BACTERIOCINS IN *S. MUTANS* STRAINS ISOLATED FROM CHILDREN WITH AND WITHOUT DENTAL CARIES: BIOTYPES AND SENSITIVITY TO ANTIBIOTICS

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ABSTRACT

The aim of this study was to determine the production of bacteriocins in the Streptococcus mutans strains isolated from children with and without dental caries. With this purpose the dmft index was determined and non-stimulated saliva was collected from 53 3- to 5-year-old children. The samples were cultured on Mitis Salivarius Bacitracin agar and incubated anaerobically for two days at 37°C. The isolates were biotyped using the Api-ZYM enzymatic system (bioMérieux; Marcy-lE´toile, France). Bacteriocin was detected using the double layer onto brain heart infusion agar technique and the minimal inhibitory concentrations of the isolates were evaluated against penicillin, amoxycillin, cefazolin, erythromycin, clindamycin, imipenem and vancomycin using an agar dilution method. The dental caries experience in these children was 66% (35/53) and dmft index average was 3.2 (range 2-6). S. mutans was found in the saliva of 33 children (62%). In the 33 strains of S. mutans, 10 biotypes were found. Eight (24%) of the 33 strains evaluated produced bacteriocins, 6 of these strains came from patients with dental caries and the other two from patients without dental caries. All isolates were highly sensitive to the antibiotics tested.

Key words: *dental caries, S. mutans, bacteriocins, inhibitory action.*

BACTERIOCINAS EN CEPAS *S. MUTANS* AISLADAS DE NIÑOS CON Y SIN CARIES DENTAL: BIOTIPOS Y SUSCEPTIBILIDAD ANTIBACTERIANA

RESUMEN

El objetivo de este estudio fue determinar la producción de bacteriocinas en cepas de Streptococcus mutans aisladas de niños preescolares con y sin caries dental. Con este fin en se determinó el índice ceod y se tomó saliva no estimulada en 53 niños con edades entre 3 y 5 años. Las muestras de saliva se cultivaron en Agar Mitis Salivarius Bacitracina para el aislamiento selectivo y recuento de S. mutans, y se incubaron en anaerobiosis durante 2 días a 37°C. Las cepas de S. mutans aisladas se biotipificaron con el sistema enzimático Api-ZYM (bioMérieux; Marcy-lE´toile, Francia). La detección de bacteriocinas se realizó de acuerdo a la técnica de doble capa en agar infusión cerebro corazón y las concentraciones mínimas inhibitorias de los aislamientos fueron evaluados contra la penicilina, amoxicilina, cefazolina, eritromicina, clindamicina, imipenem y vancomicina, por el método de dilución en agar. La experiencia de caries dental en esta población fue de 66% (35/53) y el índice ceod promedio fue de 3.2 (rango 2-6). S. mutans fue aislado en 33 de los 53 niños incluidos en el estudio (62%). Las 33 cepas de S. mutans aisladas se agruparon en 10 biotipos. Ocho (24%) de las 33 cepas evaluadas produjeron bacteriocinas, 6 de estas cepas provinieron de pacientes con caries y las otras dos de pacientes sin caries. Todos los 33 aislamientos de S. mutans fueron altamente sensibles a los antibacterianos evaluados.

Palabras clave: *caries dental, S. mutans, bacteriocinas, acción inhibitoria.*

INTRODUCTION

Dental caries is a localized, transmissible infectious pathological process that leads to the destruction of the hard dental tissue[1]. *Streptococcus mutans,* an acidogenic and aciduric microorganism that colonizes the oral cavity is recognized as the main causal agent of the disease[1]. Different studies have shown a strong correlation between the number of *S. mutans* in the oral cavity and prevalence and incidence of caries[1-3]. In addition to participating in dental caries and tooth-related pyogenic infections, *S. mutans* is also a major cause of endocarditis[4]. Recognition of *S. mutans* as the most important microorganism initiating dental caries has led to the design of preventative and control measures aimed at eliminating or reducing it in the oral cavity[5].

Several factors influence the way *S. mutans* becomes established and multiplies in the oral cavity[6]. Its

metabolic capacity to synthesize glucans from saccharose and produce bacteriocins is very important in the process of initiation and development of dental caries[6].

Hamada and Ooshima[7] showed that many *S. mutans* strains produce bacteriocins. The bacteriocins or mutacins produced by *S. mutans* have a wide range of activity against gram-positive microorganisms and closely related species[7-9].

Bacteriocins are strongly bactericidal peptides or protein antibiotics[10], which can provide advantages to certain species of bacteria in colonizing the oral cavity[11-14]. Fabio et al.[14] showed that bacteriocins production can increase the proportion of *S. mutans* in the oral cavity.

The aims of this study were: 1) to determine the profile of biotypes in the *S. mutans* strains isolated, 2) to identify the bacteriocin-producing *S. mutans* strains, and 3) to determine the sensitivity to antibiotics of the *S. mutans* strains isolated.

MATERIALS AND METHODS

Clinical examination, sampling and bacteriological procedures

A transversal observational study was conducted, which finally included 53 3- to 5-year-old children from the primary section of the Diego Torres School in Turmequé (Boyacá, Colombia). Before the children were included in the study, informed consent was obtained from their parents or guardians. None of the children included had received anti-bacterial treatment during the 7 days immediately prior to the sampling. Caries experience in each child was determined by means of clinical examination during which the examiner determined the dmft index (decayed, missing and filled teeth) according to World Health Organization criteria[15]. No radiography was taken of any of the children. To start the microbiological study, a non-stimulated saliva sample was collected from each child (2 to 5 milliliters) by means of gentle suction with a plastic pipette, and placed in a sterile tube[16]. The samples were immediately shaken in a vortex for 30 seconds and serially diluted in 0.05M phosphate tampon. In order to isolate *S. mutans* selectively and count it, 100 ul of the serial dilutions were inoculated on Mitis Salivarius Bacitracin Agar (MSB). MSB agar (Difco Laboratories; Detroit, MI) contains digested pancreatic casein, proteose peptone No. 3, proteose peptone, dextrose, saccharose 20%, dipotassium phosphate, trypan blue, crystal blue, agar, Chapman tellurite and 0.2 U/ml bacitracin. The Petri dishes with MSB agar were incubated anaerobically (H_2:CO_2:N_2 10:10:80) for 2 days at 37°C. After growth, the colonies with characteristic *S. mutans* morphology [17] were counted and expressed as colony-forming units (CFU) per ml of non-stimulated saliva. The colonies with *S. mutans* characteristics were examined by Gram stain and subject to the following biochemical tests: fermentation of raffinose, manitol, mellobiose, trehalose and inulin; hydrolysis of aesculin in presence and absence of bile; urease; hydrolysis of arginine, and resistance to bacitracin. *S. mutans* has the following biochemical profile: positive fermentation of raffinose, manitol, mellobiose, trehalose and inulin; negative hydrolysis of aesculin in presence of bile and positive hydrolysis of aesculin in absence of bile; negative urease; negative hydrolysis of arginine; and resistance to 2 U of bacitracin. The Chi square test was used to establish differences in *S. mutans* counts in the groups with and without caries.

Strain biotyping

All the *S. mutans* strains that were isolated were biotyped using the api-ZYM system (bioMérieux, Marcy-létoile, France) according to the manufacturer's instructions. The api-Zym system is a semi-quantitative investigation micro-method that allows the quick, simultaneous detection of 19 enzymatic activities from small quantities of bacterial inoculum. The system consists of a strip of 20 cupules (1 control and 19 tests) on a base containing the enzymatic substrates and the buffer. The base allows contact between the enzyme of the microorganism and the usually insoluble substrate. The substrates are inoculated with a dense suspension of bacteria (McFarland turbidity 5-6) which rehydrates and exerts enzymatic action on the substrates contained. The end products generated during a 4-hour incubation period are detected by means of color reactions produced after adding reagents. Biotyping was done in duplicate and the biotypes were assigned according to the action effected by the *S. mutans* strains on the 19 substrates in the system.

Bacteriocin detection in *S. mutans* strains

Bacteriocins were detected using the double layer assay in BHI (Brain Heart Infusion) agar, on which bacteriocin-producing strains are inoculated, showing their direct action on indicator strains.

Part a. ***Preparation of producing strains***

The producing strains are those that will act on the indicator strains. For this purpose, 2 or 3 colonies of each *S. mutans* strain cultured in BHI Agar were re-suspended in BHI (Brain Heart Infusion) broth and incubated at 37°C in anaerobiosis (H_2:CO_2:N_2 10:10:80) for 48 hours. From this suspension, 2ul were inoculated with a micropipette on the BHI Agar (1.5% agar and 2% yeast extract) and incubated at 37°C in an anaerobic atmosphere (H_2:CO_2:N_2 10:10:80) for 48 hours. Following this incubation period, the indicator strains were placed (part b) on the producing strains.

Part b. ***Preparation of the indicator strains***

The indicator strains are the ones that will be subject to the action of the producing strains. This study included two groups of indicator strains: 1) indicator strains obtained from this study and selected according to the frequency of the biotypes present and 2) the reference strains *S. mutans* MT 8148 (HG455), *S. mutans* HG 979, *S. mutans* HG 982, *S. mutans* OMZ176 (HG596) and *S. mutans* GS5 (HG715) and *S. sobrinus* HG 976, which were kindly donated by Dr. J. J. de Soet of the Department of Oral Biology at ACTA (Academisch Centrum Tandheelkunde Ámsterdam; The Netherlands).

The strains used as indicators were cultured in BHI Agar and incubated at 37°C in anaerobiosis (H_2:CO_2:N_2 10:10:80) for 48 hours. Following this, Gram and catalase assays were conducted to confirm the purity of the cultured strains, and 2-3 colonies were re-suspended in BHI broth and incubated at 37°C in anaerobiosis (H_2:CO_2:N_2 10:10:80) for 24 hours. Then 0.5 ml of this suspension was mixed with 5 ml BHI Agar (0.75% agar and 2% yeast extract) and added immediately to the BHI agar (1.5% agar and 2% yeast extract) in which the producing strains had grown (part a). These Petri dishes with double layer of BHI Agar, in which both the producing and the indicating strains had been inoculated, were incubated at 37°C in anaerobic atmosphere (H_2:CO_2:N_2 10:10:80) for 48 hours. Following these last 48 hours, bacteriocin production is reflected by the presence of an inhibition halo of the producing strain on the indicator strain. To establish bacteriocin production, inhibition halos greater than 4 mm were considered.

Sensitivity to antibiotics

The minimal inhibitory concentrations (MIC) were determined against penicillin, amoxycillin, cefazolin, erythromycin, clindamycin, imipenem and vancomycin, using the agar dilution technique, with concentrations ranging from 0.003 to 32 ug/ml[18]. The antibiotics penicillin, amoxycillin, cefazolin, erythromycin, clindamycin and vancomycin were from Sigma Chemical (St Louis, MO, USA), and imipenem from Merk (Germany). Using a replicator device, 10^5 UFC/ml suspensions of the strains being assessed were inoculated on Wilkins-Chalgren agar. Following 48 hours incubation at 37°C in anaerobic atmosphere (H_2:CO_2:N_2 10:10:80), the MIC was determined.

RESULTS

Thirty five of the 53 children included in the study had dental caries, therefore caries experience in this population was 66% and the average dmft index was 3.2 (range 2-6). The prevalence of infection shows that 62% (33/53) of the population under study was infected by this microorganism. Only 60% (21/35) of the children with caries had detectable levels of *S. mutans* and 21 of the 33 children (64%) in whom *S. mutans* was found had caries. The *S. mutans* count in the population in general was varied, ranging from 10^3 to over 10^7 UFC/ml. By means of the Chi squared statistical test it was established that there was no statistically significant difference in the *S. mutans* count between the two groups (healthy and with caries) ($p>0.05$).

Table 1 describes the behavior of the 33 *S. mutans* strains against the api-Zym system substrates and the biotypes to which they correspond. The biotypes were assigned according to the standardization by Lamby et al.[19]. The *S. mutans* strains were grouped into 10 biotypes altogether. Table 2 shows the frequency of the biotypes found in the 33 strains. The most frequent biotypes were 10, 15 and 11, with 9, 8, and 4 strains, respectively. In *S. mutans* strains from patients with caries the most frequent biotype was 10 (n=7), and among the *S. mutans* strains from patients without caries the most frequent biotype was 15 (n=3).

In order to detect bacteriocin production in all 33 *S. mutans* strains isolated, 12 indicator strains were used. Six indicator strains were selected at random and as representatives of each of the 6 most frequent biotypes found in the study population. We used strain 1 for biotype 10, strain 5 for biotype 17, strain 11 for biotype 15, strain 12 for biotype 11, strain 9

TABLE 1. Biotypes in the 33 S. mutans strains isolated

Strain	Api Zym system substrates used																			Biotype*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
2	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	17
3	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
4	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
5	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	17
6	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	11
7	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	11
8	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
9	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	14
10	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
11	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
12	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	11
13	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
14	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	16
15	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
16	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
17	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
18	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	17
19	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
20	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
21	-	-	+	+	-	+	+	+	-	+	+	+	-	-	-	+	+	-	-	-	6
22	-	-	+	+	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	7
23	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	16
24	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
25	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
26	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	10
27	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	14
28	-	-	+	+	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-	+	1
29	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
30	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	11
31	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	15
32	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	13
33	-	-	+	+	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	7

* Biotypes were assigned according to the standardization by Lamby et al. (18).

TABLE 2. Frequency of biotypes among the 33 S. mutans strains

Biotype	S. mutans in patients with caries n=21	S. mutans in patients without caries n=12	Total
10	7	2	9
15	5	3	8
11	3	1	4
17	3	0	3
14	1	1	2
16	1	1	2
7	0	2	2
6	1	0	1
1	0	1	1
13	0	1	1

TABLE 3. Bacteriocin production in the 33 *S. mutans* strains isolated.

	*Indicator strains (inhibition in mm**)											
	Strain 1	Strain 5	Strain 11	Strain 12	Strain 9	Strain 14	HG 455	HG 979	HG 982	HG 596	HG 715	HG 976
1	-	-	-	-	-	-	-	-	-	-	-	-
2	10	10	11	9	9	10	11	12	10	11	10	10
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-
7	11	11	11	10	9	10	11	10	-	11	10	11
8	-	-	-	-	-	-	-	-	-	-	-	-
9	9	10	10	11	10	10	12	12	10	-	10	10
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	10	12	11	10	9	12	11	12	-	11	10	10
16	-	-	-	-	-	-	-	-	-	-	-	-
17	9	11	9	11	12	10	11	12	10	10	9	11
18	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-
20	13	11	14	9	11	10	11	12	9	11	10	10
21	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-
25	12	11	12	9	12	10	12	12	9	11	9	10
26	-	-	-	-	-	-	-	-	-	-	-	-
27	11	13	12	11	12	10	12	11	9	11	10	10
28	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-

* Indicator strains: Strain 1 (Biotype 10), Strain 5 (Biotype 17), Strain 11 (Biotype 15), Strain 12 (Biotype 11), Strain 9 (Biotype 14), Strain 14 (Biotype 16), *S. mutans* MT 8148 (HG455), *S. mutans* HG 979, *S. mutans* HG 982, *S. mutans* OMZ176 (HG596), *S. mutans* GS5 (HG715) and S. sobrinus HG 976.

** Inhibition haloes in mm

- : Negative, inhibition halo < 4 mm

for biotype 14 and strain 14 for biotype 16. The other 6 indicator strains were reference strains donated by Dr. J. J. de Soet of the Department of Oral Biology of ACTA (Academisch Centrum Tandheelkunde Ámsterdam; The Netherlands). Only 8 of the 33 strains evaluated (24%) produced bacteriocins (Table 3). Of these 8 strains, strains 2 (Biotype 17), 7 (Biotype 11), 9 (Biotype 14), 15 (Biotype 15), 17 (Biotype 10) and 20 (Biotype 10) came from patients with caries; while the 2 remaining strains, 25 (Biotype 10) and 27 (Biotype 14), came from patients without caries. Of the 8 bacteriocin-producing strains, biotype 10 was present with 3 strains.

Sensitivity to antibiotics of the *S. mutans* strains isolated in this study is illustrated in Table 4. All isolates were highly sensitive to penicillin, amoxycillin,

TABLE 4. Values of MIC range, MIC mean, MIC_{50} and MIC_{90} average (in ug/ml) in the 33 *S. mutans* strains.

Antibiotic evaluated		
Penicillin	Range	0.007-0.06
	Mean	0.024
	MIC_{50}	0.030
	MIC_{90}	0.030
Amoxycillin	Range	0.03-0.50
	Mean	0.15
	MIC_{50}	0.12
	MIC_{90}	0.25
Cefazolin	Range	0.06-1
	Mean	0.20
	MIC_{50}	0.12
	MIC_{90}	0.25
Erythromycin	Range	0.015-050
	Mean	0.15
	MIC_{50}	.12
	MIC_{90}	0.50
Clindamycin	Range	0.015-0.50
	Mean	0.14
	MIC_{50}	0.12
	CMI_{90}	0.25
Imipenem	Range	0.015-050
	Mean	0.12
	CMI_{50}	0.12
	CMI_{90}	0.25
Vancomycin	Range	0.03-1
	Mean	0.25
	CMI_{50}	0.12
	MIC_{90}	0.50

cefazolin, erythromycin, clindamycin, imipenem and vancomycin; 50 and 90% of the *S. mutans* strains were inhibited by all the antibiotics in concentrations lower than 0.12 and 0.5 ug/ml, respectively.

DISCUSSION

The non-presence of *S. mutans* in all children with caries (60%) is probably due to the fact that the number of microorganisms was much lower than the detection limits of current bacteriological culture techniques. Another explanation for its absence could be the presence of other microorganisms (*Lactobacillus* and/or *Actinomyces)* which are responsible for producing more advanced dental caries, and for which isolation strategies were not designed in this study.

A microorganism may become established, survive and proliferate in an ecological niche where there is strong competition due to the diversity of species present if it manages to displace or eliminate the competent microorganism[20]. For many years work has been conducted in search of *S. mutans* strains that can displace native, virulent *S. mutans* dental caries-producing strains[21-23]. Several studies indicate that the displacement capacity of *S. mutans* strains is due to the production of mutacin-type bacteriocins which provide advantages in the complex ecology of oral microbes[13, 24, 25].

In this study, 8 bacteriocin-producing *S. mutans* strains were found, representing 24% of the 33 strains evaluated. Moreover, the 8 bacteriocin-producing *S. mutans* strains had a wide spectrum of action on the 12 strains used as indicators. The article "Diverse activity spectra of bacteriocin-like inhibitory substances having activity against Mutans Streptococci" by Balakrishnan et al.[23], reports finding 39 strains of the bacteriocin-producing mutans group. The 39 bacteriocin-producing strains represent 14.3% of the 272 strains evaluated. This low bacteriocin-producing frequency is consistent with the observation that *S. mutans* strains are usually resistant to the bacteriocins derived from streptococcal strains. Another study reports finding 254 bacteriocin-producing *S. mutans* strains (79.62%) isolated from patients with and without caries, which have diverse action on the 12 *S. mutans* strains used as indicators[26]. The diversity in bacteriocin production among the *S. mutans* strains evaluated in the different studies is probably due to the differing conditions under which the assays are conducted and the sensitivity of the indicator strains used.

To find out the phenotypic differences among the *S. mutans* strains isolated, they were biotyped using the api-ZYM system, which has been extremely valuable for typing *S. mutans* strains based on their enzymatic action against the 19 substrates in the system[27]. The study by Lamby et al.[19] on 3- to 6-year-old children with incipient dental caries identified 17 biotypes, and the most frequent was biotype 15, with 10 strains. In our study, the

S. mutans strains were grouped into 10 biotypes, of which the two most frequent were biotypes 10 and 15, with 9 and 8 strains, respectively. The api-ZYM system enabled inter and intra-individual differences to be established quickly and clearly.

In the search for bacteriocin-producing *S. mutans* strains, 12 indicator strains were used, 6 of which were representatives of the most frequent biotypes (biotypes 10, 15, 11, 17, 14 and 16) in the population studied, and 6 reference strains, with the purpose of gaining a more realistic idea of the effect of bacteriocins on the strains in our environment.

Bacteremia originating in the oral cavity is common and sometimes causes endocarditis. *S. mutans* as a result of dental treatments produces bacteremia, systemic infections and sub-acute endocarditis[6]. To treat these infections adequately, the sensitivity to antibiotics of the *S. mutans* strains from the oral cavity needs to be known. The results of this study, which show the high sensitivity of the *S. mutans* strains to the antibiotics evaluated, closely match observations by other authors[18, 27].

Although when we began this study we believed we would find a greater number of bacteriocin-producing strains, it has nevertheless been very important to find 8 of them. Theoretically, bacteriocin-producing strains have greater capacity for displacing other native *S. mutans* strains, which makes them candidates for use as probiotics in the control of dental caries.

Having found these 8 bacteriocin-producing *S. mutans* strains, further work is needed. Future studies involve molecular typing of the strains, chemical and molecular characterization of the bacteriocins and assessment of their activity in animal and human models, in order to determine their action in the eradication or displacement of other *S. mutans* strains.

CONCLUSIONS

1. caries experience in this population was 66% (35/53) and *S. mutans* was present in 33 of the 53 children (62%); 2. the average dmft index was 3.2 (range 2-6); 3. among the 33 strains isolated, 10 different biotypes were found; 4. the most frequent biotypes were 10, 15 and 11, with 9, 8 and 4 strains respectively; 5. only 8 of the 33 *S. mutans* strains isolated (24%) were bacteriocin-producing; and 6. The *S. mutans* strains isolated in this study were highly sensitive to the antibiotics evaluated.

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CORRESPONDENCE

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