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Prevalence of Transformable *Streptococcus mutans* in Human Dental Plaque

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A total of 100 strains of *Streptococcus mutans* serotypes c/e/f and d/g, freshly isolated from dental plaque, were screened for their ability to undergo genetic transformation to streptomycin resistance. Of the serotype c/e/f strains, 28% were found to be transformable, whereas none of the serotype d/g strains could be transformed by donor DNA from streptomycin-resistant *S. mutans* strains of either serotype c or d/g. Two of the transformable serotype c/e/f strains were transformed by DNAs from a variety of oral streptococcal species commonly found in the microflora.

We have previously reported on attempts to transfer streptomycin resistance to strains of *Streptococcus sanguis*, *Streptococcus mutans*, and *Streptococcus salivarius* freshly isolated from human dental plaque by genetic transformation (10). Of the clinical isolates tested, only strains of *S. sanguis* were found to be transformable. Recently, however, Perry and Kuramitsu (8) reported on the successful transformation of three laboratory strains of *S. mutans*. The experimental conditions in their study were somewhat different from ours. We therefore reconsidered the possibility that transformable strains could, in fact, be found among fresh clinical isolates of *S. mutans*.

In the present study, 100 clinical isolates of *S. mutans* were screened for their ability to undergo transformation to streptomycin resistance. The strains were obtained from the teeth of 70 subjects, using cotton applicators. The subjects had not received any antibiotics in the past 6 months. The swab samples were serially diluted in 0.05 M potassium phosphate buffer (pH 7.1) and plated on mitis salivarius bacitracin agar supplemented with 15% sucrose (4). The agar plates were incubated in 5% CO_2 in N_2 at 37°C for 48 h. Characteristic colonies of *S. mutans* serotypes c/e/f and d/g were isolated and transferred to 9 ml of brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) and incubated overnight. The isolates were identified by biochemical (9) and serological (1) tests. Of the 100 tested strains, 71 were serotype c/e/f and 29 were serotype d/g. The isolates were also streaked on mitis salivarius agar containing 200 μg of streptomycin per ml for checking their inherent resistance to the drug. All strains were sensitive to 200 μg of streptomycin per ml.

As DNA donors in the screening experiments, variants resistant to 200 μg of streptomycin per ml, *S. mutans* Ingbritt of serotype c (5) and *S. mutans* B13 of serotype d (3), were used. DNA was isolated from bacterial cells grown in BHI broth containing 20 mM DL-threonine (2) and 20 mM D-glucose. The cells were treated with 2 mg of lysozyme (Sigma Chemical Co., St. Louis, Mo.) and 1 mg of protease (Pronase; Merck, Darmstadt, West Germany) per ml and finally lysed by the addition of sodium lauryl sulfate (1%). The DNA was purified by extraction with both phenol and chloroform-isoamyl alcohol (24:1) and precipitated with ice-cold 99.8% ethanol at −20°C overnight. From overnight cultures of the freshly isolated *S. mutans* strains, 10 ml of BHI broth supplemented with 5% heat-inactivated (60°C for 30 min) horse serum was inoculated to contain approximately 6×10^8 CFU per ml. The experimental procedure was then essentially the same as that described by Perry and Kuramitsu (8). After 2, 3, 4, 5, and 6 h of incubation at 37°C, 2-ml portions were removed and exposed to an excess amount of DNA (5 to 10 μg/ml) and incubated for an additional 2 h for phenotypic expression. Approximately 12 strains were screened at a time and 25-μl samples were plated directly on mitis salivarius agar containing 200 μg of streptomycin per ml by means of a micropipette and incubated in 95% N_2 and 5% CO_2 at 37°C for 48 h.

In this initial screening experiment, no attempts were made to estimate the exact transformation rates or spontaneous mutation rates. However, since incubation of the recipient strains without DNA in no case revealed more than two colonies in one 25-μl spot on the streptomycin-containing mitis salivarius agar plates, i.e., ≤80 spontaneous mutants per ml, only strains which produced ≥200 transformants

TABLE 1. Transformation of clinical isolates of *S. mutans* serotype c/e/f and d/g to streptomycin resistance

No. of tested recipient strains	DNA donor	Transformable strains[a]	
		No.	%
71 (serotype c/e/f)	Ingbritt (Str^r)	20[b]	28
29 (serotype d/g)	Ingbritt (Str^r)	0	0
29 (serotype d/g)	B13 (Str^r)	0	0

[a] Strains which produced 200 or more transformants per ml. None of the controls, where DNA had not been added, revealed more than 80 streptomycin-resistant mutants per ml.

[b] Median transformants per ml, 960; range of number of transformants per ml, 200 to 17,720.

per ml were considered transformable. All strains were tested with DNA from *S. mutans* Ingbritt (Str^r). Twenty of the serotype c/e/f strains (28%) were found to be transformable, whereas none of the serotype d/g strains could be transformed to streptomycin resistance (Table 1). The 29 serotype d/g strains were also tested with DNA from *S. mutans* B13 (Str^r) of the same serotype. Neither of these transformation crosses revealed transformants.

Two of the transformable serotype c/e/f strains, LP and CA, were selected and used as recipients in additional experiments. Transformation frequency and time of optimal competence in strains LP and CA were determined (Fig. 1). Similarly, as in the screening experiment, serum-supplemented BHI broth was inoculated with approximately 6×10^8 cells and incubated at 37°C. However, after the addition of DNA at different times and incubation for 30 min, 100 μg of DNase was added per ml and incubation was continued for 2 h. The peaks of optimal competence in strains LP and CA occurred after 3 to 4 h and 2 h, respectively, when the inoculum culture had been allowed to grow for 11 h. This is important to stress since the times required for development of maximum competence in these two *S. mutans* strains were strongly related to the age of the inoculum cultures, something we have not experienced in *S. sanguis*.

Since in our earlier study DNA had been present during the whole competence development according to the method of Lawson and Gooder (6), we thought it possible that endonucleases could have caused degradation of the DNA, resulting in a loss of its transforming ability. To test this hypothesis, an additional portion of cells was exposed to DNA at the start of the screening experiments. The transformation frequencies in these tests were, however, either equal to the frequency at the respective peak of competence or higher. A more likely explanation for the earlier negative transformation results in *S. mutans* might, therefore, be that the total incubation time of the recipient cells with DNA had not been sufficient to allow phenotypic expression of the newly integrated marker.

Strains LP and CA were exposed to DNAs from streptomycin-resistant strains of a variety of oral streptococcal species commonly found in the microflora. The method of Lawson and Gooder (6) was used, where DNA (5 to 10 μg/ml) was added to the recipient cells at the start of the experiment. Representative results of these transformation crosses are shown in Table 2. Strains LP and CA were transformed by each of the DNA preparations. For both recipient strains, the highest level of transformation was obtained with DNA from *S. mutans* Ingbritt (Str^r) of serotype c. The frequency was more than 100-fold higher than with any of the other DNAs. Transformation with DNA from *S. mutans* B13 (Str^r) of serotype d was less efficient than with DNA extracted from the streptomycin-resistant strains of *S. sanguis* and *S. salivarius*. Controls, where DNA was not added to strains LP and CA, were always run simultaneously.

For comparison, the transformable *S. sanguis* Challis-6 (provided by J. Ranhand, National Institutes of Health, Bethesda, Md.) was tested with the same DNA preparations. The homologous transformation was the most efficient. The rate of transformation with most of the other DNAs was 100-fold higher than for any of the *S.*

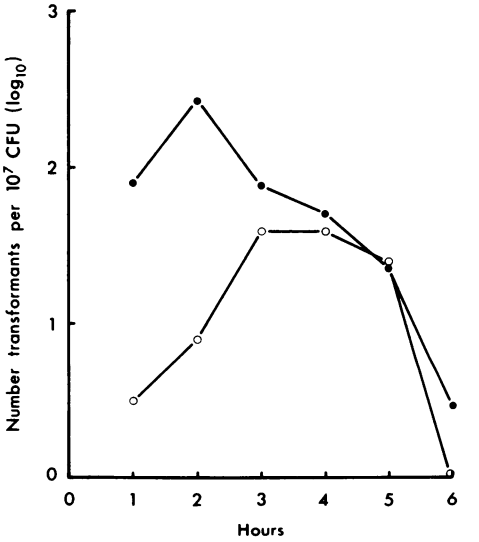

FIG. 1. Competence development in two clinical isolates of *S. mutans* serotype c/e/f: strain LP (○) and strain CA (●). Donor of DNA was streptomycin-resistant *S. mutans* Ingbritt (serotype c).

TABLE 2. Transformation of two *S. mutans* strains and *S. sanguis* Challis-6 to streptomycin resistance by DNA from various streptococcal donor strains

DNA donor	Transformants per viable cell with recipient strain:		
	S. mutans LP	*S. mutans* CA	*S. sanguis* Challis-6
S. mutans Ingbritt (Str^r)	1.2×10^{-4}	3.8×10^{-4}	1.6×10^{-4}
S. mutans B13 (Str^r)	0.04×10^{-6}	0.78×10^{-6}	2.5×10^{-4}
S. sanguis Challis-6 (Str^r)	0.44×10^{-6}	1.50×10^{-6}	250×10^{-4}
S. miteor ATCC 9811 (Str^r)	0.05×10^{-6}	0.68×10^{-6}	36×10^{-4}
S. salivarius MSS2 (Str^r)	0.24×10^{-6}	2.0×10^{-6}	1.8×10^{-4}
S. milleri NCTC 10708 (Str^r)	0.02×10^{-6}	0.67×10^{-6}	0.76×10^{-4}
Control[a]	$<6.8 \times 10^{-8}$	$<5.6 \times 10^{-8}$	$<5.8 \times 10^{-8}$

[a] No DNA added.

mutans recipient strains, except when DNA from *S. mutans* Ingbritt (Str^r) was employed. In this case, strains LP, CA, and Challis-6 were transformed to the same degree. In view of the above mentioned low transformation efficiency in strains LP and CA with the strain B13 (Str^r) DNA, it should be noted that the same DNA preparation was as efficient as the Ingbritt (Str^r) DNA in strain Challis-6 (Table 2). Thus, the low transformation rate in strains LP and CA with DNA from strain B13 (Str^r) was not due to low biological activity of the DNA but is suggestive of nonhomology between strains Ingbritt and B13.

In vivo transformation between pneumococci and streptococci has been shown to occur in mice, and transformants were isolated from the heart blood of the animals (7). Although as yet not demonstrated, the possibility of in vivo transformation in dental plaque is a most intriguing question. Our study has shown that transformable strains of *S. mutans* serotype c/e/f frequently occur in the human mouth and that the strains can be transformed with DNAs from a variety of oral streptococcal species. Thus, a significant fraction of these organisms have the potential to acquire new genetic information in nature.

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