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Interspecies Transformation of Streptomycin Resistance in Oral Streptococci

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Donor deoxyribonucleic acid extracted from streptomycin-resistant (Str^R) mutant derivatives of a variety of strains of *Streptococcus mutans*, *S. salivarius*, and *S. sanguis* was used to transform streptomycin resistance into competent *S. sanguis* strain Challis. Transfer of genetic markers for sorbitol and mannitol fermentation and for extracellular polysaccharide as demonstrated by colonial morphology was not detected in this study. Reciprocal transformation between strain Challis and other oral streptococci could not be demonstrated. Transformation frequencies for Str^R were relatively efficient among *S. sanguis* strains, with lower but significant frequencies demonstrated with strain Challis and donor deoxyribonucleic acid derived from other oral streptococci.

The etiological role of oral streptococci in dental caries has been of great interest in the past (1, 5, 6, 8). The species most often associated with dental caries in experimental animals is *Streptococcus mutans* (5, 8), which differs from other oral streptococci in fermentation patterns and several other characteristics. For example, *S. sanguis* and *S. salivarius* strains, which are reported to make up over 50% of the oral streptococcal flora (1, 3), do not ferment mannitol and sorbitol as do *S. mutans* strains (8). *S. mutans* synthesizes extracellular polysaccharides from sucrose. This together with the biologically relatively inert and insoluble glucans induces an increase in adhesion and bacterial aggregation (8, 13), thus contributing to plaque formation on teeth.

The association of *S. mutans* with other streptococci in the oral cavity presents the possibility of transfer of cariogenic potential from *S. mutans* to other streptococci. The transfer of genetic markers via transformation in streptococci has been demonstrated in vivo previously (7). However, this possibility has not been explored with respect to *S. mutans*. It has been shown (11) that *S. sanguis* strain Challis is capable of incorporating biologically active deoxyribonucleic acid (DNA) extracted from a wide variety of streptococcal species. Because of the close association between *S. mutans*, *S. salivarius*, and *S. sanguis* in human dental plaque, it was of interest to determine whether genetic markers such as antibiotic resistance, form of carbohydrate fermented, and the synthesis of specific dextrans and levans could be transferred from *S. mutans* or *S. salivarius* to *S. sanguis* by biologically active DNA in vitro. This study demonstrates genetic transformation of *S. sanguis* strain Challis by donor DNA extracted from various streptomycin-resistant derivative strains of *S. salivarius* and *S. mutans*.

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MATERIALS AND METHODS

Cultures. The bacterial strains used in this study are listed in Table 1, along with the %G+C (guanine plus cytosine) of each strain. Transformable group H *S. sanguis* strain Challis and a streptomycin-resistant derivative of strain Challis were obtained from H. Gooder (University of North Carolina, Chapel Hill). *S. mutans* strains NTCC 10449, KIR, and E49 were obtained from A. L. Coykendall (Naval Medical Research Institute, Bethesda, Md.). *S. mutans* strains BHT and FA-1 and *S. salivarius* strain HHT were obtained from R. J. Fitzgerald (National Institute of Dental Research, Bethesda, Md.). *S. sanguis* ATCC 10556, *S. sanguis* ATCC 10558, *S. salivarius* ATCC 9222, and *S. salivarius* ATCC 13419 were obtained from the American Type Culture Collection, Rockville, Md.

Media. Streptomycin-resistant derivatives of strains used in this study were grown overnight in APT broth (Difco Laboratories, Detroit, Mich.) containing 300 μg of streptomycin sulfate (Sigma Chemical Co., St. Louis, Mo.) per ml. APT agar containing 300 μg of streptomycin per ml was used to score frequency of transformation in all experiments. Mitis-salivarius agar (Difco) was used for

TABLE 1. *Oral streptococci surveyed for biologically active DNA*

Strain	%G+C[a]
S. mutans NTCC 10449	37.5
S. mutans BHT	42.2
S. mutans FA-1	42.2
S. mutans KIR	45.2
S. mutans E49	43.7
S. salivarius HHT	
S. salivarius ATCC 9222	38–40
S. salivarius ATCC 13419	
S. sanguis ATCC 10556	45.5
S. sanguis ATCC 10558	
S. sanguis (Challis)[b]	36.0

[a] Reference 4.
[b] Original designation *S. sanguis* NTCC 7868.

detection of extracellular polysaccharide formation and to determine colonial morphology. In all transformation experiments, brain heart infusion broth (BHI; Difco) containing 10% horse serum (Grand Island Biological Co., Grand Island, N.Y.) was used for growth and development of competence of strain Challis. Phenol red broth (Baltimore Biological Laboratories, Cockeysville, Md.) containing either 10% mannitol or 10% sorbitol was used as a preliminary screen for the transfer of the capacity to utilize these compounds as substrates.

Isolation of streptomycin-resistant derivatives. Spontaneous streptomycin-resistant mutants of all strains listed in Table 1 with the exception of *S. mutans* strain KIR and *S. mutans* strain E49, which proved to be initially resistant to 500 μg of streptomycin per ml, were obtained by spreading 0.2 ml of an 18-h BHI culture onto APT agar containing 500 μg of streptomycin per ml. All Str^R mutants were tested to ensure that other physiological properties corresponded to those of their respective parent strains.

Extraction of DNA. A modification of the method of Hotchkiss (9) was used to extract DNA from streptomycin-resistant mutants and strain Challis. Cultures were grown for 24 h at 37 C in APT broth containing 300 μg of streptomycin per ml, centrifuged, and washed twice with a solution of sodium chloride (0.15 M) and sodium citrate (0.15 M) (SSC solution). The washed pellet was resuspended in 15 ml of SSC solution containing 2.0% sodium dodecyl sulfate, and the cells were ruptured by using an Aminco French pressure cell and Wabash hydraulic press. The extract was treated with chloroform-isoamyl alcohol (24:1) for 30 min at room temperature with vigorous shaking. After centrifugation, the nucleic acids were spooled onto a glass rod from the aqueous layer after precipitation with cold ethanol and suspended in 2.0 M sodium chloride and 0.05 M sodium citrate. To this suspension was added 50 μg of pancreatic ribonuclease (Sigma, type II-A) per ml that had been previously heated at 90 C for 10 min to inactivate any contaminating deoxyribonuclease (DNase). The mixture was incubated for 30 min at 37 C. Nuclease-free Pronase (*Streptomyces griseus* protease, B grade; Calbiochem, LaJolla, Calif.) at a concentration of 50 μg/ml was then added, and the mixture was incubated for a further 30 min at 37 C. The entire DNA precipitation and purification technique was repeated three times. The final DNA concentration was determined spectrophotometrically according to the method of Hotchkiss (10). DNA preparations were checked for sterility by plating a sample onto APT agar.

Intraspecific and interspecific transformation of strain Challis. In all experiments, strain Challis (Str^S) was grown to maximum competence by diluting an overnight BHI broth culture 1:10 with additional BHI broth containing 10% horse serum and incubating at 37 C for 90 min. Individual Str^S strain Challis cultures then received 5 μg of DNA per ml extracted from each of the streptomycin-resistant strains. After a further 15 min of incubation at 37 C, pancreatic DNase (Sigma, type I, 1× crystallized without additional purification) was added at a final concentration of 100 μg/ml to hydrolyze any unbound DNA. Incubation was continued for 90 min to allow for integration and phenotypic expression of the transforming marker. Each sample was plated onto APT agar containing 250 μg of streptomycin per ml and scored for transformants after incubation for 24 h at 37 C. Total viable counts were determined by plating the sample on APT agar without streptomycin. A strain Challis culture not exposed to transforming DNA was used as the control and was plated onto APT agar containing streptomycin.

RESULTS

Development of competence in strain Challis. The time required for development of peak competence was determined in Str^S strain Challis. A strain Challis culture in BHI broth inoculated at a 1:10 dilution into BHI broth containing 10% horse serum was incubated at 37 C. At various time intervals samples of 0.1 ml were transferred to 1.8 ml of BHI plus 10% horse serum containing DNA (5 μg/ml) from Str^R strain Challis. After 15 min, DNase was added and incubation was continued for 75 min. Samples plated onto APT agar containing 250 μg of streptomycin per ml were incubated overnight and scored for transformants. Viable counts were also determined on each sample by plating onto APT agar. Maximum competence occurred 90 min after inoculation into the BHI-horse serum broth (Fig. 1). This time of incubation was used in subsequent experiments involving interspecies donor DNA.

Effect of DNA concentration on frequency of transformation. After growth of strain Challis to peak competence, 0.1-ml samples were added to 1.8 ml of BHI plus 10% horse serum containing various amounts of DNA extracted from Str^R strain Challis. After incubation at

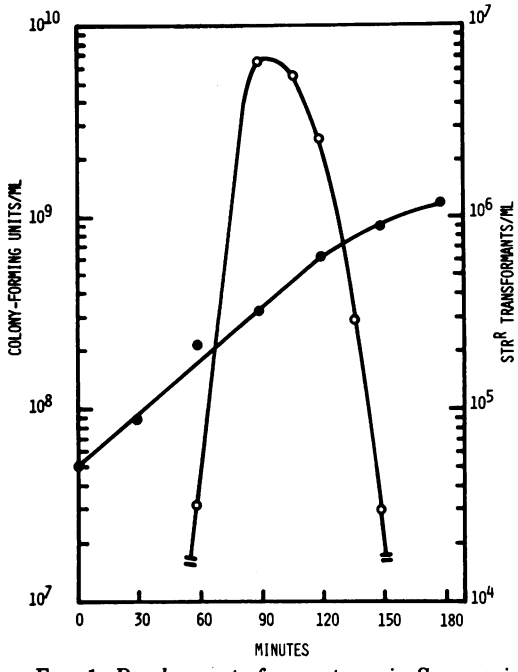

FIG. 1. *Development of competence in S. sanguis strain Challis. Symbols: ○, number of transformants per milliliter; ●, total viable units per milliliter.*

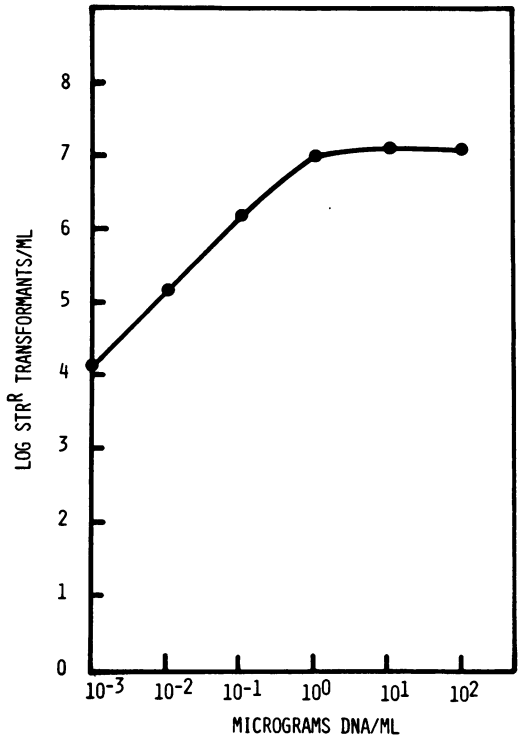

FIG. 2. *Relationship of donor DNA concentration to frequency of transformation of strain Challis.*

37 C for 15 min, DNase was added, and incubation was continued for 75 min. Transformants were scored after incubation of cultures on APT agar containing streptomycin. Results (Fig. 2) indicated that the frequency of transformation approached a peak at approximately 10 μg of DNA per ml. As little as 10^{-3} μg of DNA per ml gave significant transformant numbers, and the frequency increased linearly up to 1 μg of DNA per ml.

Effect of time of exposure to DNA on frequency of transformation. Samples of a culture of Str^S strain Challis were added to BHI plus horse serum containing DNA (5 μg/ml) from Str^R strain Challis, and separate tubes were incubated for various times ranging from 5 to 60 min. After DNase addition and a subsequent 90-min incubation period, samples were plated to streptomycin-APT agar and scored for transformants after incubation for 24 h. Peak frequency of transformation occurred after approximately 15 min of exposure time (Fig. 3). The time required for phenotypic expression after addition of DNase is shown in Fig. 4. As demonstrated, maximal numbers of transformants were achieved after 75 min.

Intraspecific and interspecific transformation of strain Challis. Experiments involving incubation of recipient wild-type strain Challis with donor DNA extracted from Str^R mutants

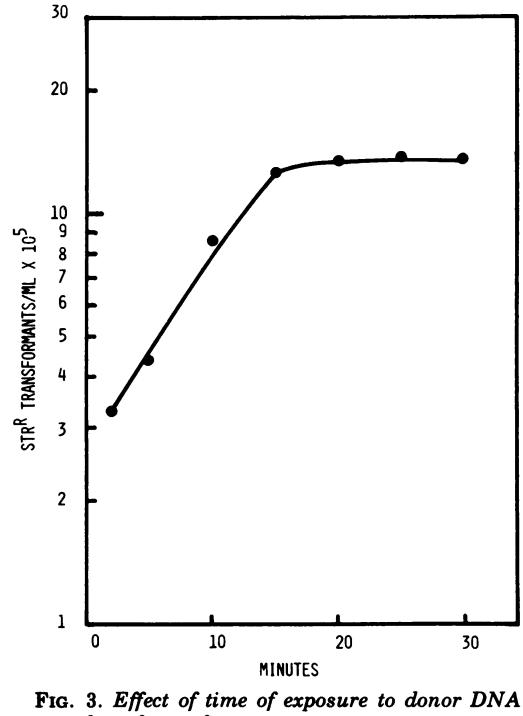

FIG. 3. *Effect of time of exposure to donor DNA on number of transformants.*

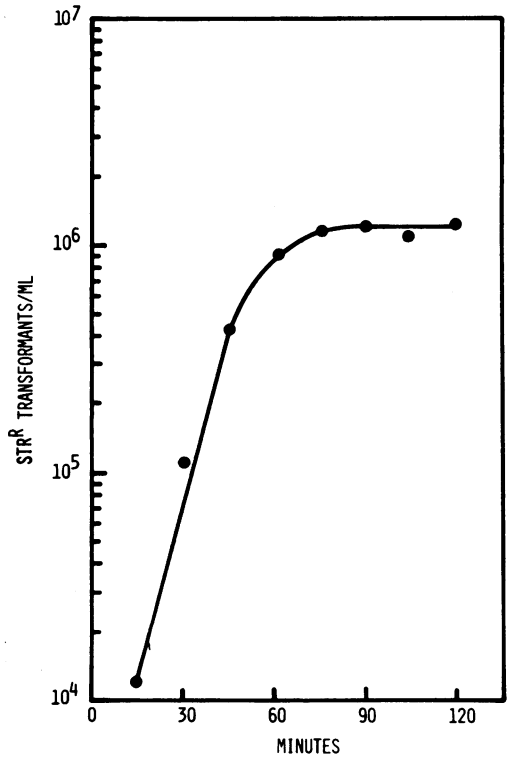

FIG. 4. *Relationship of incubation time required after addition of DNase for phenotypic expression to frequency of transformation.*

of strain Challis and other species of oral streptococci were performed. Transformation of the streptomycin resistance marker occurred between strain Challis (*S. sanguis*) and different species of oral streptococci (Table 2). Greater frequency of transformation occurred between homologous *S. sanguis* strains. However, the frequency of transformation from *S. mutans* and *S. salivarius* to strain Challis is significant in that controls showed no spontaneous streptomycin-resistant clones. *S. salivarius* HHT (0.044%), *S. mutans* KIR (0.01%), and *S. mutans* BHT (0.034%) showed a much higher frequency of transformation of the Str^R marker into strain Challis than other nonhomologous strains tested.

Stability of the streptomycin-resistant marker. After transformation experiments involving donor DNA from each streptomycin-resistant strain listed in Table 1 and Str^S strain Challis as recipient, six to eight transformants were isolated from each cross in pure culture on APT agar containing 250 μg of streptomycin per ml. Each of these transformants was grown through five successive transfers in APT broth not containing streptomycin, and appropriate dilutions were then plated to both APT agar and APT-streptomycin agar. Viable counts on the streptomycin-agar were the same as those on plain agar, indicating that the transformed Str^R marker was stably integrated into the recipient genome.

Reciprocal transformation. Each strain listed in Table 1 was grown under the same conditions favorable for development of maximum competence in strain Challis. Reciprocal transformation studies were made by using DNA extracted from streptomycin-resistant strain Challis and DNA extracted from other homologous streptomycin-resistant strains. *S. sanguis* ATCC 10556 and *S. sanguis* ATCC 10558 were the only strains in which reciprocal transformation with strain Challis could be demonstrated.

Transformation of unselected markers. All *S. mutans* used in this study can ferment mannitol and sorbitol, whereas strain Challis can not. Transformants that had been selected for streptomycin resistance in which the donor DNA was from *S. mutans* strains were tested for the capacity to ferment mannitol and sorbitol. Phenol red broth was supplemented with either 10% mannitol or 10% sorbitol. None of the transformants formed acid within 24 h, sug-

TABLE 2. *Efficiency of strain Challis transformation with DNA extracted from streptomycin-resistant derivatives of oral streptococci*

Source of donor DNA	Str^R transformants/ml		Transformation (%)[a]
	Control[b]	Experimental[c]	
S. mutans NTCC 10449	0	2.0×10^4	0.002
S. mutans BHT	0	3.4×10^5	0.034
S. mutans FA-1	0	4.0×10^2	0.00004
S. mutans KIR	0	1.0×10^5	0.010
S. mutans E49	0	8.0×10^3	0.0008
S. salivarius HHT	0	4.4×10^5	0.044
S. salivarius ATCC 9222	0	8.0×10^3	0.0008
S. salivarius ATCC 13419	0	1.0×10^3	0.0001
S. sanguis ATCC 10556	0	3.2×10^5	0.032
S. sanguis ATCC 10558	0	1.4×10^7	1.40
S. sanguis (Challis)	0	2.4×10^7	2.40

[a] Percentage of transformation = [(number Str^R transformants per milliliter)/(number Challis colony-forming units per milliliter at t_0) × 100.

[b] Control tubes contained 5 μg of donor DNA per ml with DNase added to give 100 μg/ml 10 min prior to addition of competent Challis cells; the mixture was incubated at 37 C for 105 min after addition of cells before plating for transformants.

[c] Experimental tubes contained 5 μg of donor DNA per ml; after adding competent Challis cells, incubation was continued for 15 min at 37 C; DNase was then added to give 100 μg/ml and incubated for an additional 90 min before plating for transformants.

gesting that the genes for mannitol and sorbitol fermentation had not been integrated into the recipient genome.

Transformants were also plated onto mitis-salivarius agar plates containing 40% sucrose, incubated, and examined for the colonial morphology (small, irregular, frosted-glass appearance) typical of *S. mutans*. Only small, compact, flat colonies with raised centers typical of *S. sanguis* were seen, indicating that there was no transfer of markers involved with colonial morphology.

DISCUSSION

Data obtained in this study indicate that *S. sanguis* strain Challis is transformable by biologically active DNA extracted from other oral streptococci, especially other *S. sanguis* strains. The organisms used in this study have similar DNA base ratios with a %G+C range of 36.0 to 45.5. Some degree of homology in the DNA of these organisms would increase the likelihood of interspecies transfer of genetic markers in oral streptococci. It has been reported that *S. sanguis* constitutes approximately 50% of the oral streptococcal flora (1, 3). Evidence from this study emphasizes the possible ecological significance of interspecies transfer of genes among oral streptococci in that a few strains of *S. mutans*, notably strains BHT, KIR, and 10449, and *S. salivarius* strains were capable of transforming the streptomycin resistance marker into strain Challis at relatively high frequencies.

Perry and Slade (11) reported that transformation occurred between most groups of streptococci and group H *S. sanguis* strain Challis. However, the strains used and the frequencies of transformation were not reported. Of relevance to dental microbiology is the possibility of transfer of caries-inducing properties from *S. mutans* to other oral streptococci. This would be feasible assuming that a recipient oral streptococcus could achieve the competent state in the ecological environment of the oral cavity. The possibility of stable integration of genetic markers is demonstrated by the stability of the Str^R marker in the transformants obtained in this study. Although unselected markers for such functions as colonial morphology and sorbitol or mannitol fermentation were not shown to be transformed in this study, further work should be done to determine whether these markers as well as others might also prove to be transferred by a transformation system. Regarding cariogenic potential, most strains of *S. sanguis* do not initiate significant tooth decay when tested in gnotobiotic animals. In plaque associated with caries, the numbers of *S. sanguis* are proportionately lower than those of *S. mutans*. Transfer of caries-inducing markers from *S. mutans* to *S. sanguis* could be epidemiologically significant, since it has been shown that transformation can occur in vivo.

Reciprocal transformation was not demonstrated to occur between strain Challis and *S. mutans* strains. It has been shown that the growth requirements for *S. mutans* vary greatly among strains and are different from those of *S. sanguis* (1-3); therefore the parameters for the development of competence may be quite different from those characteristic for strain Challis. Perry and Slade (12) reported the ability of group H *S. sanguis* strain Wicky to undergo transformation when the filtrate from the competent strain Challis was added. Efforts to induce transformation in other serological groups of streptococci with the use of group H competence factor were unsuccessful. In the present study, no reciprocal transformation was attainable when filtrates from the competent strain Challis were used.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Carlsson, J. 1965. Effect of diet on presence of *Streptococcus salivarius* in dental plaque and saliva. Odontol. Revy 16:336-347.
2. Carlsson, J. 1970. Nutritional requirements of *Streptococcus mutans*. Caries Res. 4:305-320.
3. Carlsson, J. 1972. Nutritional requirements of *Streptococcus sanguis*. Arch. Oral Biol. 17:1327-1332.
4. Coykendall, A. L. 1970. Base composition of deoxyribonucleic acid isolated from cariogenic streptococci. Arch. Oral Biol. 15:365-368.
5. Edwardsson, S. 1968. Characteristics of caries-inducing human streptococci resembling *Streptococcus mutans*. Arch. Oral Biol. 13:637-646.
6. Fitzgerald, R. J., and P. H. Keyes. 1960. Demonstration of the etiologic role of streptococci in experimental caries in the hamster. J. Am. Dent. Assoc. 61:9-19.
7. Griffith, F. 1928. Significance of pneumococcal types. J. Hyg. 27:113-118.
8. Guggenheim, B. 1968. Streptococci of dental plaque. Caries Res. 2:147-163.
9. Hotchkiss, R. D. 1957. Isolation of sodium deoxyribonucleate in biologically active form from bacteria, p. 692-696. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.
10. Hotchkiss, R. D. 1957. Methods for characterization of nucleic acid, p. 708-713. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.

11. Perry, D., and H. D. Slade. 1962. Transformation of streptococci to streptomycin resistance. J. Bacteriol. 83:443–449.
12. Perry, D., and H. D. Slade. 1966. Effect of filtrates from transformable and non-transformable streptococci on the transformation of streptococci. J. Bacteriol. 91: 2216–2222.
13. Wood, J. M., and P. Critchley. 1966. The extracellular polysaccharide produced from sucrose by a cariogenic streptococcus. Arch. Oral Biol. 11:1039–1042.