Purification and properties of extracellular mutacin, a bacteriocin from *Streptococcus sobrinus*

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Mutacin MT6223, a cell-free bacteriocin produced by *Streptococcus sobrinus* MT6223, was purified by ammonium sulphate precipitation, chromatofocusing with PBE 94 and column chromatography on SP Sephadex C-25. The specific activity of the purified mutacin was increased 1950-fold with a recovery of 9.7%. The molecular mass of the purified mutacin preparation was estimated to be 6.5 kDa. The mutacin activity was stable from pH 2–7, and was resistant to treatment at 100 °C for 20 min. It was inactivated by papain or ficin digestion, and was partially inhibited by α -chymotrypsin. The mutacin was found to be active against strains of serotypes *c*, *e* and *f* of *Streptococcus mutans* and the addition of purified mutacin MT6223 to growing cells of *S. mutans* MT8148 resulted in a rapid inhibition of incorporation of [³H]thymidine, [³H]uracil or L-[³H]glutamic acid into DNA, RNA or protein, respectively. Specific pathogen-free Fischer rats fed diet 2000 and infected with *S. mutans* MT8148R showed significantly fewer caries and lower plaque scores when mutacin was administered through drinking water. The present study demonstrates that mutacin MT6223 inhibited the growth of mutans streptococci. Thus, mutacin MT6223 may be a candidate for use in dental caries prevention.

Introduction

Bacteriocins are antimicrobial polypeptides produced by some strains of various bacterial species. Typical bacteriocins, particularly those derived from Gram-negative bacterial species, have a narrow inhibitory spectrum of activity against homologous or related species. Clinically isolated strains of mutans streptococci have been reported to produce bacteriocin-like substances that exhibit a wide spectrum of antibacterial activity, and the term mutacin has been proposed to describe these antibacterial substances (Hamada & Ooshima, 1975*a*). The mutacin activity of mutans streptococci has been well-recognized on agar plates, but only a few strains of *Streptococcus mutans* have been reported to produce cell-free mutacin in liquid culture (Hamada & Ooshima, 1975*b*; Delisle, 1986). Bacteriocin production is affected by cultural conditions such as composition of the culture medium, incubation time, and pH. Paul & Slade (1975) found a bacteriocin from *S. mutans* GS5 (serotype *c*) with a molecular mass of more than 20 kDa which was non-diffusible. Hillman *et al.*

(1984) reported that a serotype *c* strain of *S. mutans* produced a mutacin with a molecular mass < 1 kDa, and Ikeda *et al.* (1982) described a mutacin from *S. mutans* C3603, a serotype *c* strain, with a molecular mass estimated to be 4.8 kDa.

It has been suggested that strains of *S. mutans* belonging to serotypes *c* or *e* are more potent producers of bacteriocin-like inhibitory factors against other mutans streptococci (Hamada & Ooshima, 1975*a*). The bacteriocinogeny of *S. mutans* may modulate local bacterial ecology in dental plaque on tooth surfaces. In fact, using the experimental caries system in rats or hamsters, it was shown that isolated mutacin C3603 and a bacteriocin from *Staphylococcus epidermidis* 1580 (i.e. staphylococcin 1580) exerted cariostatic activities (Ikeda *et al.*, 1982; Fitzgerald *et al.*, 1986). More recently, we reported that a mutacin-producing strain, *Streptococcus sobrinus* MT6223, clearly inhibited colonization on tooth surfaces by *S. mutans* MT6222, a mutacin-sensitive serotype *f* strain (Kitamura *et al.*, 1989). This study deals with the purification and characterization of extracellular bacteriocin from *S. sobrinus* MT6223 (mutacin MT6223) and its caries inhibitory activity *in vivo*.

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Methods

Bacterial strains and growth conditions. *S. sobrinus* MT6223, a serotype g strain of mutans streptococci, was used as a producer of mutacin (Kitamura *et al.*, 1989). MT6223 was grown in a diffusate medium of TTY broth (Hamada & Torii, 1978) at 37 °C for 24 h. Culture supernatant was obtained by centrifugation (10000 g, 20 min, 4 °C). Indicator bacterial strains used for determining sensitivity to the mutacin MT6223 included *S. mutans* MT8148, MT8148R (the suffix R refers to a streptomycin-resistant mutant), MT4263, NG71, Ingbritt and GS5 (serotype c); MT703R, MT3567R and LK3 (serotype e); MT6222, OMZ 175 and MT3940 (serotype f); and *S. sobrinus* OMZ 176, B13R and MT4502 (serotype d); 6715, MT6223, and MT4532 (serotype g). *Streptococcus cricetus* E49 and HS6 (serotype a); *Streptococcus rattus* FA1 and BHT (serotype b); and *Streptococcus downei* MFe28 (serotype h) were also included in this study. These organisms were grown on Trypticase soy agar (TSA; BBL Microbiology Systems) and were subcultured once a week. Other bacterial strains were selected from the culture collection at the Department of Oral Microbiology, Osaka University Faculty of Dentistry, Osaka, Japan.

Bacteriocin assay. The activity of mutacin MT6223 was estimated by using twofold dilutions of the test preparations and aseptically dispensing 100 µl of each dilution into wells of sterile flat-bottomed 96-well microtitre plates (Corning). Indicator organisms (6×10^4 cells ml⁻¹) of *S. mutans* MT8148R were then inoculated into the wells in 100 µl Brain Heart Infusion broth (BHI, Difco) with 1% (w/v) sucrose and 250 µg streptomycin ml⁻¹. The plates were incubated at 37 °C for 18 h, and the supernatant was removed from the wells. The plates were then fixed with 0.1% (v/v) glutaraldehyde for 5 min at room temperature, and stained with 0.5% (w/v) crystal violet for 5 min. The wells were then washed with water and dried. An arbitrary unit (AU) of mutacin was defined as the reciprocal of the highest dilution showing visible inhibition of the growth of the indicator organisms (Dajani *et al.*, 1976). Specific activities were expressed in terms of AU (mg protein)⁻¹, after quantification of protein in the samples by Lowry's method.

The bactericidal activity of mutacin MT6223 was determined by measuring the decrease over time in the number of c.f.u. of the sensitive indicator strains grown in BHI broth. Various concentrations (2.5–100 AU ml⁻¹) of purified mutacin MT6223 were added to 5 ml BHI broth. Then 1×10^4 c.f.u. of the indicator strains were added, and the suspension was incubated at 37 °C. The viable counts in the reaction mixture were determined by colony counting using Mitis Salivarius (MS; Difco) agar plates at 6, 12, 18 and 24 h incubation periods. The growth inhibition was determined as the difference in viable counts at 24 h between the samples with or without mutacin plated on MS agar and incubated at 37 °C for 48 h. Production of mutacin in the agar medium was examined by the stab culture technique (Hamada & Ooshima, 1975b).

Purification of mutacin. Mutacin was purified from the culture supernatant of *S. sobrinus* MT6223 grown in TTY (8 l) broth. Solid ammonium sulphate (351 g l⁻¹) was added to the supernatant to 55% saturation. The precipitate was collected by centrifugation, dissolved in 25 mM-imidazole/HCl buffer (pH 6.2), and applied to a column of Polybuffer PBE 94. Fractions (15 ml) showing mutacin activity were demonstrated in the void volume portions. Active mutacin fractions were pooled, concentrated by precipitation with 55% saturated ammonium sulphate and dialysed overnight against 10 mM-sodium phosphate buffer (pH 5.0). The concentrated mutacin from the chromatofocusing column was applied to an SP Sephadex C-25 (Pharmacia) column (1.6 × 10 cm) equilibrated with 50 mM-sodium phosphate buffer (pH 5.0). The column was eluted with a linear

gradient of 50 mM-sodium phosphate buffer (pH 5.0; 150 ml) to 50 mM-sodium phosphate buffer (pH 7.0; 150 ml) and 15 ml fractions were collected. All buffers used for purification were sterilized by filtration through a membrane filter (0.45 µm pore size).

Physico-chemical properties. To determine the heat stability of the purified mutacin, mutacin MT6223 was kept at 37, 60 and 80 °C for 1 h, 100 °C for 20 min, and at 120 °C for 5 or 20 min. The pH stability was estimated by incubating mutacin for 2 h at 37 °C in filter-sterilized 50 mM buffers covering the following ranges: glycine/HCl buffer (pH 2–3), sodium acetate buffer (pH 4–5), sodium phosphate buffer (pH 6–8) and sodium carbonate buffer (pH 9–11). The effect of various enzyme treatments on mutacin MT6223 activity was determined by incubating mutacin (640 AU) with the enzyme to be tested at 37 °C for 1 h. The following filter-sterilized enzymes were examined: papain type III and ficin (dissolved in 1 mM-EDTA, 67 mM-2-mercaptoethanol, and 5.5 mM-cysteine/HCl buffer, pH 2.8), and trypsin and lipase (in Tris/HCl buffer, pH 7.0 and 6.0). Phospholipase A, catalase, α-chymotrypsin, pronase P, protease XIV, protease XVII (B) and protease XXVI were dissolved in 10 mM-potassium phosphate buffer (pH 7.0). All reagents were purchased from Sigma. After treatment with the enzyme, the remaining mutacin activity was estimated in 96-well plates as described above.

SDS-PAGE. Slab gels were prepared as described by Laemmli & Favre (1973). Mutacin MT6223 (20 µg) was heated at 100 °C for 5 min in 10 mM-Tris/HCl buffer (pH 6.8), containing 1% (w/v) SDS, 1% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol, and 10% (w/v) sucrose. Electrophoresis was done at 4 °C for 3 h using a 15% (w/v) running gel and a 4% (w/v) stacking gel (8.5 × 7.7 × 0.1 cm) with and without 0.1% SDS. Ovalbumin (43 kDa), carbonic anhydrase (29 kDa), β-lactoglobulin (18.4 kDa), lysozyme (14.3 kDa), bovine trypsin (6.2 kDa), insulin B and C (3.4 kDa), and insulin A (2.3 kDa) were used as calibration markers for determination of molecular mass (BRL). The mutacin activity was located on the gel after electrophoresis by soaking the unstained gel for 18 h in a saline streptomycin solution (250 µg ml⁻¹) to remove SDS, and then by embedding the gel in Trypticase soy soft agar (0.8%, w/v, agar) containing *S. mutans* MT8148R as the indicator strain (Hamada & Ooshima, 1975b). Streptomycin (250 µg ml⁻¹) was included in the wash buffer to prevent growth of contaminating bacteria. Identification of the position showing the inhibition zone of the bacterial growth was then compared with the protein band which developed in the gel stained with Coomassie blue run at the same time.

Incorporation of radioactive substrates. DNA, RNA and protein syntheses were measured by the incorporation of radioactive precursors [³H]thymidine, [³H]uracil or L-[³H]glutamic acid into the TCA-precipitable fraction in *S. mutans* MT8148 grown in synthetic FMC broth (Terleckyj *et al.*, 1975) at 37 °C. Addition of 10 µCi (0.37 MBq) [³H]thymidine, 5 µCi (0.185 MBq) [³H]uracil, or 5 µCi (0.185 MBq) L-[³H]glutamic acid ml⁻¹ was made to cultures with an OD₆₄₀ of 0.4. After 30 min incubation at 37 °C, the culture was divided into three fractions which were incubated with mutacin MT6223 (10 or 25 AU ml⁻¹) or buffer without mutacin to serve as the control. Samples (1 ml) of the cultures were taken at intervals and mixed with 0.5 ml cell suspension containing 1.5 mg (dry mass) cells of unlabelled *S. mutans* MT8148. Then, 1 ml 10% (v/v) TCA solution was added to the above cell suspension, and the precipitate was collected by centrifugation (5000 g, 20 min, 4 °C). All the samples were washed extensively in saline to remove free radioactive substrate and the radioactivity of the precipitate was quantified by an LRB 1214 RackBeta liquid scintillation counter. All experiments were repeated at least three times and assays were run in triplicate.

Experimental dental caries induction in rats. Specific pathogen-free Fischer rats (Charles River Japan), 15-d old, were used. Four groups of rats (12 rats per group) were given powdered diet CE-2 containing tetracycline [4 mg (g diet)⁻¹], and deionized water containing penicillin G (4000 U ml⁻¹) *ad libitum* for 2 d. Group A non-infected rats were fed caries-inducing diet 2000 containing 56% sucrose. Group B, C and D rats were infected orally on two successive days with *S. mutans* MT8148R (0.1 ml 1×10^9 c.f.u. ml⁻¹, twice a day) and given diet 2000. Then, group C and D rats were administered mutacin MT6223 (50 and 100 AU ml⁻¹, respectively) in drinking water. The animals remained on this regimen for 55 d. At the end of the experiment the rats were killed under ether anaesthesia in a CO₂ atmosphere to prepare mandibular and maxillary specimens for the following examinations. Dental plaque accumulation on the molar teeth was evaluated by the procedure of Regolati & Hotz (1972). The attached bacteria on the mandible were dispersed in saline using a sonicator, then ten-fold dilutions of the bacterial suspension were prepared with sterile saline, spread on MS agar plates containing streptomycin (500 µg ml⁻¹) for counting total c.f.u. of *S. mutans* MT8148R, as described previously by Ooshima *et al.* (1991). Dental caries were scored by the method of Keyes (1944) as modified by Ooshima *et al.* (1981). Caries scores were analysed by Student's *t* test.

Results

Purification of mutacin MT6223

The concentrate of culture supernatant of *S. sobrinus* MT6223 obtained by ammonium sulphate precipitation was applied on a Polybuffer PBE 94 column equilibrated with 25 mM-imidazole/HCl buffer (pH 6.2). The mutacin was eluted without adsorbing to the column. The fractions containing mutacin activity were pooled and concentrated by ammonium sulphate precipitation. The concentrated mutacin was further purified by SP Sephadex C-25 column chromatography. A summary of a typical experiment is presented in Table 1. The specific activity of mutacin was increased to 1950 AU (mg protein)⁻¹ and the final purification was over 2000-fold with a recovery of 9.7%. Furthermore, SDS-PAGE analysis revealed that mutacin MT6223 was purified to a single band with an apparent molecular mass of 6.5 kDa. The growth inhibition of the purified mutacin MT6223 was located on an unstained gel run at the same time

Fig. 1. SDS-PAGE of various preparations of mutacin MT6223. Lanes: 1 and 4, molecular mass markers; 2, mutacin after chromatofocusing; 3, mutacin after SP Sephadex C-25 column; 5, mutacin activity, i.e. growth inhibition of the indicator strain on unstained gel.

after embedding the gel in soft agar inoculated with indicator organisms, i.e. *S. mutans* MT8148R (Fig. 1).

Characteristics of purified mutacin MT6223

Storage of purified mutacin preparations at various temperatures indicated that the activity was stable at 4 °C or in a frozen state (–20 to –70 °C) for at least 3 months. At room temperature, however, preparations of mutacin MT6223 lost some activity, decreasing in titre from 50 to 25 AU ml⁻¹ after 4 weeks. The purified mutacin was stable to heating at pH 5.0, with no apparent decrease in the activity after 20 min at 100 °C.

Table 1. Purification of mutacin from culture supernatant of *S. sobrinus* MT6223

Step	Total protein (mg)	Total activity (AU)	Specific activity (AU mg ⁻¹)	Recovery (%)	Degree of purification
Culture supernatant	84000	80000	0.95	100	1
Ammonium sulphate	1680	52800	31.4	66	33
Chromatofocusing	52	38400	738	48	775
SP-Sephadex C-25	4	7800	1950	9.7	2048

However, mutacin activity was partially inactivated by heating at 120 °C for 5 min, and was completely destroyed by heating at 120 °C for 20 min.

The mutacin was stable in neutral and acidic conditions (pH 2–7), but was inactivated rapidly in alkaline conditions (pH 8–11). Purified mutacin was completely inactivated after digestion at 37 °C for 1 h by ficin and papain, while protease XVI (B) and α -chymotrypsin partially inhibited mutacin activity (results not shown). The effect of mutacin MT6223 on the viability of *S. mutans* MT8148 was examined by counting c.f.u. on MS agar after incubation in the presence of mutacin (Fig. 2). Concentrations of mutacin over 10 AU ml⁻¹ clearly reduced the viable count of the indicator organisms, while smaller amounts of mutacin (5 or 2.5 AU ml⁻¹) retarded the cell growth of the indicator strain as compared to the control without mutacin. A selection of strains of mutans streptococci were examined for their susceptibility to the purified mutacin MT6223 by the broth assay and by the stab culture assay in agar plates (Table 2). A significant growth inhibition of *S. mutans* strains (serotypes *c*, *e* and *f*) was found by both methods. However, most strains of *S. cricetus*, *S. rattus* and *S. sobrinus* were insensitive to the purified mutacin by the broth assay. The mutacin also exhibited growth inhibition of strains of *S. salivarius*, *S. milleri* and

Fig. 2. Susceptibility of growing cells of *S. mutans* MT8148 to increasing concentrations of mutacin MT6223 (2.5–50 AU ml⁻¹). □, Control (no mutacin); ■, 2.5; ○, 5; ●, 10; △, 25; and ▲, 50 AU mutacin ml⁻¹.

Table 2. Inhibitory activity of mutacin MT6223 against strains of mutans streptococci

The bactericidal activity of mutacin was determined by measuring c.f.u. in BHI broth containing the mutacin. The inhibition of indicator strains in agar was determined by the stab culture technique and the results are expressed as the diameter (mm) of the inhibition zone.

Species	Serotype	Strain	Percentage inhibition in broth				Inhibition zone diameter (mm)
			Mutacin concn (AU ml ⁻¹)				
			10	25	50	100	
<i>S. cricetus</i>	<i>a</i>	E49	19.8	58.8	86.2	100	18
		HS6	0	0	0	0	0
<i>S. rattus</i>	<i>b</i>	FA1	0	0	0	0	12
		BHT	0	0	0	0	11
<i>S. mutans</i>	<i>c</i>	NG71	33.4	78.1	100	100	19
		GS5	38.2	83.2	100	100	21
		MT8148	35.4	82.4	100	100	20
		Ingbritt	36.2	78.6	100	100	20
		MT4263	35.4	82.2	100	100	20
	<i>e</i>	MT703R	31.0	74.0	100	100	20
		MT3567R	35.6	79.0	100	100	20
		LK3	34.2	81.5	100	100	20
	<i>f</i>	MT6222	35.2	79.0	100	100	19
		OMZ175	35.4	82.6	100	100	20
<i>S. sobrinus</i>	<i>d</i>	MT3940	35.0	82.2	100	100	20
		B13R	0	0	0	0	11
		OMZ176	0	0	0	0	16
	<i>g</i>	MT4502	0	0	0	0	10
		MT6223	0	0	0	0	0
		6715	0	0	0	0	10
		MT4532	0	0	0	0	9
<i>S. downei</i>	<i>h</i>	MFe28	15.8	41.0	74.3	100	17

Fig. 3. Effect of the addition of mutacin MT6223 (indicated by arrow) on DNA, RNA and protein syntheses of *S. mutans* MT8148 grown in FMC broth containing [^3H]thymidine (a), [^3H]uracil (b) or L-[^3H]glutamic acid (c) as described in Methods. Samples (1 ml) of the cultures were taken in triplicate over time to estimate the incorporation of radioactivity into cells. ●, Control (no mutacin); ○, 10; and ■, 25 AU mutacin ml^{-1} .

S. sanguis, but not against *Staphylococcus* sp., *Actinomyces* sp. and *Lactobacillus* sp. or *Escherichia coli* (data not shown). Addition of purified mutacin MT6223 to growing cells of *S. mutans* MT8148 resulted in a rapid inhibition of incorporation of the radioactive precursors of DNA, RNA and protein synthesis (Fig. 3).

Inhibition of experimental caries development in rats

Specific pathogen-free Fischer rats fed diet 2000 and infected with *S. mutans* MT8148R showed significantly fewer caries and lower plaque scores when mutacin (50 or 100 AU ml^{-1}) was administered to these rats through drinking water (Table 3). These results indicated that

administration of mutacin reduced the level of infecting *S. mutans* MT8148R in the oral cavity of rats, and that caries inhibition was dependent on the dose of mutacin.

Discussion

Mutacin MT6223 is the only bacteriocin isolated in a cell-free state from culture supernatant of *S. sobrinus*. Using chromatofocusing and cation exchange chromatography, we purified mutacin MT6223 to a single protein band in SDS-PAGE (Fig. 1). The purified mutacin was calculated to have a molecular mass of 6.5 kDa, and was stable chemically and physically. Treatment of the mutacin with papain or ficin resulted in a complete loss of activity. This indicates that mutacin MT6223 is protein/polypeptide in nature. However, the mutacin activity was not affected by the treatment of some proteases such as trypsin, pepsin, pronase P and other enzymes, i.e. catalase, phospholipase C, and lipase. We are currently investigating the detailed chemical structure of mutacin MT6223 in our laboratory.

In an earlier paper, we demonstrated that simultaneous inoculation of 10^5 c.f.u. of *S. sobrinus* MT6223 inhibited colonization in rats by *S. mutans* MT6222, while a non-bacteriocinogenic mutant of MT6223 did not inhibit the colonization by *S. mutans* MT6222 (Kitamura *et al.*, 1989). This result strongly suggested that *in situ* production of bacteriocin may modify microbial ecology *in vivo*, and that susceptible organisms of mutans streptococci and other related species can be suppressed by the mutacin locally produced *in vivo*. In the present study, cell-free mutacin MT6223 given to rats through drinking water did prevent the development of dental caries in rats that had been infected with *S. mutans* MT8148R, a serotype *c* strain of mutans streptococci (Table 3). Similar findings were reported by others using bacteriocin from different origins. Fitzgerald *et al.* (1986) showed that daily application of

Table 3. Effects of mutacin MT6223 on caries development in the rat model

Specific pathogen-free Fischer rats fed diet 2000 and infected with *S. mutans* MT8148R were given drinking water with mutacin (50 or 100 AU ml^{-1}) or without mutacin. Dental plaque accumulation, caries scores and the *S. mutans* MT8148R attached on the mandible were evaluated as described in Methods.

Group	Treatment		Recovery of MT8148R (c.f.u.)	Plaque score	Caries score
	Infection	Mutacin (AU ml^{-1})			
A	No	0	0	0.73 ± 0.16	6.8 ± 3.1
B	Yes	0	2×10^6	1.69 ± 0.14	49.7 ± 9.5
C	Yes	50	6×10^4	$0.99 \pm 0.12^*$	$26.6 \pm 11.4^*$
D	Yes	100	2×10^4	$0.92 \pm 0.15^*$	$13.4 \pm 3.7^*$

* $P < 0.0001$ by Student's *t* test against group B.

staphylococcin 1580 in the cheek pouches of Syrian hamsters inhibited caries development by approximately 50%. This bacteriocin was obtained from a clarified culture supernatant of *Staphylococcus epidermidis* 1580 (Jetten *et al.*, 1972) and was found to be effective *in vitro* against various strains of *S. mutans* and other Gram-positive bacterial species (Fitzgerald *et al.*, 1986). Mutacin C3603 derived from *S. mutans* C3603, a serotype *c* strain of mutans streptococci, was examined for *in vivo* caries inhibition abilities (Ikeda *et al.*, 1982; Ooshima *et al.*, 1985). In these experiments rats infected with an *S. mutans* strain and given mutacin C3603 developed less caries than the controls. When compared with these findings, mutacin MT6223 was shown to be an effective agent, in terms of *in vivo* activities, to inhibit dental caries in animal experiments.

Earlier studies indicated that few strains among many clinical isolates of *S. mutans* produced a cell-free mutacin, although 80% of the strains tested were demonstrated to produce some bacteriocin-like substance when grown in agar medium (Hamada & Ooshima, 1975a; Rogers, 1976). *S. mutans* GS5 (Paul & Slade, 1975), C3603 (Ikeda *et al.*, 1982) and JH1000 (Hillman *et al.*, 1984), which are all serotype *c* strains of mutans streptococci, were reported to synthesize a cell-free mutacin. However, the properties of mutacin MT6223 were clearly different from these preparations. The molecular mass (6.5 kDa) of mutacin MT6223 was higher than that of mutacin C3603 (4.8 kDa) and JH1000 (under 1 kDa), and lower than that of mutacin GS5 (20 kDa), although the estimation method of molecular mass was different in each case. Further, papain treatment of mutacin C3603 did not inactivate its activity (Ikeda *et al.*, 1982), but did inactivate mutacin MT6223 completely as shown in this study. Differences in the sensitivity spectrum of the indicator strains were also noted among these three mutacins. Notwithstanding the differences in various properties, we now have strong evidence to indicate that bacteriocins, including mutacins, can affect microbial ecology in the oral flora and some bacteriocins may function to suppress caries development if they have the ability to inhibit the growth of mutans streptococci *in vitro* (Fitzgerald *et al.*, 1986; Ikeda *et al.*, 1982; Ooshima *et al.*, 1985; Weerkamp *et al.*, 1977).

In conclusion, the present study demonstrates that mutacin MT6223 selectively inhibits the growth of mutans streptococcal strains, especially in strains belonging to serotypes *c*, *e* and *f* *in vitro* and *in vivo*. This bacteriocin may be a good candidate for use as an anticaries agent.

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