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Mutacin Production by *Streptococcus mutans* May Promote Transmission of Bacteria from Mother to Child

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The production of bacteriocin-like inhibitory substances, mutacins, by mutans streptococci varies among isolates. To find if the degree of mutacin activity of an isolate was related to its transmission between mother and her child, 19 mothers and their 18-month- to 3-year-old children were sampled for their oral mutans streptococci. In addition, the stability of mutacin activity was studied with isolates from the mothers and with isolates from five unrelated 5-year-old children in 5- to 7-year follow-up studies. A total of 145 oral mutans streptococcal isolates were serotyped by immunodiffusion, ribotyped, and mutacin typed by the stab culture technique. Mutacin was produced by 88% of the strains against more than 1 of the 14 indicator strains, representing mutans streptococci, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus oralis*, *Streptococcus gordonii*, and *Streptococcus pyogenes*. *Streptococcus mutans* isolates showed more inhibitory activity than did *Streptococcus sobrinus* isolates. Identical ribotypes had similar mutacin activity profiles within a subject, initially and in the follow-up studies, in all but two cases. The mothers harbored a total of 37 different mutans streptococcal ribotypes. Six children were negative for mutans streptococci. Transmission was probable in 9 of 20 mother-child pairs on the basis of the presence of identical strains, as determined by ribotyping and bacteriocin (mutacin) typing. *S. mutans* strains shared between a mother and her child showed a broader spectrum of inhibitory activity than did nontransmitted strains. In conclusion, the mutacin activity of clinical isolates is reasonably stable, and this virulence factor seems to be of clinical importance in early colonization by *S. mutans*.

Bacteriocins are by definition proteinaceous antibacterial substances that some bacteria produce to interfere with the growth of other, generally closely related bacteria. Bacteriocins are produced in addition to other inhibitory substances, including bacteriolytic enzymes and metabolic by-products such as organic acids, diacetyl, and hydrogen peroxide, which are formed during bacterial growth (23). Also nongrowing cells can produce bacteriocins (36). Bacteriocins produced by mutans streptococci, the facultatively anaerobic gram-positive cocci that are implicated as the principal initiator microorganisms in dental caries (20, 27), are designated mutacins (18). Mutacin typing, including both susceptibility to and production of mutacin, has been used for epidemiological typing of isolates, and maternal transmission of the microorganism to the child was first suggested by Berkowitz and Jordan (6) on the basis of a mutacin typing method. Clinically, mutacins have been considered important for the establishment and equilibrium of bacteria in dental plaque: the mutacin-producing strains might colonize more easily and suppress nonproducing strains (21, 38). Most strains of mutans streptococci produce mutacins on agar, but very few produce mutacins in liquid culture (11, 19, 31, 40). It has been shown that mutacin production usually is not plasmid encoded (9). Several mutacins have been purified and biochemically characterized (10, 12, 15, 22, 28–30).

The aim of this study was to determine the mutacin activity of genotypically characterized mutans streptococcal isolates from young children and their mothers in order to examine the possible role of mutacins in transmission. To demonstrate transmission between a mother and her young child, the clonal identity of mutans streptococcal isolates was determined by ribotyping. The ribotyping results were complemented by the results of phenotypic analysis by mutacin typing. In addition, the stability of mutacin production by mutans streptococcal strains, fingerprinted by ribotyping, was studied in follow-up samples obtained from mothers and from five children who were 5 years old at the start of the experiment.

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MATERIALS AND METHODS

Subjects. The study population consisted of 19 mothers aged between 18 and 34 years and their 18-month- to 3-year-old children (one twin pair). Thirteen of the children were caries free, and seven had nursing caries, diagnosed on the basis of their history and typical clinical features, including rampant caries of the maxillary incisors (33). For the children with nursing caries, the range of the number of decayed, missing, and/or filled tooth surfaces was 17 to 63.

Bacterial isolates. For the 18-month- to 3-year-old children, mutans streptococci were isolated from pooled plaque samples. For the mothers, isolates were obtained from salivary samples on one to four occasions within 4 years (14). The samples were cultured on MSB agar (13).

Colonies of mutans streptococci were examined under a dissecting microscope and identified by their distinctive colony morphology. At each sampling time, at least four *Streptococcus mutans* isolates and four *Streptococcus sobrinus* isolates, if available, were picked and tested for the ability to ferment mannitol and sorbitol. When necessary, the fermentation profile of the isolate was determined by the API 20 Strep system (Bio Mérieux, Marcy-l'Étoile, France). A total of 115 isolates from the 18-month- to 3-year-old children and 180 isolates from the mothers (295 isolates altogether, or an average of 9 isolates per culture-positive individual) were stored at −70°C until tested.

For mutacin activity testing, the following 14 stock culture strains were used as indicator strains: *S. mutans* MT 8148 (serotype *c*), *S. mutans* LM7 (*e*), *S. mutans* OMZ175 (*f*), *S. sobrinus* 6715 (*g*), *S. sobrinus* B13 (*d*), *Streptococcus rattus* FA-1 (*b*), *Streptococcus sanguis* ATCC 10556, ST3, ST202, and B220, *Streptococcus oralis* ATCC 10557, *Streptococcus gordonii* ATCC 10558, *Streptococcus salivarius* HHT, and *Streptococcus pyogenes* SV. The stock culture strains were kindly provided by S. Hamada (Osaka University).

One clinical strain exhibiting very distinct antibacterial activity was used as a control in the stab culture assay on each plate.

The control strain and the indicator strains were revived from the frozen stocks

about every 3 weeks. The strains were subcultured on brucella base blood agar and stored at 4°C between experiments.

To augment the number of samples studied for the stability of mutacin production and to aid in the estimation of likelihood of ribotype matching between family members, 38 *S. mutans* isolates from five epidemiologically unrelated children, 5 years old at the start of the experiment, were included in the study. The children had attended a longitudinal study on dental health (2). The isolates had been serotyped and ribotyped as part of a study on the stability of oral *S. mutans* infection in children (1). The isolates had been obtained in 5- to 7-year follow-up studies, and isolates within a subject exhibited the identical ribotype: the follow-up isolates were of the same ribotype as the baseline isolates for all five children. Isolates obtained from four children were of serotype *c* (total of 31 isolates), and isolates from one child (7 isolates) were of serotype *f*; and they represented a total of five different ribotypes. The isolates were stored at −70°C until tested.

Serotyping. All isolates from the mothers and their children (a total of 295 isolates) were serotyped by the immunodiffusion technique (17). Antigen extracts were prepared by autoclaving the bacterial cells (32). Antisera against representative strains of *S. mutans* and *S. sobrinus* were prepared in rabbits by using lyophilized whole-cell antigens. The stock culture strains used were MT8148 (serotype *c*), B13 (serotype *d*), MT703R (serotype *e*), OMZ175 (serotype *f*), and 6715 (serotype *g*). Antisera against serotypes *d* and *g* showed cross-reactions, and serotype-specific antisera were prepared by absorption with cross-reactive strains (16).

Ribotyping. Representative isolates of each serotype from an individual were ribotyped. A total of 81 isolates from the 18-month- to 3-year-old children and 110 isolates from the mothers (a total of 191 isolates) were ribotyped as described previously (35). Briefly, chromosomal DNA of isolates was isolated by the method of Ushiro et al. (37). Chromosomal DNA of isolates (2 to 3 μg) was digested to completion with the restriction endonuclease *Hin*dIII (Promega, Madison, Wis.) as specified by the manufacturer. In two cases, where two nonrelated mothers were found to harbor identical ribotypes by analysis with *Hin*dIII, the isolates were also ribotyped with *Eco*RI. The restriction fragments were separated by electrophoresis through 0.9% (wt/vol) agarose gels, and lambda DNA (marker III; Boehringer GmbH, Mannheim, Germany) was used as the molecular size marker for gel electrophoresis. DNA fragments were transferred to a positively charged nylon membrane (Boehringer GmbH) and fixed by UV irradiation at 302 nm for 4 min. Fragments were hybridized to recombinant plasmid pKK3535 (7), which contains the *rrnB* rRNA operon of the *Escherichia coli* chromosome. Hybridization, labelling, and detection were performed with the nonradioactive DIG DNA-labeling and detection kit (Boehringer GmbH).

Ribotypes were considered identical when they exhibited the same numbers and sizes of hybridizing fragments. Strains with closely resembling hybridization patterns were always compared after the digests were run in the same gel.

Isolates included in the evaluation of mutacin production. Isolates from the mothers and their children were mutacin typed by evaluation of mutacin production. The isolates were selected as follows. From each mother-child pair, every distinct ribotype was represented by two isolates. When a ribotype was shared by a mother and her child, four isolates, if available, exhibiting the same ribotype were selected, two isolated from the mother and two isolated from the child.

The 38 isolates representing five different ribotypes from the 5-year-old children were also included in evaluation of mutacin production.

Mutacin production testing. Mutacin production by clinical isolates was tested by a modification of the deferred-antagonism method of Fredericq, the stab culture method (18). The strains were grown from frozen stocks on mitis salivarius agar. About 20 colonies were inoculated in 4 ml of brain heart infusion broth and grown overnight in candle jars. The strains were inoculated into Trypticase soy agar (TSA) (2% agar; BBL Microbiology Systems) with a 0.6-mm-thick needle. The clinical mutans streptococcal control strain was included into each TSA plate. After a 48-h incubation at 37°C in candle jars, the plates were overlaid with 4 ml of soft TSA (0.8%) containing 0.5 ml (about 10^7 CFU) of an overnight Trypticase soy broth (TSB) culture of the indicator strain. After overnight incubation at 37°C, the diameter of the inhibition zone was measured. The isolate was recorded as mutacin active against the indicator strain if the diameter was 4 mm or greater. The breakpoint of 4 mm was based on earlier studies (4, 19).

The isolates were tested in duplicate for mutacin activity at least twice. The mean size of the inhibition zone was used in the statistical analyses. A difference in the level of mutacin production between two isolates against indicator strains was interpreted when the mean size of the inhibition zone diameter differed by more than 4 mm. Mutacin production profiles were considered different when the results between isolates differed for more than one indicator strain.

The surface pH of the TSA inside the inhibition zone was measured with a Ross combination flat-surface electrode (model 8135; Orion, Boston, Mass.) before the surface was covered with the soft indicator agar. The surface pH of the agar after a 48-h culture in a candle jar did not fall below 6.0 around any of the stab-inoculated strains.

Statistics. The Mann-Whitney U test was used to analyze differences between the sizes of inhibition zone produced against single indicator strains by isolates derived from different subject groups. A strain was considered transmitted if representative isolates from a mother and her child had similar ribotype profiles and were identical in mutacin typing. When the numbers of indicator strains inhibited by transmitted strains and nontransmitted strains were compared, the equality of medians was tested by Fisher's exact test. The total inhibitory activity of isolates among subject groups was compared by the Wilcoxon signed-rank test for mean inhibition zone sizes. $P < 0.05$ was considered statistically significant. The odds ratio was used to estimate the likelihood of ribotype matching between family members.

RESULTS

Detection of mutans streptococci and serotype distribution. Among the mothers and their children, all subjects except six caries-free children harbored mutans streptococci. Of the 33 mutans streptococcus-positive subjects, all harbored *S. mutans* isolates and 8 also harbored *S. sobrinus* isolates. A total of 29 subjects (16 mothers and 13 children) had serotype *c* isolates, 7 (5 mothers and 2 children) had serotype *e* isolates, 8 (5 mothers and 3 children) had serotype *g* isolates, and 1 (a mother) had serotype *f* isolates. A total of 12 mothers and 10 children (67% of the subjects) harbored one serotype only, whereas 6 mothers and 4 children (30%) harbored two serotypes and 1 mother (3%) harbored three serotypes.

Ribotype distribution and comparison of ribotypes between separate subjects. From the 18-month- to 3-year-old children, 20 mutans streptococcal ribotypes were detected, and from the mothers, 37 ribotypes were detected. A total of 14 subjects harbored one ribotype, 12 subjects harbored two ribotypes, 6 subjects harbored three ribotypes, and 1 subject harbored four ribotypes.

Identical ribotypes were detected in 9 of 14 mutans streptococcus culture-positive mother-child pairs. One mother shared two ribotypes with her child, and eight mothers shared one ribotype with their children. Isolates were unique for nonrelated individuals except in two cases, where isolates from two mothers could not be distinguished by ribotyping with *Hin*dIII or by ribotyping with *Eco*RI (Table 1).

The isolates from the study represented in total 52 different ribotypes of mutans streptococci: mothers and their children harbored 47 different ribotypes of mutans streptococci, and the isolates from the initially 5-year-old children represented 5 different ribotypes.

Mutacin production. On the basis of ribotyping results, 145 isolates representing the 52 ribotypes were included in testing for bacteriocinlike inhibitory activity; 87% of the ribotypes produced mutacin against more than one of the indicator strains. The inhibition zone sizes for producer strains varied from 4 to 26 mm in diameter. On average, the isolates produced mutacins against 7 of the 14 indicator strains. Four isolates, representing two ribotypes of *S. mutans* serotype *e*, were active against all the indicator strains. *S. sobrinus* serotype *g* strains produced mutacins against only one or no indicator strain. On average, serotype *c* isolates produced mutacins against 8 indicator strains (range, 1 to 13), serotype *e* produced mutacins against 10 (range, 3 to 14), and serotype *f* isolates produced mutacins against 6 (range, 3 to 6).

Strains of identical ribotype within a subject, isolated on the same occasion, were used for evaluation of mutacin activity within ribotypes. The data included 43 cases of two or more isolates of identical ribotype within a subject, obtained from the same sample. In 40 of these comparable cases, the sizes of the inhibition zones were identical, i.e., differed by less than 4 mm. With two isolate pairs, the sizes of the inhibition zones differed for one indicator strain. This difference for 1 indicator out of 14 was considered within the accuracy of the method. One isolate pair (ribotype R17) differed in mutacin activity profile for four indicator strains. Thus, the mutacin activity of mutans streptococcal isolates, defined by the stab culture

TABLE 1. Strains used in this study[a]

Subject	No of different ribotypes[b]	Ribotype designation[c]
18-mo to 3-yr-old children		
I	1 (10)	R1
II[d]	1 (6)	R1
III	3 (9)	**R2**, R3, R4
IV	1 (5)	R5
V	2 (5)	**R6**, **R7**
VI	2 (12)	R8, **R9**
VII	2 (6)	R10, **R11**
VIII	2 (3)	**R12**, R13
IX	2 (7)	**R14**, R15
X	1 (4)	**R16**
XI	1 (6)	**R17**
XII	1 (3)	R18
XIII	1 (4)	R19
XIV	1 (1)	**R20**
Mothers of mutans streptococcus-positive children		
XV (m[e]I, mII)	2 (6)	R21, R22
XVI (mIII)	1 (8)	**R2**
XVII (mIV)	3 (9)	R23, R24, R25
XVIII (mV)	3 (5)	**R6**, **R7**, R26
XIX (mVI)	2 (6)	R27, **R9**
XX (mVII)	2 (5)	**R11**, R28
XXI (mVIII)	1 (4)	**R12**
XXII (mIX)	4 (5)	**R14**, R29, R30, R31
XXIII (mX)	3 (13)	**R16**, R32, R33
XXIV (mXI)	1 (4)	**R17**
XXV (mXII)	1 (4)	R34
XXVI (mXIII)	2 (4)	R35, R36
XXVII (mXIV)	1 (4)	**R20**
Mothers of mutans streptococcus-negative children		
XXVIII	2 (4)	R37, R38
XXIX	3 (10)	R39, R29′, R40
XXX	3 (6)	R41, R42, R43
XXXI	2 (6)	R32′, R44
XXXII	2 (5)	R45, R46
XXXIII	1 (2)	R47
5-yr-old children in follow-up		
XXXIV	1 (13)	R48
XXXV	1 (6)	R49
XXXVI	1 (7)	R50
XXXVII	1 (6)	R51
XXXVIII	1 (6)	R52

[a] Mutans streptococcus ribotypes of 14 18-month- to 3-year-old children and their mothers, of 6 mothers whose young children did not harbor mutans streptococci, and of 5 5-year-old children in a follow-up study initially and 5 to 7 years later.

[b] The total number of isolates ribotyped is given in parentheses.

[c] Ribotypes shared by mother and her child are printed in boldface type. Isolates were unique for epidemiologically unrelated persons except in two cases, where two mothers shared one ribotype, designated R29/R29′ and R32/R32′.

[d] Twin brother of subject I.

[e] m, mother of.

method, was ribotype specific in 42 of 43 cases. Mothers and children shared 10 ribotypes, and the isolates representing these ribotypes exhibited a similar mutacin activity profile. Also, all four isolates of ribotype R1, isolated from subjects I and II (Table 1), who were twin brothers, showed an identical mutacin activity profile.

Strains of the same serotype within a subject, isolated on the same occasion, showed heterogeneity of mutacin activity in eight subjects (subjects III, XXIII, XXIV, XXVIII, XXIX, XXX, XXXI, and XXXII [Tables 1 and 2]). In total, 12 pairs of isolates of the same serotype from a single subject, exhibiting variability in mutacin activity profile, could be compared. In seven subjects, or 11 isolate pairs, these isolates had different ribotypes. On the other hand, in four subjects, concomitantly isolated strains exhibiting different ribotypes could not be differentiated by mutacin typing.

In two cases, two mutans streptococcal isolates from nonrelated mothers had similar ribotype profiles (Table 2). The mutacin activity profiles of the isolates were not identical. For the analysis of the clinical implications of the mutacin activity of mutans streptococci, strains R29, R29′, R32, and R32′ were considered to be different.

Strain matching between mothers and children, and probability of transmission. Ribotyping and mutacin typing was used to study the transmission of mutans streptococci between mothers and their children. A ribotype match for mothers and children was found for 10 of 36 ribotypes detected. When comparing ribotypes of all 19 adult women and the 5 initially 5-year-old children who were included without their mothers (a total of 42 ribotypes), identical ribotypes were detected in two cases, where two mothers had the same ribotype. The odds ratio of the occurrence of shared identical strains, determined by ribotyping with one enzyme, in mothers and children living in the same household (10 of 36) compared to that in unrelated subjects not living in the same household (2 of 42), was 7.69. When the results of ribotyping were complemented with the results of mutacin typing, no case of identical strains detected in epidemiologically unrelated persons was revealed. In conclusion, the used typing methods could reveal transmission of mutans streptococcal strains with good fidelity. The presence of identical ribotypes in 9 of 14 mother-child pairs indicated maternal transmission of mutans streptococci in 64% of mutans streptococcus culture-positive children.

Mutacin activity in relation to transmission of isolates from mother to child. *S. mutans* isolates harbored by the mothers represented 35 distinct types, determined by ribotyping and mutacin typing (Table 2). Eight of these had been transmitted to the child. The transmitted strains inhibited a mean of 10.6 indicator strains (standard deviation [SD], 1.9; median, 10; range, 9 to 14) and nontransmitted types inhibited a mean of 7.0 indicator strains (SD, 3.4; median, 6; range, 1 to 14); the difference was statistically significant (equality of medians tested by Fisher's exact test, $P < 0.002$). When the sizes of inhibition zone against single indicators were compared, for all indicator strains the zones were bigger for transmitted strains than for nontransmitted strains; the difference was statistically significant for 8 of the 14 indicator strains: MT 8148 (Mann-Whitney U test, $P < 0.01$), OMZ175 ($P < 0.01$), FA-1 ($P < 0.01$), ST202 ($P < 0.02$), B220 ($P < 0.03$), ATCC 10558 ($P < 0.02$), HHT ($P < 0.02$), and SV ($P < 0.03$). The 8 *S. mutans* types that had been transmitted, compared to the 27 types that had been available from the mothers but had not been transmitted, showed significantly more mutacin activity (the mean zone sizes against the indicator strains were compared by the Wilcoxon signed-rank test, $P < 0.003$).

Five ribotypes representing *S. sobrinus* isolates harbored by the mothers inhibited only one or none of the indicator strains. The *S. sobrinus* mutacin production activity could not be related to transmission.

Mutacin activity of clinical isolates in follow-up. The stability of mutacin production was studied by comparing the spectrum of mutacin activity of baseline and follow-up isolates of identical ribotypes within a subject. The isolates were obtained at 1- to 7-year intervals (mean, 3.1; SD, 2.3) from 10 mothers and 5 initially 5-year-old children, a total of 19 comparable isolate pairs. The mutacin activity of the isolates initially and in the follow-up study was similar in 14 subjects. The isolates

TABLE 2. Inhibition zone sizes of maternally derived *S. mutans* isolates (35 distinct types) against 14 indicator strains

Subject	Serotype/ ribotype[a]	Inhibition zone size (mm) against indicator strain[b]:													
		S. mutans MT8148 (c)	*S. mutans* OMZ175 (f)	*S. mutans* LM7 (e)	*S. sobrinus* 6715 (g)	*S. sobrinus* B13 (d)	*S. rattus* FA-1 (b)	*S. sanguis* ATCC 10556	*S. sanguis* ST3	*S. sanguis* ST202	*S. sanguis* B220	*S. oralis* ATCC 10557	*S. gordonii* ATCC 10558	*S. salivarius* HHT	*S. pyogenes* SV
XV	*c*/R21	3	3	3	1	3	4	6	6	6	5	5	4	5	6
XV	*c*/R22	3	2	3	1	2	3	5	7	8	6	8	5	4	4
XVI	*c*/**R2**	**4**	**2**	**5**	**1**	**3**	**4**	**5**	**6**	**7**	**4**	**5**	**5**	**4**	**6**
XVII	*e*/R23	3	3	3	3	3	2	5	5	5	4	5	3	2	3
XVII	*e*/R24	2	3	3	2	3	2	5	5	5	3	5	3	2	3
XVIII	*c*/**R6**	**3**	**3**	**3**	**1**	**0**	**5**	**6**	**6**	**9**	**5**	**7**	**5**	**5**	**8**
XVIII	*e*/**R7**	**12**	**5**	**4**	**7**	**8**	**11**	**15**	**18**	**19**	**17**	**18**	**14**	**11**	**22**
XVIII	*e*/R26	12	6	4	7	8	11	14	19	19	18	18	14	11	22
XIX	*c*/R27	1	2	2	0	3	1	4	3	5	3	4	2	1	4
XX	*c*/R28	0	2	2	1	1	2	5	7	6	3	4	2	1	3
XXI	*c*/**R12**	**3**	**3**	**3**	**0**	**1**	**5**	**5**	**5**	**7**	**6**	**5**	**5**	**4**	**9**
XXII	*c*/**R14**	**4**	**3**	**5**	**10**	**4**	**12**	**24**	**17**	**19**	**19**	**14**	**16**	**13**	**16**
XXII	*c*/R29	4	3	5	8	4	12	23	17	19	18	11	16	10	15
XXII	*e*/R30	3	3	6	6	3	10	19	10	15	13	11	11	10	13
XXIII	*c*/**R16**	**4**	**2**	**3**	**2**	**2**	**4**	**8**	**8**	**8**	**6**	**5**	**9**	**8**	**16**
XXIII	*c*/R32	4	2	3	2	1	4	8	8	7	6	5	9	8	16
XXIII	*c*/R33	2	1	1	3	0	2	2	3	3	3	3	2	2	4
XXIV[b]	*c*/R17	2	3	3	0	0	3	4	4	6	3	4	3	3	6
XXIV[b]	*c*/**R17**	**3**	**3**	**3**	**0**	**1**	**4**	**5**	**4**	**6**	**4**	**4**	**4**	**4**	**7**
XXV	*e*/R34	3	4	2	2	2	2	5	5	5	4	5	3	1	5
XXVI	*e*/R35	4	3	4	7	4	10	18	9	15	14	11	11	9	11
XXVII	*c*/**R20**	**3**	**2**	**3**	**5**	**1**	**7**	**15**	**9**	**10**	**11**	**12**	**9**	**6**	**9**
XXVIII	*c*/R37	2	2	2	1	0	2	4	4	7	4	5	3	3	4
XXVIII	*c*/R38	3	2	2	1	2	4	4	5	7	4	4	4	4	5
XXIX	*c*/R29′	3	3	3	3	2	6	13	9	10	10	7	8	6	9
XXIX	*c*/R39	3	3	2	1	1	3	11	9	7	5	5	8	7	16
XXIX	*f*/R40	2	3	2	2	1	2	3	4	3	3	4	2	2	5
XXX	*c*/R41	3	2	2	0	0	5	5	6	6	5	6	6	4	11
XXX	*c*/R42	1	2	2	0	1	1	5	6	5	3	5	3	2	8
XXX	*c*/R43	1	2	2	0	0	1	3	3	5	3	5	3	2	7
XXXI	*c*/R32′	1	3	3	0	1	4	5	5	6	5	5	4	4	8
XXXI	*c*/R44	2	2	2	0	1	1	6	6	5	4	5	3	1	4
XXXII	*c*/R45	2	3	2	0	1	2	17	20	6	3	5	3	0	4
XXXII	*c*/R46	2	3	2	0	0	1	4	3	6	3	5	2	1	4
XXXIII	*c*/R47	1	2	2	0	0	1	4	4	5	3	5	2	2	3

[a] For ribotyping, bacterial chromosomal DNA was digested with *Hin*dIII. Ribotypes shared by the mother and her child are given in boldface type. In two cases two mothers shared the same ribotype of mutans streptococci; the ribotype profiles of isolates obtained from mothers XXII (*c*/R29) and XXIX (*c*/R29′) and mothers XXIII (*c*/R32) and XXXI (*c*/R32′), respectively, were identical. Mutacin activity profiles of the isolates were, however, not identical. R29, R29′, R32, and R32′ were considered as distinct types of *S. mutans.*

[b] Inhibition zone sizes corresponding to ribotypes shared by the mother and her child are given in boldface type. Isolates representing ribotype R17 showed two different mutacin typing patterns.

from one child obtained 5 years apart showed a difference in mutacin activity against two indicator strains.

DISCUSSION

The mutacins have been implicated as virulence factors in dental caries (25, 38). In the present study, we investigated the mutacin activity of clinical mutans streptococcal isolates in relation to the transmission of the isolates between mothers and their young children. We considered that transmission had taken place if the mother and her child harbored mutans streptococcal isolates with identical ribotype profiles and similar mutacin types. We found that *S. mutans* isolates that produced mutacins against several indicator strains and yielded bigger inhibition zones were more easily transmitted than were isolates producing mutacins against fewer indicator strains and giving smaller inhibition zones.

The importance of mutacin production in strain colonization in the oral cavity was also shown by Hillman et al. (21). In their study, an *S. mutans* strain with increased mutacin activity could colonize the oral flora of adults even after a single application. In young children, during acquisition of the microorganisms (8, 39), mutacins may promote *S. mutans* colonization of tooth surfaces, in competition with other microorganisms. When a child is exposed to infection by an *S. mutans* strain exhibiting an increased level of mutacin production, it can be presumed that under favorable circumstances the strain will colonize, especially if the flora has not yet reached stability.

Maternal transmission of mutans streptococci was suggested in 64% of the children. This result agrees with previous reports

of the application of DNA fingerprinting in transmission studies (26). In the study by Li and Caufield (26), oral mutans streptococci of mothers and their infants were monitored from birth for approximately 3 years at 3-month intervals, and this monitoring showed identity in restriction endonuclease analysis banding patterns of isolates in 71% of mother-child pairs. Our study population was fairly small, and we examined only a limited number of isolates. From one child (XIV), only one isolate in total was obtained from the plaque sample cultured on MSB agar. Thus, it is likely that if more isolates had been collected from the mothers and children, more distinct ribotypes would have been found, including more transmitted strains. This could have increased the percentage of children with maternal transmission of mutans streptococci, but since the isolates were randomly chosen, it is not likely that the proportion of ribotypes found to be "transmitted" or "nontransmitted" would have changed.

Variability in the mutacin activity of mutans streptococci of the same serotype obtained from a single subject was detected in an earlier study (4). Our material included eight subjects harboring isolates of the same serotype but with different mutacin activities. In seven cases, the isolates had different ribotype profiles, indicating that the subjects were colonized with two or three different strains. In four subjects, concomitantly isolated strains exhibiting different ribotypes could not be differentiated by mutacin typing. This finding of ribotyping being more discriminative than bacteriocin typing is in accordance with the findings of Alonso et al. (5), who compared the discriminative ability of the two techniques in typing of *Serratia marcescens* (discrimination index, 0.92 and 0.74 for ribotyping and bacteriocin typing, respectively).

The present material included follow-up samples from 15 subjects for a 1- to 7-year evaluation. Among the follow-up strains, only one ribotype differed in mutacin production in the 5-year follow-up. Strains shared by mothers and their children had similar mutacin activity profiles. This indicates that the property is fairly stable. In 1976, Rogers (34) also reported finding stability in mutacin synthesis of mutans streptococci in in vitro experiments.

Our present results suggest that the bacteriocinogenecity of *S. mutans* strains is reasonably stable and that strains producing increased levels of mutacin are more easily transmitted to young children than are strains with lower mutacin activity. Earlier studies have shown that early establishment of mutans streptococci in the mouths of infants increases the risk of caries (3, 24). Consequently, it is possible that bacteriocinlike inhibitory activity of *S. mutans* will indirectly increase the risk for early childhood caries.

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