]	V679	V685	V721
V288	0	0	12.5 ± 0.7	0	0	0
V380	0	0	19.0 ± 1.0	0	0	0
V403	0	0	0	0	0	0
V679	0	0	12.9 ± 0.9	0	0	0
V685	0	0	13.5 ± 0.9	0	0	0
V721	0	0	12.7 ± 0.8	0	0	0

[a] The diameter of the zone of inhibition surrounding the producing colony was measured to the nearest millimeter. The results represent the average of three independently performed experiments. Bacteriocin assays were performed as previously described (16, 17).

[b] *S. mutans* V403 produces a broad-spectrum, protease-sensitive bacteriocin (17).

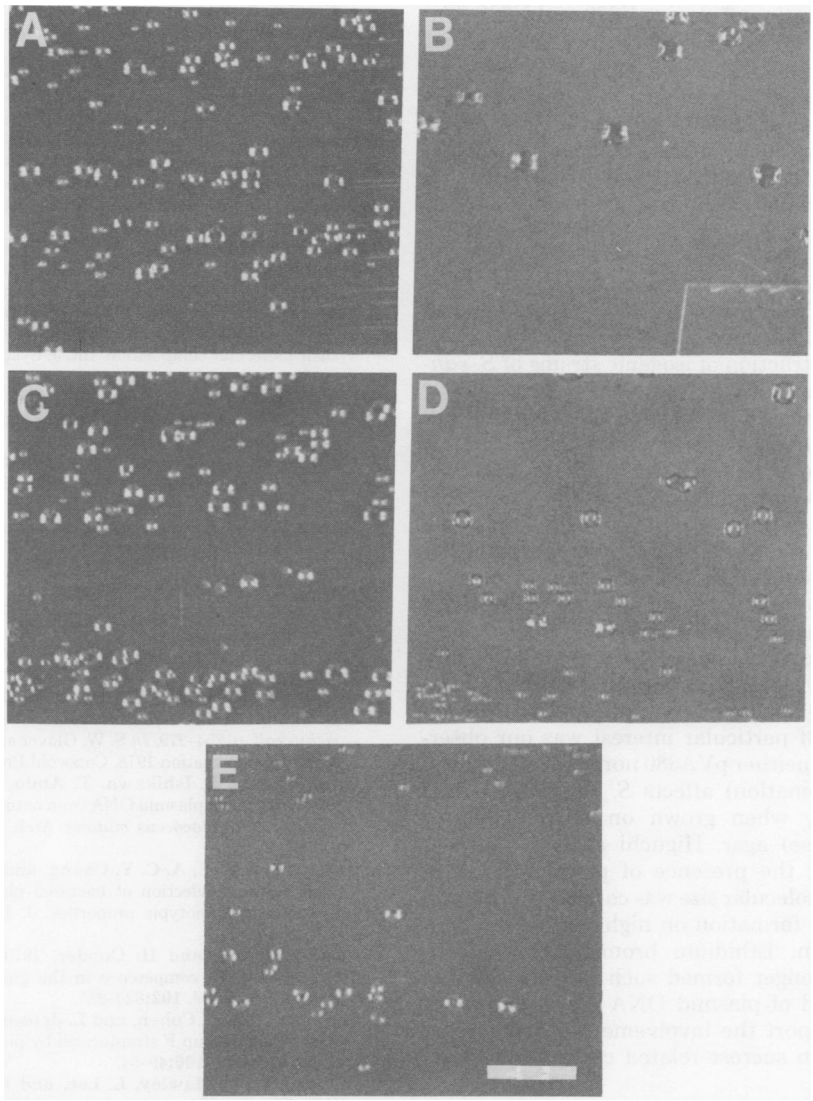

FIG. 3. *Colonial morphology of transformed and parental strains. TH broth cultures of each strain were streaked on mitis salivarius agar and grown anaerobically for 30 h at 37°C. The plates were then allowed to incubate aerobically at room temperature (20 to 22°C) for 12 h before photographing. (A) S. sanguis V288; (B) S. sanguis V679; (C) S. sanguis V685; (D) S. sanguis V721; and (E) S. ferus V380. The scale in (E) represents 4 mm and applies to all panels.*

ants in our experiments were significantly lower than those reported for the *E. coli* system (11). Kretschmer et al. (11) reported cotransformation at frequencies of up to ~70% when a molar ratio (indicator plasmid/cryptic plasmid) of 10^{-5} was employed. In the experiments reported here (Table 2) our highest frequency of cotransformants was ~8%. This value, although low, still enabled the ready detection of double transformants in the streptococcal system (cotransformation frequency never exceeded 10% in three independent experiments). In fact, the rapid screening technique used in this work (18a) enabled us to examine up to 50 transformants per day. The detection of double transformants thus could be accomplished conveniently even at their relatively low frequency of occurrence.

The protocol described in this paper should be useful for constructing *S. sanguis* strains bearing any phenotypically cryptic streptococcal plasmid. The construction of *S. sanguis* strains bearing pVA380 and pVA380-1 (alone or in combination) has afforded us the means to study these plasmids both physically and genetically.

The construction of strains V679 and V685 (Table 1 and Fig. 1) enabled us to establish that pVA380 and pVA380-1 are able to replicate independently of one another. This was of interest since they were initially isolated as part of the genetic complement of the same host cell. Moreover, restriction endonuclease analysis (Fig. 2) of the individual plasmids isolated from strains V679 and V685 suggests that these two plasmids do not share a common ancestry. Their grossly different restriction fragment fingerprints indicate that pVA380 and pVA380-1 have evolved as independent replicons.

The construction of isogenic strains of *S. sanguis* bearing pVA380, pVA380-1, or pVA380/pVA380-1 has enabled us to meaningfully address the question of plasmid phenotype. In this context, the possibility that *S. ferus* plasmids might not be expressed in *S. sanguis* does exist, but it appears unlikely. Specifically, R-plasmid expression in a variety of oral streptococci (including *S. sanguis* and *S. mutans*) is known to occur, as is heterospecific transformation with chromosomal markers (13, 14, 22). Our comparative physiological studies (Tables 3 through 5 and Fig. 3) failed to reveal any differences between V288 and its isogenic plasmid-bearing derivatives. Of particular interest was our observation that neither pVA380 nor pVA380-1 (alone or in combination) affects *S. sanguis* colonial morphology when grown on mitis salivarius (high-sucrose) agar. Higuchi et al. (7, 8) suggested that the presence of plasmid DNA of unknown molecular size was correlated with mucoid colony formation on high-sucrose-containing medium. Ethidium bromide-treated cells which no longer formed such mucoid colonies were devoid of plasmid DNA (7, 8). Our work fails to support the involvement of pVA380 or pVA380-1 in sucrose-related colonial morphology (Fig. 3).

Finally, the introduction of the small cryptic plasmids of *S. ferus* into *S. sanguis* provides a means for the development of useful molecular cloning vectors in this host. Such a recombinant DNA system in *S. sanguis* could be used to clone chromosomal gene sequences which are important in oral colonization. Work in this area is currently under way in our laboratory.

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