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Frequency and expression of mutacin and putative bacteriocin genes in isolated *Streptococcus mutans* and its antimicrobial activity against gram-positive and gram-negative bacteria

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Abstract

Background: *Streptococcus mutans* in the oral cavities sable to produce mutacin (bacteriocin-like substances) with antibiotic properties. The aim of this study was to investigate the frequency and expression of genes encoding mutacins typeI, II, III and IV and also two of 8 genes in a cluster encoding the putative bacteriocins, the designated bsm 283, bsm 299, bsm 423, bsm 1889c, bsm 1892c, bsm 1896, bsm 1906c and bsm 1914, were also screened by PCR and specific primers for each type of mutacin biosynthesis gene and then mutacin activity against the indicator strains determined.

Methods: In this study, dental clinic samples were collocated; *Streptococcus mutans* was detected using biochemical tests and molecular methods (PCR). Frequency of mutacin biosynthesis genes types I, II, III and IV, bsm299 and bsm1899 were measured by PCR, using specific primers for each type of mutacin biosynthesis gene. Furthermore, the antimicrobial spectra of *Streptococcus mutans* isolates against other indicators, including *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Salmonella typhi, Pseudomonas, Escherichia coli* were evaluated using well diffusion, disk diffusion and the minimal inhibitory concentrations (MICs) methods.

Results: Out of 56 samples collected from patients referred to Milad Hospital dental clinic on October 2011 and three private dental clinics on November 2011, 24 strains of *Streptococcus mutans* produced mutacins. 67.52% of the strains had a wide antimicrobial spectrum and 37.5% of 67.5% had a high frequency of genes with more inhibitory activity against, *Staphylococcus epidermidis, Staphylococcus aureus* and *Enterococcus faecalis* respectively that are more related to putative bacteriocins. The expression frequency of the bsm gene (putative bacteriocins) was higher than that of the characterized mutacins types (I–IV). The lowest dilution rate mutacin was found against *Staphylococcus epidermidis* (0.0625 unit/mL).

Conclusion: These findings suggested that all putative bacteriocins may represent a large repertoire of inhibitory substances produced by *Streptococcus mutans*. Therefore, the high diversity of mutacin-producing phenotypes, associated with high frequency of expression of the biosynthesis genes screened and wide antimicrobial activity against *Staphylococcus epidermidis* could be used as safe antimicrobial agents in treatment of superficial infections such as, removing the pimple caused by *Staphylococcus epidermidis*.

Keywords: Antimicrobial, Mutacin, *Streptococcus mutans*, Gram-Positive Bacteria

Introduction

Streptococcus mutans is the causing agent of dental caries (tooth decay) worldwide, and is considered as the most cariogenic oral streptococci, which is the biggest challenge in many developing countries.

The high amount of sucrose in daily diet

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changes the pH level of the mouth, which provides a stable condition for growth of certain bacteria that tolerate the highly acidic environments (Bowden et al., 1998 & Facklam et al., 2002).

The ability of *Streptococcus mutans* in producing mutacins (bacteriocin-like substances) is recognized as an important causative agent of dental plaque and tooth decays. *Streptococcus mutans* is considered as a major etiological agent of human dental caries due to its capacity to adhere, acid resistance, resistance to other stress conditions and mutacins production (Bowden GHW et al., 1998 &Napimoga MH et al., 2005).

In addition, *Streptococcus mutans* produces mutacins, which have bactericidal effects on other bacteria of the same or closely related species, as well as other Gram-positive micro-organisms, which provides an ecological advantage in diverse bacterial communities such as saliva and dental biofilms. (Parrot et al., 1990).

Streptococcus mutans secretes a wide variety of mutacins with a diverse inhibitory spectrum (Kamiya et al., 2005). In addition, the strains of *Streptococcus mutans* isolated from caries-active individuals produced a broader spectrum of mutacins in comparison with those from caries-free individuals (Kamiya et al., 2005), suggesting that isolates from subjects with high caries activity were better at colonizing and accumulating on teeth and consequently, inducing caries. Based on the chemical structure,bacteriocins are generally classified into two groups including class I (the lantibiotics) and class II (the heat-stable non lantibiotics).

The class I lantibiotics (mutacins I, II, III,1140, BNy-266, and SmbA and SmbB). Class I bacteriocins comprise two subgroups according to their primary peptide sequences. Subgroup AI contains the nisin-like lantibiotics, as well as subtilin,epidermin, pep5, and mutacins I and III as the most thoroughly characterized members. Subgroup AII consists of lantibiotics including lacticin 481, SA-FF22, salivaricin,variacin and mutacin II (Hillman et al.,1998; Qi et al., 1999a, b, 2000; Yonezawa & Kuramitsu, 2005).

Class II bacteriocins include non-lantibiotics, divided into subclass IIa, which contains the pediocin-like substances,

And subclass IIb, which is composed of two synergic peptides the non-lantibiotic mutacin IV, a non-lantibiotic class IIb bacteriocin encoded by the nlmA and nlmB genes (de Vos et al., 1995; Sahl &Bierbaum, 1998; Qi et al., 2001).

Furthermore, analysis of the Streptococcus mutans genome sequence has revealed ten small open reading frames with high similarity to the leader peptides of NlmA and NlmB that encode class IIb bacteriocins (nonlantibiotics).

The aim of this study was to evaluate the frequency and expression of genes encoding

mutacin type I, II, III and IV as well as two of eight genes of a cluster encoding putative bacteriocins known as *bsm299* and *bsm1889* by PCR and the antimicrobial spectra of *streptococcus mutans* isolates against indicator strains determined.

Material and Methods

In this study 56 samples of dental plaque and caries lesions were collected from patients referred to Milad Hospital dental clinic on October 2011 and three private dental clinics from on November 2011. Samples were collected using sterile swabs and then transported to the laboratorial ethylene glycol container (Merck) (Flo´ rio et al., 2004; Klein et al., 2004). First, the samples incubated in BHI broth (brain heart infusion) for 48 hours at 37°C. Then, the hemolysis was assessed on blood agar. The cultured isolates on blood agar with pinpoint colonies were cultured and incubated for 48 hours in streptococcus selective agar. Finally, the colonies were cultured and incubated on bile esculin agar for 48. The colonies which were grown in streptococcus selective medium, were selected and then, Gram staining, biochemical tests such as catalase test, carbohydrate fermentation: (mannitol, sorbitol, raffinose, melibiose, inulin, lactose), Urease test, VP test, the hydrolysis of Arginine, antibiotic sensitivity and also molecular biology analysis using PCR and specific primers were conducted to identify *Streptococcus mutans* (Table1).

Table 1 PCR Primers

Protein	Primer	Primer sequences	GenBank accession no.	Amplicon (bp)
Sm479	F R	5'-TCGCGAAAAAGATAAACAAACA-3' 5'-GCCCCTTCACAGTTGGTTAG-3'	NC_004350 NC_004350	(433bp)
Mutacin I/ III	MutAI/ III _F MutAI/ III _R	5'-AGTTTCAATAGTTACTGTTGC-3' 5'-GCCAAACGGAGTTGATCTCGT-3'	AF238860	(750/450 bp)
Mutacin II	mutAII_F mutAII_R	5'-CAGTAACGCAGTAGTTCCTT-3' 5'-TTAACAGCAAGTGAAAACAT-3'	U40620	(444bp)
Mutacin IV	nlmA_F nlma-R	5'-CAATTTGATGTAATGGACAG-3' 5'-CTACACAATATGGGGTAACA-3'	NC_004350	(1344 bp).
Bsm_299	Bsm_299F Bsm_299R	5'-AAACTATGGATGCTGAAAC-3' 5'-GAAGCCCAGCTATTTACTA-3'	NC_004350	(160bp)
Bsm_1889	Bsm_1889cF Bsm_1889cR	5'-GCTCCTGATATAGCTCCTA-3' 5'-AATGTATTGTAGGGACAGG-3'	NC_004350*	(154bp)

Extraction of chromosomal DNA

DNA from strains was extracted using a simple DNA preparation, modified from Welsh & McClelland (1990) and Saarela et al. (1996), in which the cells from an overnight culture are washed and boiled for 10 min with TE buffer (10 mM Tris/ HCl, 1 mM of EDTA, pH 8.0), the debris was pelleted and the supernatant was used for identification by PCR and genotyping by arbitrarily primed (AP)-PCR.

PCR identification

The detection of frequency and expression of mutacin biosynthesis genes of *Streptococcus mutans* isolates with different mutacin-producing phenotypes were analyzed by PCR, using specific primers (2 pM per mL) for each biosynthesis gene. Primers for the genes encoding mutacins or bacteriocins were designed based on sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) PCR amplification was performed using a GeneAmp PCR System 2400 (Perkin Elmer). The PCR was performed in a final volume of 25 µ.l (Table 2).

The 50 ml reactions consisted of 16PCR buffer containing 2.5 mM MgCl2, 200 mM each dNTP, 0.3 mM each oligonucleotide primer, 1.25 U Taq DNA polymerase (Life Technologies) and 50 ng template DNA. In addition to the strains being tested, purified genomic DNA from *Streptococcus mutans* UA159 was used as a positive control for mutacin gene type IV and bsm genes. Distilled water was used as a negative control in each PCR. The PCR products were analysed by electrophoresis in a 1.0% agarose gel stained with 0.3 mg ethidium bromide ml21. A 100 bp DNA ladder was included in each gel.

Table 2 for Identification of *Streptococcal* mutans

Master Mix	Amount, mL
H2O	16.95
Taq	0.3
Primer R	1
Primer F	1
dNTP	0.5
Mgcl2	0.75
PCR Buffer	2.5

Strains and Streptococcus mutans isolates

In order to accurately determine and confirm the strains of *Streptococcus mutans* from other oral streptococci, PCR were carried out using specific Smu49 primer (Table1) and sm479 programme (Table 3) (Klein et al., 2004).

Table 3 PCR Sm479 Program

Steps	Temperature, °C	Time, min
Pre denaturation	94	5
Denaturation	94	1
Annealing	56	1
Extension	72	2
Repeat	35	-
Final Extension	72	7
Final hold	4	-

PCR program to determine the presence of the genes mut I / III, mut II, nlmA, nlmB, bsm299 an bsm1892

Frequency Identification genes mut I / III, mut II, nlmA, nlmB, bsm299 an bsm1892 genes, using specific primers in listed in Table 1 and gene mut I / III, mut II, nlmA, nlmB, bsm299 an bsm1892 program as described in Tables 4 to 9.

Table 4 PCR mut I / III Program

Steps	Temperature, °C	Time, min
Pre denaturation	94	5
Denaturation	94	1
Annealing	54	1
Extension	72	2
Repeat	35	-
Final Extension	72	7
Final hold	4	-

Table 5 PCR mut II Program

Steps	Temperature, °C	Time, min
Pre denaturation	94	5
Denaturation	94	1
Annealing	56	1
Extension	72	2
Repeat	35	-
Final Extension	72	7
Final hold	4	-

Table 6 PCR nlmA Program

Steps	Temperature, °C	Time, min
Pre denaturation	94	4
Denaturation	94	1
Annealing	51	1
Extension	72	2
Repeat	35	-
Final Extension	72	5
Final hold	4	-

Table 7 PCR nlmB Program

Steps	Temperature, °C	Time, min
Pre denaturation	94	4
Denaturation	94	1
Annealing	48	1
Extension	72	2
Repeat	35	-
Final Extension	72	5
Final hold	4	-

Table 8 PCR bsm299 Program

Steps	Temperature, °C	Time, min
Pre denaturation	94	4
Denaturation	94	1
Annealing	49	1
Extension	72	2
Repeat	35	-
Final Extension	72	5
Final hold	4	-

Table 9 PCR bsm1894 Program

Steps	Temperature, °C	Time, min
Pre denaturation	94	4
Denaturation	94	1
Annealing	49	1
Extension	72	1
Repeat	35	-
Final Extension	72	5
Final hold	4	-

To evaluate the effect of bacteriocins, indicator bacteria such as *Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium* and *Pseudomonas aeruginosa* were used. Suspension of the indicator strains was prepared with 0.5 McFarl and turbidity (usually 2 to 6 hours).

To detect the bacteriocins activity, well diffusion method, disk diffusion method and MIC test were used. Isolated *Streptococcus mutans* reactivated in brain heart infusion broth (BHI) and incubated at 37°C for 48 hours. Afterwards, the culture media centrifuged for 20 minutes at 4000 rpm. Supernatant (containing mutacin) were studied to evaluate the antagonistic activity.

Well Diffusion Method

First wells with 6 millimeters in diameters drilled by a Pasteur pipette in BHI agar. 30µL of the supernatant obtained by centrifugation was poured into the wells (the middle well containing methanol is considered as control) and incubated for 48 hours at 37°C (Van Loveren et al., 2000).

Disk Diffusion Method

30µL the supernatant of 24 isolates was poured into a sterile test tube and then paper discs were placed in tubes for 120 minutes. Afterwards, the dried discs were placed on BHI agar containing bacteria with appropriate the distance. Vancomycin disks (30 µg) as positive control and a negative control of sterile saline were used. After incubation, the ability of each isolate to prevent bacterial growth and inhibition zone formation markers, were measured by a military ruler, The experiments were repeated at least 3

times (CLSI et al., 2006).

Determination of MIC by agar plate dilution method

Agar dilution involves the incorporation of different concentrations of the antimicrobial substance into a nutrient agar medium followed by the application of a standardized number of cells to the surface of the agar plate. Growth is assessed after incubation for a defined period of time (16-20 h) and the MIC value is read. To standardize the inoculum des a suspension of bacteria in sterile saline was prepared. For this purpose, 1 mL of the supernatant was added to MRS broth (deMan, Rogosa and Sharpe) (1 mL), doubling dilutions (serial two-dilution) were prepared and using the wells diffusion method (the AU/mL arbitrary unit per mL) turbidity.

Results

A total number of 56 selected samples were cultured on different and specific media such as Blood, Strep selective agar, Bile Esculin Agar biochemical tests, showed that 28 of 56 samples were *streptococci mutans*. Using PCR for determination of the exact strain of oral streptococci revealed that 24 of the total *Streptococcus mutans* isolates were positive for the presence of *Sm479* gene (Fig1).

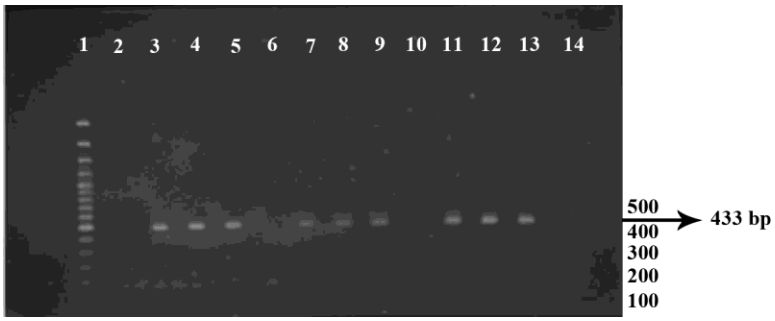

Fig1. Electrophoresis results of *Streptococcus mutants Sm479* gene (433 bp) on a 1% agarose gel size markers. Lanes: 1, 250 bp marker ladder; 2, 6, 10 and 14, negative control (distilled water); 3, 4, 5, 7, 8, 9, 11, 12, 13 (Sm479, 433bp).

The results of the molecular analysis showed that among 24 strains of *Streptococcus mutans* isolated rom the oral cavity, 25% were positive for the mut I/III and 37.5% showed positive results for the mut IV (nlmA, nlmB). On the Other hand, the analysis of the *Streptococcus mutans* genome sequence confirmed the presence of a putative bactriocin (*bsm299*gene) in all isolated strains. We also found that 37.5% of the isolates carried *Bsm1889* mutacin gene (Fig 2 and 3).

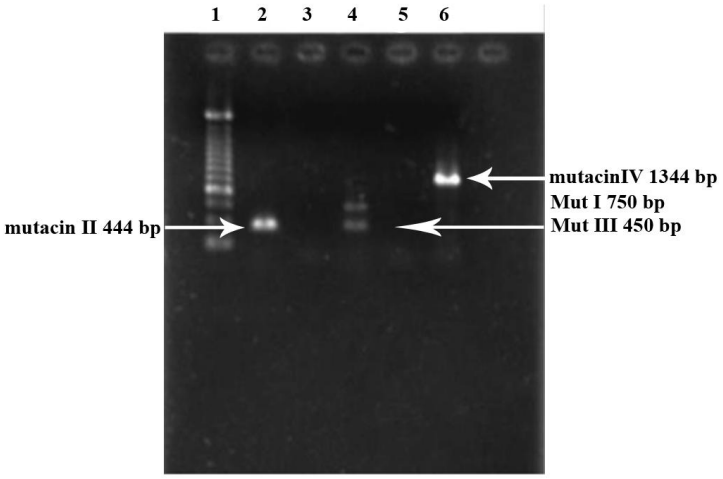

Fig 2. PCR Products of Four Different Mutacin Genes isolated from *S. mutans* Lanes: 1; 250 bp marker ladder; 3 and5, negative control (distilled water); 2, mutacin II (444 bp); 4, mutacin I/III (750/450 bp); 6, mutacinIV (1344 bp). On a 1% agarose gel.

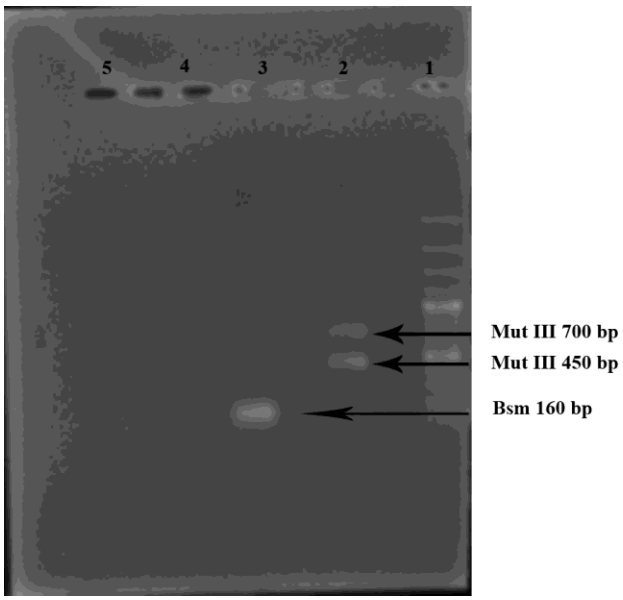

Fig 3 PCR Products of three Different Mutacin Genes isolated from *S. mutans* Lanes: 1; 250 bp marker ladder; 2 and 4, negative control (distilled water); 3, mut I/III (450 bp - 700 bp); 5, bsm299 gene (160bp), on a 1% agarose gel.

The detection of biosynthesis genes encoding mutacin types I, II, III and IV and different bsm genes was performed by PCR using primers specific to each type of gene. Primers for the genes encoding mutacins or bacteriocins were designed based on sequences obtained from GenBank (Table 1). For each gene, the bands observed on the gel were studied. The molecular

analysis of the data showing genetic profile and inhibition effects are summarized in Table 10.

Table 10 Frequency and expression of biosynthetic genes mutacin classified (types I, II, III and IV) and non-classified samples (bsm genes) from the tooth decay.

Isolates	Genetic profiles						Inhibited zones (mm)		
	Bsm299	Bsm1889	nlmA	nlmB	mutII	mutI/III	MIN (Minimum)	MAX (Maximum)	MID (middle)
Mut4	+	+	+	+	-	-	8	**20**	14
Mut6	+	-	-	-	-	-	5	10	7/5
Mut7	+	-	-	-	-	-	4	10	7
Mut8	+	-	-	-	-	+	5	10	7/5
Mut12	+	-	-	-	-	+	٧	9	8
Mut15	+	+	+	+	-	-	8	15	11/5
Mut18	+	-	-	-	-	-	5	10	7/5
Mut23	+	-	-	-	-	-	3	9	6
Mut24	+	-	-	-	-	+	4	11	7/5
Mut29	+	-	-	-	-	+	7	9	8
Mut31	+	+	+	+	-	-	7	19	11/5
Mut32	+	+	+	+	-	-	7	20	13/5
Mut35	+	+	+	+	-	-	10	20	15
Mut38	+	+	+	+	-	-	8	20	14
Mut39	+	-	-	-	-	+	5	10	7/5
Mut40	+	-	-	-	-	-	3	8	5/5
Mut41	+	-	-	-	-	-	7	15	11
Mut42	+	+	+	+	-	-	7	20	13/5
Mut47	+	-	-	-	-	+	7	10	8/5
Mut48	+	+	+	+	-	-	9	19	14
Mut51	+	-	-	-	-	-	7	15	11
Mut52	+	-	-	-	-	-	5	10	7/5
Mut55	+	+	+	+	-	-	7	18	13/5
Mut56	+	-	-	-	+	-	8	15	11/5

It was shown that the spectrum of bactriocins inhibition of these strains were very diverse, The inhibition zone size for the producer isolates varied from 6 to 30 mm in diameter. Approximately, 62.5% of the isolates showed a broad antimicrobial spectrum and 37.5% showed a lower spectrum of antimicrobial activity against the indicator strains. Approximately 37.5% of isolates containing bsm299, bsm1889, nlmA, nlmB genes with the inhibition zone size ranged from 8.7-18 mm against strains are considered as indicators (*Staphylococcus epidermidis*, *Enterococcus faecalis* and *staphylococcus aureus*), 25% of these isolates containing mut III, bsm299, mut I genes, had an inhibition zone of about 6.1-10 mm and 37.5% of strains containing the bsm299 gene, showed an inhibition zone of 4.9-10.8 mm. There was no antimicrobial activity against *Streptococcus gordonii* and Gram-negative bacteria, *P. aeruginosa* and *Escherichia coli*.

Streptococcus mutans pathogenic strains, isolated from caries and caries-free individuals were studied to evaluate the relationship between the number of genotypes isolated from caries and caries-free individuals.

In this research, 66.67% of the strains isolated from caries, 33.33% out of 66.67% isolates were positive for the genes encoding mutacin Bsm299, Bsm1892, NlmA, NlmB, 16.67% of strains containing the genes encoding mutacin I, III Bsm299, Mut, and 16.67% of strains contained genes encoding mutacin Bsm299. On the other hand, 33.3% of strains were isolated from individuals without caries that 4.17% of those were positive for the genes encoding mutacin Bsm299, Bsm1889, NlmA, NlmB, 8.33% of the isolates containing the genes encoding mutacin III Mut I and 20.83% isolates containing the mutacin biosynthesis genes of Bsm299.

The inhibition zones showed that only 24 of 28 isolates (85.71%) showed inhibition zones against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, but none of these 24 isolates produced inhibition zones against *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*.

The lowest dilution of mutacin against bacterial indicator (*Staphylococcus epidermidis*) in isolates containing bsm299, bsm1889, nlmA, nlmB, were 0.0625 mL/AU, this amount in isolates with mut I/III and bsm299 genes were 0.125 AU/mL and for the isolates containing bsm299 gene, were only 0.25 AU/mL as shown in Fig. 4. (Dilution mutacin terms of arbitrary units/mL).

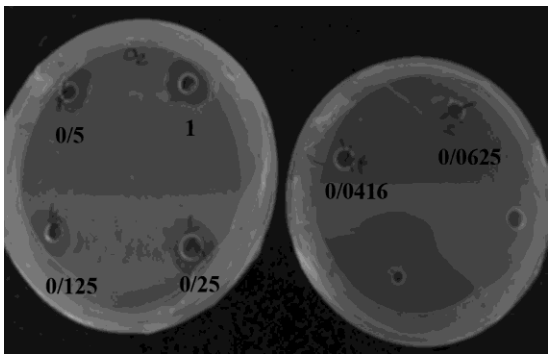

Fig. 4 Strain containing Mutacin Bsm299, Bsm1892, NlmA, NlmB showed The Lowest Dilution(MIC) inhibitory was 0.0625 mL/AUA gainst *Staphylococcus epidermidis.*

Discussion

Oral cavity provides favourable condition for the growth of microorganisms. However natural mechanisms including the champ, continuous secretion of saliva and gingival reticular fluid (such as lysozyme and lactoferrin-containing enzymes and proteins) and immune system activity can prevent bacterial growth and colonization. Therefore the microorganisms tend towards tooth cavities and cracks to be located (Suzuki, 1988; Bowden, 1998; Napimoga et al., 2005).

There are several factors that affect the balance of oral microorganisms. Several factors have been identified to inhibit microbial growth, mutaci being one of those (Fukushima et al.,

1985; Berkowitz et al., 1975; Hillman et al., 1987). Mutacin antimicrobial peptides or proteins are the property of antagonism against other strains of the same or a similar occupied ecological niche (Fukushima et al., 1985; Longo., 2003).Mutacin can play a wide range of important biological roles in complex microbial communities particularly in colonization of *Streptococcus mutans* (Kamiya et al., 2005). So, mutacin is considered as one of the most important virulence factors in dental caries that are produced by some *Streptococcus mutans* strains that enable those microorganisms to occupy positions more competitive than other types of oral streptococci on the teeth. However, the low frequency of mutacins genes that previously characterized in these isolates suggested a high diversity of bacteriocin genetic determinants in *Streptococcus mutans* (Kamiya et al., 2005). Despite this great diversity, little has been revealed about the biochemical structures of mutacins. In addition, many mutacins have not yet been identified. In the characterized mutacins (I, II, III IV), the most frequent gene was *mutIV* (29.1 %), (Longo et al., 2003; Kamiya et al., 2005).

Mutacin IV was produced by 17% of isolates, although it presented low antimicrobial spectra with activity mainly against initial colonizers, as well as *Streptococcus sanguinis* and *Streptococcus oralis*. In contrast, Hale et al. (2005a) demonstrated the broad antimicrobial spectra of mutacin IV produced by Streptococcus mutans UA159 against a panel of 84 mutacin-sensitive indicator bacteria consisting of 74 streptococcal strains, eight strains of *Lactococcus lactis* and two strains of *Micrococcus luteus*.

In our study, the expression frequency of the mut*IV gene was higher than the characterized mutacins* (I /III). the high frequent gene of the isolates was *IVmut* (37.5%) so they are likely to have broad antimicrobial spectra against the bacteria *Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecalis*, respectively rather than the stains without this gene.

Recently, analysis of *Streptococcus mutans* genetic sequence revealed ten small open reading frames that could encode class II mutacins. In the present study, the *bsm* genes of the uncharacterized mutacins (putative bacteriocins) were more frequent than the characterized types namely; I, II, III IV. The frequencies varied between 4 to 95% and the gene expression varied between 2 to 60% among clinical isolates. The *bsm 299* and *bsm 1889* genes were more frequently expressed in comparison to other bacteriocins. These findings suggest that all putative bacteriocins may represent a wide range of inhibitory substances produced by *Streptococcus mutans*. According to van der Ploeg (2005), different

combinations of bacteriocins have different antimicrobial spectra. Finally, these peptides were considered as the main antimicrobial agent produced by *Streptococcus mutans* and their role in the pharmaceutical industry as well as their use as a safe antimicrobial encourage researchers to study more in this field .(Hale et al.,2005; Vander Ploeg., 2005).

In our study the presence of *299bsm* gene in all strains (100%) and 1889 bs*m* gene in 37/5% of strains were approved, which indicating that there is a high frequency of these genes among other mutcins producers which is in accordance with the results of other researchers(Kamiya et al., 2005; Hale et al.,2005; Vander Ploeg., 2005)..

According to Kreth et al.(2006), some *Streptococcus mutans* isolates showed a broad-spectrum antimicrobial effect against other clinical isolates, including antibiotic resistance bacteria, as well as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Streptococcus saliyarius* 34.1% (16/47) of these isolates which had a broad-spectrum antimicrobial effects, inhibited more than 43% of the indicator strains, including *Streptococcus mutans isolates*, and 48.9% (23/47) of them with a low spectrum antimicrobial effect, inhibited 3 to 36% of the *Streptococcus* spp as the indicators.

In this study, all *Streptococcus mutans* strains isolated from caries and dental plaque were able to produce mutacins against one or more strains, which were considered as indicators (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium*). The inhibitory zones produced by the strains were very diverse. On average, 62.5% of these isolates presented a broad-antimicrobial spectrum and 37.5% showed a low-antimicrobial spectrum against the indicator strains. Strains with broader antimicrobial spectrum had the greater effect on *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Enterococcus faecalis*, respectively. The size of the inhibition zones ranged 7.5 to 20 mm.

In this study, 37.5% of strains with different gene frequency were chosen and their antagonistic effect on *Staphylococcus epidermidis* considered as indicators which showed that the lowest dilution of inhibitory activity against the bacterial indicator, the AU 0625/0, was expressed in mL.

According to van der Ploeg (2005), different combinations of bacteriocins showed different antimicrobial effects. No correlation between the number of indicator strains (antimicrobial spectra) and bacteriocine genes was observed (Spearman correlation test, r5”0.03, P.0.05). While our studies showed that the higher genetic diversity of strains which had a broader antimicrobial spectrum of strains were

considered as indicators.

A high diversity of mutacin-producing phenotypes is associated with high frequency of the isolated genes that reveals a broad range of genetic determinants encoding antimicrobial peptides, which can act in different combinations. However, differences in gene expression levels would depend on many factors, including genetic mutations not identified which may modify the proteins and their activities. Low gene expression may hinder protein detection, as at lower levels there will be insufficient antimicrobial activity of the protein and little effusion in the antagonism assay. However, it seems the range of indicator strains selected for this study was not sufficiently diverse to detect any activity conferred by different Bsm proteins. On the other hand, the regulatory or transport mechanisms of these peptides may be defective (Kamyiar et al, .2005).

According to Napimoga et al., 2005, a statistically significant positive association between the level of synthesis of water-insoluble glucan by S. mutans clinical isolates and the frequency of adherent cells in the presence of sucrose in caries-active subjects, but not in caries-free subjects In addition, the strains of S. mutans isolated from caries-active individuals produced a wide spectrum of mutacins in comparison with those from caries-free individuals Kamiya et al., 2005, suggesting that isolates from subjects with high caries activity were better at colonizing and accumulating on teeth, and, consequently, inducing caries.

There was a high phenotypic diversity; some *Streptococcus mutans* isolates showed abroad inhibition zone against other *Streptococcus mutans* clinical isolates, including antibiotic resistant bacteria, as well as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Streptococcus pyajeneus*. There was no correlation between the number of indicator strains (antimicrobial spectra) and the number of mutacin genes (Spearman correlation test, r5”0.03, P.0.05).In conclusion, the high diversity of mutacin-producing phenotypes is associated with the high frequency of biosynthesis genes expression, which shows a broad range of genetic determinants encoding antimicrobial peptides that can act in different combinations (Hale et al., 2005).

The phenotypic group was not similar to the genotypic group. Genotypes with the same qualitative gene expression profiles did not present the same mutacin production profiles, and distinct genotypes were grouped.

In the same phenotypic profile of production, it was shown that the patterns of inhibitory spectra reduced by distinct Streptococcus mutans isolates were independent of the degree of genetic similarity of the tested strains. These

results may suggest that the genetic and phenotypic traits of mutacin production are related to the genetic background of each tested isolate, or the genetic polymorphism or other mutacins are as-yet-unidentified.

The high diversity of mutacin-producing phenotypes suggests that mutacin production could be an important virulence factor for colonization and establishment of *Streptococcus mutans* in the oral cavity. Furthermore, the high frequency of the biosynthesis genes represent broad antimicrobial spectra determinants encoding antimicrobial peptides; however, further studies are necessary to determine the role of each bacteriocin and to explain how mutacin production is regulated and how mutacins are secreted (Lembo et al., 2007).

the mutacin-producing isolates can be used to inhibit staphylococcus epidermidis growth. These data also suggest that mutacin is probably useful in treatment of superficial infections associated with *Staphylococcus epidermidis*.

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Authors, Contribution:

All authors have worked equally.

Conflict of Interest:

There is no conflict of interest for this study.

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