J. Microbiol. Biotechnol. (2002), **12**(1), 18–24

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Anticaries Activity of Antimicrobial Material from *Bacillus alkalophilshaggy* JY-827

CHUN, JU-YEAN[1], IL-HWAN RYU, JUNG-SUN PARK[1], AND KAP-SANG LEE*

College of Life Science and Natural Resources, Wonkwang University, Korea
[1]Department of Dental Hygiene, Wonkwang Health Science College, Korea

Received: June 14, 2001
Accepted: October 29, 2001

Abstract The present study was performed to investigate the excellent microbial anticaries substance, aminoglycoside antibiotic, which is more effective than chlorhexidine for the treatment of dental caries. The aminoglycoside antibiotic against *Streptococcus mutans* JC-2 from a novel alkaliphilic *Bacillus alkalophilshaggy* JY-827 exhibited no significant difference at the treatment concentration of 2.5×10^{-7} M, however, it inhibited the activity of the *Streptococcus mutans* glucosyltransferase by 70.2% and 99.8% at the concentrations of 2.5×10^{-7} M and 2.5×10^{-6} M, respectively. Lineweaver-Burk plot of the inhibitory aminoglycoside antibiotic showed competitive inhibition, with K_i value of 6.4×10^{-6} M. The aminoglycoside antibiotic did not show any cytotoxicity against human gingival cells. To evaluate the industrial applicability of the aminoglycoside antibiotic, a toothpaste containing this substance was prepared and tested on the extracted human teeth. The inhibitory rate of tooth calcification and calcium ion elution by the aminoglycoside antibiotic were 50% and 2.5 times, respectively. These results suggested that the aminoglycoside antibiotic from *Bacillus alkalophilshaggy* JY-827 is an effective agent against dental caries.

Key words: *Bacillus alkalophilshaggy* JY-827, glucosyltransferase inhibitor, cytotoxicity, tooth calcification, calcium ion elution

The characteristic of the Streptococcus strain having the common immunologic antigenicity as the cause of dental caries in humans has already been identified to be the same as that of the strain discovered by the British scientist Clarke [8] in 1924. Clarke named this strain, *Streptococcus mutans*. This strain can colonize the tooth surface and builds initial plaque formation due to its ability to synthesize extracellular polysaccharide from sucrose, mainly α-1.3 linked insoluble glucan, α-1.6 linked soluble glucan, and a mixture of soluble-insoluble glucan, using glucosyltransferase (GTase) [3, 11, 13, 14, 15, 35]. This sucrose dependent adherence and accumulation of cariogenic *Streptococcus mutans* is critical for the development of a pathogenic plaque [23], in which the accumulation of acid leads to localized demineralization of the enamel surface. Most previous work developing a therapeutic agent to prevent tooth decay has been focused at disrupting the plaque matrix, 30–40% (dry wt) of which is polysaccharide, insoluble glucan [9, 18, 36]. As such, inhibiting the function of this important plaque building enzyme is of great interest as a primary means of controlling bacterial colonization and the accumulation of plaque [10].

Some of the polyphenols that have been isolated from plants exhibit anticaries activity due to either growth inhibition against *Streptococcus mutans* or the inhibition of GTase [12, 28]. Ooshima *et al.* [30] reported that oolong tea polyphenols possessed a strong anti-GTase activity and inhibited experimental dental caries in specific pathogen-free rats infected with *Streptococcus mutans*. Studies have also been actively conducted on other GTase activity inhibitors, such as chemicals and metal ions. Platte *et al.* [33] reported on the development of a new GTase activity inhibitor, N-butyldeoxynojirimycin; Ciardi *et al.* [7] reported that 0.2–2.0 mM of SDS, anionic compounds of hexylresorol, cetylpyridinium chloride, cetylaminehydrofluoride, and the guanidine dimer inhibited the enzyme activity of GTase of the type 6715 *S. mutans* by more than 90%. Salem *et al.* [34] reported that one of the most potent antiseptic drugs used in dentistry, Chlohexidin (CHX), was a cationic bisguanidine compound with bactericidal properties and GTase inhibition against oral bacteria. The MIC of Chlohexidin against *Streptococcus mutans* was 0.004 mM. The efficacy of CHX as an antiplaque agent has also been studied extensively [1]. The drug's high clinical efficacy as a plaque controlling agent has been attributed largely to its high retention on oral surfaces [4].

*Corresponding author
Phone: 82-63-850-6675; Fax: 82-63-850-6675;
E-mail: LKS6675@Wonkwang.ac.kr

Musaka *et al.* [28] reported that the activity of the GTase of *S. mutans* was inhibited with common metals such as Pb^{++} and Cu^{++} at a concentration of about 1 mM.

Interest has recently been focused on new substances that exhibit a continuous inhibitory effect on GTase activity with no side effects, therefore, many researches are being conducted in this area. However, these substances have not been put into practical use due to problems such as appearance of mottled teeth, emergence of resistance, the development of ulcers, the disquamation of oral mucous cells, and economical reasons.

Accordingly, in the present study, attempts to elucidate the anticaries activities of the antibiotic produced by *Bacillus alkalophilshaggy* JY-827, and to examine the effectiveness of a sample toothpaste containing this antibiotic, were made in a hope that the results could be applied to commercialization of the toothpaste.

MATERIALS AND METHODS

Materials

The aminoglycoside antibiotic substance used in the present study was separated and purified from *Bacillus alkalophilshaggy* JY-827, as previously reported [5, 6], and then stored at 4°C until use.

Measurement of Cell Membrane Permeability of *Streptococcus mutans* JC-2

In order to measure the effect of the aminoglycoside antibiotic on the cell permeability of *S. mutans* JC-2, *S. mutans* JC-2 was inoculated into a BHI broth (200 g/l calf brain infusion, 250 g/l beef heart infusion, 10 g/l proteose peptone, 5 g/l NaCl, 2.5 g/l Na_2HPO_4, 2 g/l glucose, pH 7.4) with the antibiotic substance added at different concentrations and cultured at 37°C in a CO incubator for 24 h. According to the method of Park and Shin [31], the cultured suspension was centrifuged (10,000 ×g, 20 min, 4°C) to recover the strain which was resuspended in 0.1 M potassium phosphate buffer solution (pH 7.0), and left at 37°C for 10 min; to measure the cell permeability, the eluted cytoplasmic substance was quantified colorimetically using a spectrophotometer at 260 nm.

Production of Glucosyltransferase and its Effect on Activity Suppression

The GTase was prepared according to the method of Hamada and Toroii [16]. *S. mutans* was inoculated into the same TTY broth (15 g/l tripicase (BBL), 4 g/l trypton (Difco), 4 g/l yeast extract (Difco), 2 g/l K_2HPO_4, 5 g/l KH_2PO_4, 2 g/l Na_2CO_3, 2 g/l NaCl, 10 mg/900 ml glucose), cultured at 37°C for 20 h, and centrifuged at 12,000 ×g for 15 min to remove the strain. The supernatant was saturated with 50% ammonium sulfate. Thereafter, it was left for 24 h at 4°C, centrifuged at 10,000 ×g for 10 min to collect the precipitate, which was dissolved in 9 ml of 0.1 M-potassium phosphate buffer(pH 6.8) and dialyzed against the same buffer for 24 h at 4°C. The crude GTase of *S. mutans*, the liquid present in the dialysis membrane after the dialysis, was stored at -20°C.

In order to observe the inhibitory effect on the crude GTase, 0.1 ml of the crude enzyme, 0.2 ml of sucrose solution, 0.2 ml of 1 mM separated antibiotic, and 0.5 ml of 0.1 M K-phosphate buffer (pH 6.8) were added into a test tube (2.0×180 mm) and reacted at 37°C for 24 h. Then, 1 ml of 2% trichloroacetate was added into the test tube, which was then heated to 100°C for 5 min to stop the reaction. The mixture was immediately centrifuged at 3,000 ×g for 10 min to collect the precipitate, which was washed with 5 ml distilled water 3 times and dissolved with 1 N-NaOH. Using the DNS method, both the amount of glucan produced and the enzyme activity were calculated from the standard curve.

Kinetic Study of Glucosyltransferase Suppression

In order to calculate the Michaelis constant (K_m) of the glucosyltransferase for sucrose, the GTase activity was measured by reacting 4.0 units of the enzyme at 37°C for 15 min. The apparent K_m value was determined using a Lineweaver-Burk plot. To study the type of inhibition for the antibiotic substance against GTase, varying concentrations of the antibiotic substance were preincubated at 37°C for 30 min without the addition of sucrose, and then reacted with varying substrate concentrations to measure the GTase activity. The inhibitory pattern was determined by a Lineweaver-Burk plot. Also, in order obtain the inhibitor constant (K_i) on GTase, the GTase activity was measured by varying substrate concentration after a 30 min prereaction at 37°C for 30 min without the addition of sucrose, for a Dixon plot. The inhibition constant was then calculated from the value of the x-coordinate, which is the intersection point of the straight lines of other substrate concentrations.

Cytotoxicity Test Against Human Gingival Cells

In order to measure the effect of the purified antibiotic substance on the activity of human gingival cells, the method of Kim [21] was used. The human gingival cells activated through subculturing were treated with a trypsin EDTA (0.05% trypsin, 0.53 mM EDTA, Gibco/BRL, U.S.A.) solution to make a suspension, which was then centrifuged to obtain single cells. The cells obtained were placed in microtest plate wells (1×10^4 cell/well) and cultured in DMEM (Dulbecco's Modified Eagle's Medium, Gibco/BRL, U.S.A.) containing 1% FBS (fetal bovin serum; Gibco/BRL, U.S.A.) in a CO_2 incubator at 37°C, CO_2 5%, and 100% humidity for 72 h. In order to remove the cells not attached in the cultured medium, the cultured cells were washed with DMEM (not containing FBS) and

cultured again with DMEM (containing 1% FBS) treated with the antibiotic substance. Then, 250 µl of MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide, Sigma Co., U.S.A.] dissolved in phosphate buffer solution was placed into each well, which was cultured in a CO_2 incubator for 4 h. In order to remove the MTT solution in the culture medium and to dissolve formazan crystals, 200 µl of DMSO (dimethyl sulfoxide, Sigma Co., U.S.A.) was added, the mixture was then moved to a 96-well plate, and the absorbance was read at 570 nm to evaluate the cytotoxicity using an ELISA analyzer (spectra MAX 250, Molecular Devices Co., U.S.A.). The cellular activity was calculated as percentile using the following formula and treated statistically according to the SAS system.

$$\text{Cellular activity}(\%) = \frac{\text{absorbance of the well}}{\text{absorbance of the control well}} \times 100$$

Preparation of Toothpaste

The toothpaste was prepared by dispersing a humectant into the isolated antibiotic substance at a concentration of $2.5\times10^{7}-10^{9}$ at 2,500 rpm for 10 min, and it was further mixed for 5 min after adding a binder. After dispersing and dissolving a sweetener, preservative, and tubiditor in distilled water, the mixture was mixed at 2,500 rpm for 10 min and then further mixed at 2,500 rpm for 20 min after the polisher was added. Then, a spice and bubble agent were added into the mixture, which was then placed under depressurized conditions to remove air and kept in an air-tight container until use.

The process of making the toothpaste is summarized in Fig. 1.

Measurement of Decalcification Ratio in Teeth Enamel

To measure the effect of the isolated antibiotic substance on existing tooth tissue, extracted teeth from healthy adults of 20–30 years of age were affixed in Epoxy resin, and the samples were prepared with the exposed surface at the length and width of 2 mm×2 mm, and the initial hardness was measured 6 times using a Knoop Hardness tester. The samples were washed with distilled water after being submerged in the toothpaste (composition not shown), and this process was repeated for a week. The decreased rate of hardness compared to the initial hardness was determined as the decalcification ratio.

The decalcification ratio was calculated using the following formula

$$\text{Decalcification ratio} = \frac{(\text{initial hardness} - \text{hardness after } t \text{ hrs})}{\text{Initial hardness}}$$

Quantification of the Calcium Ion Eluted from Teeth

In order to measure the amount of calcium ion eluted from teeth, extracted teeth from healthy adults of 20–30 years old were fixed with Epoxy resin, and the samples were prepared with the exposed tooth surface at the length and width of 2 mm×2 mm, submerged in the toothpaste for 3 min, and then washed with 1 ml distilled water 5 times. The collected distilled water was centrifuged for 20 min at 5,000 ×g, then the absorbance of the supernatant was read, and the calcium ion concentration was calculated

Humectant
↓ added $2.5\times10^{7}-10^{9}$ M aminoglycoside compound
Mixed
↓ 2,500 rpm, 10 min
↓ added binder
Mixed
↓ 2,500 rpm, 5 min
↓ added Sweetener and Tubiditor in distillied water
Mixed
↓ 2,500 rpm, 10 min
↓ added Polisher
Mixed
↓ 2,500 rpm, 10 min
↓ added Spice, Bubble agent
Mixed

Fig. 1. Procedure for toothpaste production.

RESULT AND DISCUSSION

Measurement of Cell Membrane Permeability of *Streptococcus mutans* JC-2

In order to measure the effect of the aminoglycoside antibiotic substance, produced by *Bacillus alkalophilsaggy* JY-827, on the cell membrane permeability of *S. mutans*, the extract was added at different concentrations to measure the cell membrane permeability, the eluted cytoplasm substance was quantified colorimetically by a series of methods, and the results are shown in Fig. 2. At the concentrations of 2.5×10^{-7} M and 2.5×10^{-8} M, the value increased compared with that of the control, and the value was similar with that of the control at 2.5×10^{-9} M. Other than the aminoglycoside antibiotic produced by a novel microbe inhibiting the growth of *S. mutans* JC-2 by lowering m-RNA function, the aminoglycoside antibiotics including streptomycin, kanamycin, capreomycin, and neomycin inhibit the protein synthesis of the liposomes of the examined microbe. The general results of the aminoglycoside antibiotics participating in the inhibition of protein synthesis and cell membrane permeability are based on the study by Jung [20], who reported that some aminoglycoside antibiotics act on cell membrane. Lanford *et al.* [25] reported that these antibiotics interacted with receptors on the cell surface, and Marche *et al.* [27] reported that these antibiotics play a

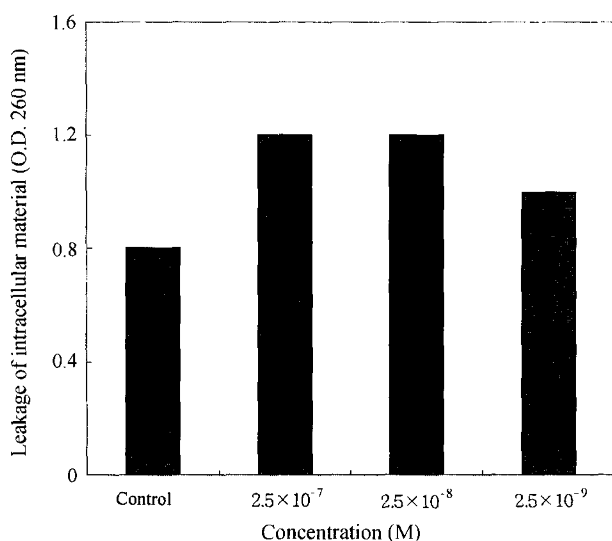

Fig. 2. Effect of aminoglycoside antibiotic on the cellular permeability of *Streptococcus mutans* JC-2.

role in transport of substances through the ion channel with the degree of participation proportional to the concentration of the antibiotic.

Suppressive Effect on Glucosyltransferase Activity

As an extracellular enzyme secreted by *S. mutans*, glucosyltransferase (GTase) forms plaque by synthesizing insoluble glucan, a type of polysaccharide having viscosity, from various sugars remaining in the mouth after food intake. Thus, since the development of dental caries could be stopped at the early stage by inactivating GTase or preventing the activity of GTase, we measured the inhibitory effect of the aminoglycoside antibiotic substance on the activity of *S. mutans* GTase. The result in Table 1 shows that the GTase activity was inhibited by the aminoglycoside antibiotic substance to reduce the amount of insoluble glucan synthesis and that the inhibitory rate increased with increasing concentration. Thus, the amounts of insoluble glucan measured by absorbance were 0.486±0.010 at 2.5×10^{-8} M, 0.198±0.005 at 2.5×10^{-7} M, and 0.001±0.001 at 2.5×10^{-6} M, and the inhibitory rates of insoluble synthesis

Table 1. Inhibitory effect of aminoglycoside antibiotic on glucosyltransferase activity from *Streptococcus mutans* JC-2.

Concentration (M)	Synthesized insoluble glucan (O.D. 550 nm)	Inhibition (%)
0	0.642±0.005	-
2.5×10^{-9}	0.524±0.010	19.39
2.5×10^{-8}	0.486±0.010	25.30
2.5×10^{-7}	0.198±0.005	70.16
2.5×10^{-6}	0.001±0.001	99.85

Mean±standard deviation.

were 25.30, 70.16, and 99.8%, respectively, compared with those in the control, thus indicating no synthesis and enzyme activity of insoluble glucan even at 2.5×10^{-6} M.

The activity of GTase is the important up-stream target in the pathological cascade. GTase participates in two reactions; it hydrolyzes sucrose into fructose and the glucozyl section that combines with enzyme, and transfers to the C-3/C-6 section of the glucose residue of glucan. According to the characteristics of the enzyme, GTase has a relatively independent domain structure [17]. *S. mutans* produces three types of enzyme including GTase-S1 that synthesizes glucan, GTase-1 that synthesizes water soluble glucan with α-1,3 binding, and GTase-3 that synthesizes water soluble glucan with α-1,6 binding [38].

GTase-1 has the signal peptide composed of 34 residues at the N-terminus and composed of 1,475 amino acids with DSIRVDAVD (446–454 residue) as the sequence participating in sucrose synthesis, and with 451 Asp as one of the main catalyst activity centers. The C-terminal glucan binding site is composed of six repetitive units having about 65 amino acid residues, and more than three units are needed for glucan synthesis [2]. Among these, aminoglycoside antibiotics are known to act on 451 Asp to lower the enzyme activity. As for inhibitors of enzyme activity, Park *et al.* [32] reported that (+)-catechin inhibits glucan synthesis by 99.8% at 1,000 g/ml, Kim [22] reported that propolis extracted and purified from honeycombs inhibited the enzyme activity by 93.9% at 1,600 μg/ml, and Park and Shin [31] reported that Phellodendin cortex. L water extract at 1,000 μg/ml inhibited the enzyme activity by 63.3%. Thus, the aminoglycoside antibiotic in the present study inhibited the enzyme activity effectively at relatively low concentration compared with these studies.

Kinetic Study of Glucosyltransferase Suppression by the Antibiotic Substance

The double reciprocal plot of Lineweaver-Burk was employed to calculate the Michaelis constant (K_m) of 6.66 mM for sucrose, (Fig. 3). Also, when the double reciprocal plot of Lineweaver-Burk was plotted to compare the affinity of aminoglycoside compounds produced by novel microbes on the glucosyltransferase, a competitive inhibition was shown, as in Fig. 4. Furthermore, a Dixon plot was drawn to calculate the K_i for GTase by varying the concentration of the antibiotic substance and the results are shown in Fig. 5. The K_i value calculated from the figure was 3.75×10^{-7} M, which was lower than the K_i value of peptide of 10.5 μM reported by Akiko *et al.* [2]. We believe that this low value was due to the different structure and characteristics of the compound.

Cytotoxicity Test Against Human Gingival Cell

The results of the cellular activity of the aminoglycoside compound determined to have an anticaries effect on

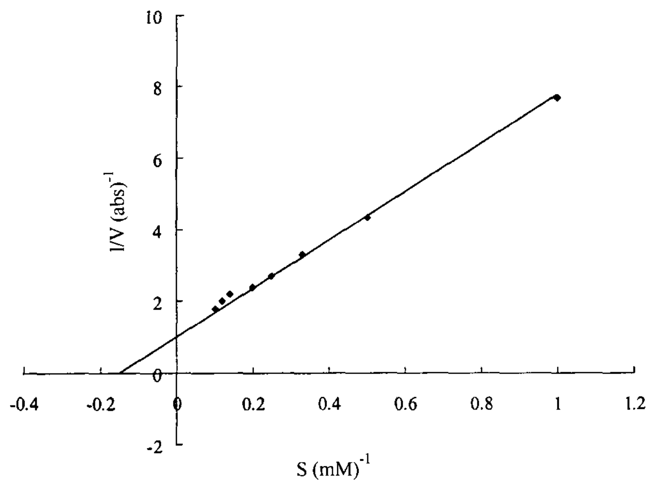

Fig. 3. Lineweaver-Burk plot of GTase on the sucrose.

human gingival cells are shown in Table 2. The cellular activity was decreased with the increase in the concentration of aminoglycoside compound, but the degree of decrease was very slight; with the addition of 5×10^{-7} M, the cellular activity was 83.33% on the first culture day, 87.5% on the second day, and 89.52% on the third day, and no significant difference was observed with the control under this concentration. The similar problem of strong cytotoxicity has also been encountered with other antibiotic substances. For example, despite the cytotoxicity on human gingival cells with 20 μg/ml, chlorhexidine is currently being widely used as the dental caries and stomatosis preventive treatment agent [24], and Jang *et al.* [19] reported that this

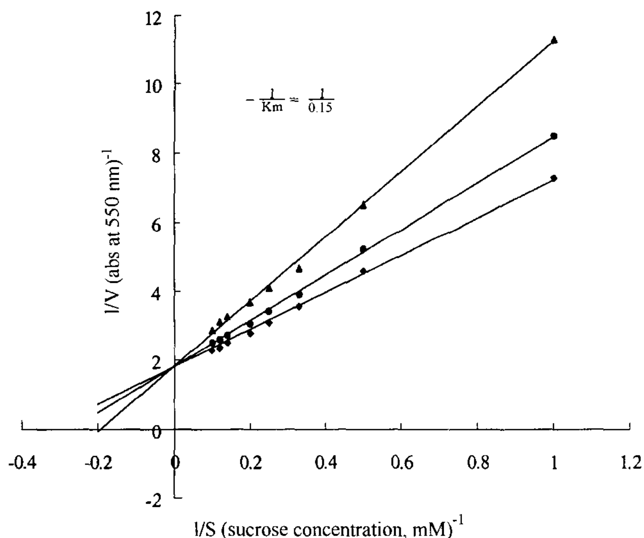

Fig. 4. Lineweaver-Burk plot of GTase inhibition by aminoglycoside compound.
Inhibition concentration: ◆ Control, ● 2.5×10^{-7} M, ▲ 2.5×10^{-8} M.

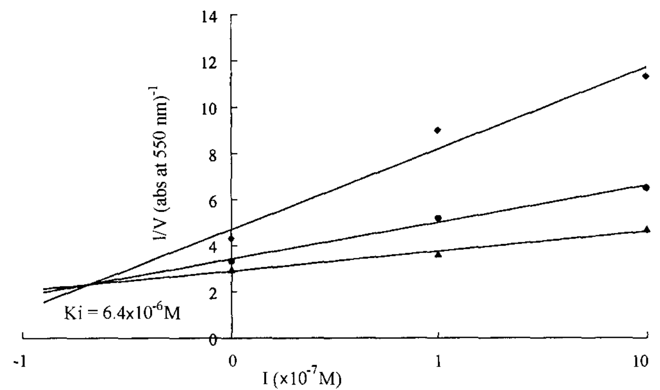

Fig. 5. Dixon plot of GTase inhibition by the aminoglycoside compound.
Sucrose concentration: ◆ 0.1 mM, ● 0.2 mM, ▲ 0.3 mM.

agent decreases the gingival cell activity to 75.2% and 99.7% at the concentrations of 1.0 μg/ml and 10.0 μg/ml, respectively.

Accordingly, it would appear that the aminoglycoside antibiotic in the present study has a high potential to replace the current commonly used dental caries and stomatosis preventive treatment agent, chlorhexidine; chlorhexidine causes various side effects such as coloration of tooth, desquamation of oral epithelical cells, and ageusia.

Manufacture of Toothpaste and Examination of its Characteristics

Measurement of the decalcification ratio of tooth enamel. The antibiotic at 2.5×10^{-7}–10^{-9} M was dispersed with humectant and decompressed after adding a binder, sweetener, preservative, tubiditor, polisher, spice, and bubble agent to remove any air, and the decalcification ratio of human extracted tooth enamelum of the toothpaste was measured. As shown in Table 3, the toothpaste with the addition of 2.5×10^{-7} aminoglycoside compound inhibited

Table 2. Cell activity of the aminoglycoside compound on human gingival fibroblast cell.

Incubation time (day)	Concentration (M)	Relative cell activity (%)
1	0	100
	5×10^{-9}	93.33
	5×10^{-8}	85.58
	5×10^{-7}	83.33
2	5×10^{-9}	94.91
	5×10^{-8}	92.13
	5×10^{-7}	87.50
3	5×10^{-9}	95.87
	5×10^{-8}	94.92
	5×10^{-7}	89.52

Table 3. Effect of the aminoglycoside compound from *Bacillus alkalophilshaggy* JY-827 on tooth decalcification.

Concentration (M)	Decalcified ratio of tooth (%)
Control	1.34
2.5×10^{-7}	0.78
2.5×10^{-8}	0.98
2.5×10^{-9}	1.06

the decalcification by about 50%, higher than the inhibition rate of 38% reported in the patent [26].

Assay of calcium ion eluted from teeth. The results of the amount of calcium ion eluted from the toothpaste, which was made by adding the separated antibiotic at the concentration of $2.5\times10^{-7}-10^{-9}$ M, on teeth extracted from humans, are shown in Table 5. The aminoglycoside compound at a concentration of 2.5×10^{-7} M prevented the elution of calcium ion by about 2.5 times.

From these results, the aminoglycoside compound, which was separated from *B. alkalophilshaggy* JY-827 and then purified, was concluded to have an excellent anticaries activity and a high potential for commercial application.

Conclusion

The aminoglycoside antibiotic substance produced by *B. alkalophilshaggy* JY-827, which is an effective strain in reducing the growth of *S. mutans* JC-2, decreased the activity of *S. mutans* JC-2 glucosyltransferase by 99.8% at a concentration of 2.5×10^{-7} M. The antibiotic also showed a competitive inhibition against glucosyltransferase, with K_i value of 6.4×10^{-6} M and exhibited no toxicity against human gingival cells. A toothpaste made with this substance inhibited tooth calcification by about 50% and reduced calcium ion elution by 2.5 fold. Since the aminoglycoside antibiotic substance produced by the novel basophilic microorganism, *B. alkalophilshaggy* JY-827, not only showed an excellent anti-dental caries activity, but also exhibited no toxicity against gingival cells with its excellent effectiveness when introduced into toothpaste, it is concluded that this substance showed a strong potential as the substance that could prevent dental caries and showed a strong possibility to replace chlorhexidine, which is currently being used in toothpaste for the prevention of dental caries.

Table 4. Effect of the aminoglycoside compound from *Bacillus alkalophilshaggy* JY-827 on calcium ion eluted from teeth.

Concentration (M)	Decalcified ratio of tooth (%)
Control	0.41
2.5×10^{-7}	0.12
2.5×10^{-8}	0.30
2.5×10^{-9}	0.33

Acknowledgment

This study was supported by a research grant (2001) from Wonkwang Health Science College.

REFERENCES

1. Addy, M. 1986. Chlorhexidin compared with other locally delivered antimicrobial, A short review. *J. Clin. Peridentol.* **13:** 957–964.
2. Akiko, E., C. Takaomi, K. F. Saido, T. Shigeo, I. Susumu, N. Tosiki, and H. Nobuhiro. 1999. Inhibitory effect of a self-derived peptide on glucosyltransferase of *Streptococcus mutans*. *J. Biol.Chem.* **274:** 15797–15802.
3. Aok, H., T. Shiroza, M. Hayakawa, S. Satto, and H. K. Kuramitsu. 1986. Cloning of *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. *Infect Immun.* **53:** 587–594.
4. Bonesvoll, P. and P. Giermo. 1978. A comparison between chlorhexidin and some quaternary ammonium compound with regard to retention, salivary concentration, and plaque inhibiting effect in the human mouth after mouth rinse. *Arch. Oral Biol.* **23:** 289–294.
5. Chun, J. Y., I. H. Ryu, S. U. Lee, and K. S. Lee. 2000. Isolation and identification of novel alkalophilic *Bacillus alkalophilshaggy* JY-827 with anticaries microbe *Streptococcus mutans* JC-2. *Kor. J. Appl. Microbial Biotechnol.* **28:** 243–250.
6. Chun, J. Y., I. H. Ryu, S. U. Lee, and K. S. Lee. 2000. Production condition, purification and properties of anticaries microbial agent by *Bacillus alkalopilshaggy* JY-827. *Kor. J. Appl. Microbial Biotechnol.* **28:** 270–278.
7. Ciardi, J. E., W. H. Bowen, and G. Rolla. 1978. The effect of antibacterial compound on glucosyltransferase activity from *Streptococcus mutans*. *Arch. Oral Biol.* **23:** 301–305.
8. Clarke, J. K. 1924. On the bacterial factor in the aetiology of dental caries. *J. Exp. Pathol.* **5:** 141–150.
9. Critchley, P. 1969. The breakdown of the carbohydrate and protein matrix of dental plaque. *Caries Res.* **3:** 249–265.
10. David, W. and W. H. Bowen. 1999. Action of agents on glucosyl-transferase from *Streptococcus mutans* in solution and adsorbed to experimental pellicle. *Arch. Oral Biol.* **44:** 203–214.
11. Gibbons, R. T. and J. van Houte. 1975. Bacterial adherence in oral microbial ecology. *Annu. Rev. Microbiol.* **29:** 19–44.
12. Hada, S., N. Kakiuchi, M. Hattori, and T. Namba. 1989. Identification of antibacterial principle against *Streptococcus mutans* and inhibitory glucosyltransferase from the seed of *Areca catechu* L. *Phytothe Research* **3:** 140–144.
13. Hamada, N. and H. K. Kumamitsu. 1989. Isolation and characterization of the *Streptococcus mutans* gtf C gene, coding for primer dependent soluble glucan synthesis. *Infect. Immun.* **57:** 2079–2085.
14. Hamada, S. and H. D. Slade. 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. *Microbiol. Rev.* **44:** 331–384

15. Hamada, N. and H. K. Kumamitsu. 1988. Isolation and characterization of the *Streptococcus mutans* gtf C gene, coding for synthesis of both soluble and insoluble glucan. *Infect. Immun.* **56:** 1999–2005.
16. Hamada, S. and S. Toroii. 1980. Interaction of glucocyltransferase from *Stereptococcus mutans* with various glucans. *J. Gen. Microbiol.* **116:** 51–60.
17. Hanada, N., Y. Isobe, Y. Aizawa, T. Katayama, S. Sato, and M. Inoue. 1993. Nucleotide sequence analysis of the gtf-1 gene from *Streptococcus sobrinus* OMZ176. *Infect. Immun.* **61:** 2096–2103.
18. Hotz, P., B. Guggenheim, and R. Schmid. 1972. Carbohydrate on pooled dental plaque. *Caries Res.* **6:** 103–121.
19. Jang, B. S., C. P. Chung, S. H. Son, and K. H. Bae. 1993. The effect of honoliol and magnolol on the antimicrobial, bacterial collagenase activity, cytotoxicity and cytokine production. *J. Korean Acad. Periodontology* **23:** 145–158.
20. Jung, D. H. 1989. *Antibiotics*. Korea Medical Publishing Co. pp. 83-167.
21. Kim, D. B. 1994. Effect of propolis against caries activity with *Streptococcus mutans*. Chunbuk Graduated thesis, Korea.
22. Kim, S. S. 1991. Effect of tannin and vitamin B6 on the growth and attachment to the test tube wall of *Streptococcus mutans* 10449. Wonkwang Graduated thesis, Korea.
23. Koo, H., B. P. F. A. Gomes, P. L. Rosalen, G. M. B. Ambrosano, Y. K. Park, and J. A. Cury. 2000. *In vitro* antimicrobial activity of propolis and *Arnica montana* against oral pathogens. *Arch. Oral Biol.* **45:** 141–148.
24. Lander, P. Z., G. M. Newcomb, G. J. Seymour, and R. N. Dowell. 1986. The antimicrobial and clinical effects of a single subgingival irrigation of chlorhexidine in advanced periodontal lesions. *J. Clin. Periodont.* **13:** 74–80.
25. Lanford, R. E., L. Estlack, and A. L. White. 1996. Neomycin inhibits secretion of apolophoprotein by increasing retention on the hephatocyte cell sulface. *J. Lipid Res.* **37:** 2055–2064.
26. Lucky Company, 1991. Toothpaste Composition, KP 91-2095.
27. Marche, P., S. Koutouzov, and A. Girard. 1983. Impairment of membrane phosphoinositide metabolism by aminoglycoside antibiotics, streptomycin, amicacin, kanamycin, dibekacin, gentamycin and neomycin. *Amer. Soc. Pharm. Exp. Therap.* **227:** 415–420.
28. Musaka, T., M. Tsunezuka, Y. Takehara, and S. Nunome. 1979. *Infect. Immun.* **23:** 564–570.
29. Nakahara, K., S. Kawabata, H. Ono, K. Ogura, T. Ooshima, and S. Hamada. 1993. Inhibitory effect of oolong tea polyphenol on glucosyltransferase of *Streptococcus mutans*. *Appl. Environ. Microbiol.* **59:** 968–973.
30. Ooshima, T., T. Minami, W. Aono, A. Izumitami, S. Sobue, T. Fujiwara, S. Kawabata, and S. Hamada. 1993. Oolong tea polyphenols inhibit experimental dental caries in SPF tat infected with *Streptococcus mutans*. *Caries Research* **27:** 124–129.
31. Park, C. S. and Y. S. Shin. 1995. Effect of *Phellodendri cortex* L. on the activity of glucosyltransferase and human Gingival cell, growth and membrane permeability of *Streptococcus mutans* JC-2. *J. Korean Acad. Dental Health* **19:** 447–456.
32. Park, C. S., Y. S. Shin, K. S. Lee, I. H. Kang, and S. S. Kim. Effect of *Phellodendri cortex* L. on the activity of glucosyltransferase and human gingival cell, growth and membrane permeability of *Streptococcus mutans*. *J. Korean Acad. Periodontology* **21:** 245.
33. Platt, F. M., G. R. Neises, R. A. Dwek, and T. D. Butters. 1994. N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. *J. Biol. Chem.* **269:** 8362–8365.
34. Salem, A. M., D. Adams, H. N. Newman, and L. W. Rawle. 1987. Antimicrobial properties of two aliphatic amines and chlohexidin *in vitro* and in saliva. *J. Clin. Periodontol.* **14:** 44–47.
35. Tanzer, J. M., M. L. Freedman, and R. J. Fitzgerald. 1985. Virulence of mutans defective in glucosyltransferase, dextran, mediated aggregation or dextranase activity. *In* Mergenhagen, S. E. and Rosan, B. (eds.). *Molecular Basis of Oral Microbial Adhesion*. ASM. Washington. USA. pp. 204–211.
36. Vacca-Smith, A. M., A. R. Venkitaraman, K. M. Schilling, and W. H. Bowen. 1996. Characterization of glucosyltransferase of human saliva adsorbed on to hydroxyapatite surfaces. *Caries Res.* **30:** 354–360.
37. Yamashita, Y., W. H. Bowen, R. A. Burne, and H. K. Kuramitsu. 1993. Role of the S*treptococcus mutans* ftf genes in caries induction in the specific-pathogen-free rat model. *Infect. Immun.* **61:** 3811–3817.