Pediococcus pentosaceus K1270에 의한 인공치태 형성억제 효과

최외임 · 한수지* · 김 신

부산대학교 치과대학 소아치과학교실,
피츠버그대학 약학대학 약학과 약물유전학센터*

국문초록

치태형성을 억제하는 유산균 K1270을 김치로부터 분리한 후 배양적, 생화학적 특징 및 16S rDNA 염기서열 분석에 의해 *Pediococcus pentosaceus* K1270으로 동정하였다. K1270 균주는 5% 자당이 함유된 배지에서 *Streptococcus mutans* Ingbritt에 의한 인공치태 형성을 대조군에 비해 94.6 % 억제하였으며, 비수용성 글루캔의 합성을 89.6 % 억제하였다. *S mutans* Ingbritt의 증식은 대조군에 비해 100배 정도 억제하였다. K1270균주는 TMB와 peroxidase가 첨가된 MRS 한천배지에서 과산화수소를 생산하였으며, 인공치태 형성에 대한 K1270균주의 억제 효과는 catalase 첨가에 의해 일부 감소되었다. K1270 균주의 배양 상청액을 10 % 자당이 함유된 2×M17 broth에 동량 가한 경우 인공치태 형성 및 *Streptococcus mutans* Ingbritt의 증식이 억제되었으며, 이 억제효과는 catalase첨가에 의해 일부 감소되었고, 열처리, 또는 trypsin 처치에 의해 완전히 소실되었다. 따라서, 본 연구에서 분리, 동정된 *P. pentosaceus* K1270은 과산화 수소와 bacteriocin 유사물질을 분비하여 *S. mutans* Ingbritt의 증식을 억제함으로써 인공치태 형성을 억제하는 것으로 사료된다.

주요어 : 인공치태, 김치, 치태형성억제, *S. mutans*, *P. pentosaceus*

Ⅰ. Introduction

Human dental plaque, which is critical in the pathogenesis of dental caries and periodontal disease, is mainly composed of oral bacteria and some of non-cellular materials such as glucan and fructan [1,2]. Among microorganisms in dental plaque, *Streptococcus mutans* is primarily a concern of the etiology of human dental caries [3,4]. *S. mutans* has a high ability to form cariogenic dental plaque on tooth surface through de novo synthesis of water-soluble and water-insoluble glucan from sucrose by the action of glucosyltransferase (GTF: EC 2.4.1.5) [5-7]. The stickiness of water-insoluble glucan promotes bacterial adherence and accumulation on tooth surface [8,9], followed by the formation and growth of dental plaque, which is a large bacterial successive community.

There have been a lot of efforts to inhibit the growth of *S. mutans* and formation of dental plaque including chlorhexidine, antibiotics, triclosan, and sanguinarine [10-15]. Also there have been reports on enzymatic reduction of dental plaque by use of glucanase, which is hydrolyzing dextran or mutan [16-19]. However, despite the significant advances in the control of dental caries and periodontal disease, these remain widespread and costly to be treated. Thus, for the effective and economic prevention of dental caries and periodontal disease, it's necessary to develop a new method for the inhibition of plaque formation.

The growth inhibition of one bacterial species by another is a well-recognized mechanism as bacterial

교신저자 : 김 신

부산시 서구 아미동 1가 10번지
부산대학교 치과대학 소아치과학교실
Tel : 051-240-7449
E-mail : shinkim@pusan.ac.kr

antagonism. Lactic acid bacteria (LAB) have been suggested to protect the gastrointestinal systems from many other pathogenic infections in human and animals. It has been reported that LAB can kill pathogens, detoxify carcinogens, metabolize cholesterol, and enhance the immune response [20-23]. Against the pathogenic bacteria, LAB shows antibacterial activity by producing the bactericidal compounds like organic acids lowering pH [24,25], low-molecular weight bacteriocins [26-30], and H_2O_2 [31-33]. Thus LAB has been vigorously challenged for the use in probiotics whose concept was evolved from a theory first proposed by Elie Metchnikoff, who suggested that the long life span of Bulgarian peasants resulted from their consumption of fermented milk products containing LAB [34]. But, there has no report on the LAB for probiotic application to *S. mutans*.

In this study, we have isolated and identified the LAB, which inhibited the biofilm formation by *S. mutans* Ingbritt, from *kimchi*, Korean traditional vegetable food that many LAB inhabit. Furthermore, the inhibitory mechanism of action was also investigated.

Ⅱ. Materials and Methods

2. 1. Bacterial strains and media

Streptococcus mutans Ingbritt was grown at 37℃ in brain heart infusion (BHI, Difco Laboratories, Detroit, MI, USA) or M17 (Difco) medium. LAB isolated from homemade *kimchi* was cultured in MRS (Difco) or Rogosa (Difco) media. These bacterial strains were stored at -70℃ in appropriate broth with 20 % glycerol and the stock cultures were propagated twice in BHI or MRS broth for 18 h before each experiment.

2. 2. Isolation of LAB from kimchi

Homemade *kimchi* was used as the source of LAB. About 1.0 g per each *kimchi* sample from total 497 samples were soaked in 10 ml of MRS broth and the supernatant fluid was serially diluted with sterile 0.9% NaCl solution and spread on MRS agar. After incubation at 37℃ for 48 h, the colonies were picked up and subcultured on fresh MRS agar plates. After grown at 37℃ for 24 h, LAB was successively transferred on MRS agar several times until pure colonies were obtained. The isolated strains were stored at -70℃ in MRS broth with 20 % glycerol. Before used in each experiment, LAB was propagated twice in MRS broth overnight.

2. 3. Agar spot test

The entire isolated LAB was tested for their capacity to inhibit the growth of *S. mutans* Ingbritt. *S. mutans* Ingbritt ($1x10^6$ CFU) was inoculated in 3ml of medium A (mixture of equal volume of BHI and MRS with 0.1 M of MES (2-(N-Morpholino) ethanesulfonic acid Monohydrate, pH 6.5) containing 0.7 % agar, and then transferred to the same medium. After the liquid of the plates was absorbed, each isolated LAB was spotted on the agar overlaid with *S. mutans* Ingbritt with toothpicks. These plates were incubated at 37℃ for 24 h and the inhibition zone around the LAB spot was examined. The LAB colonies surrounded by clear zone were selected for next experiments.

2. 4. Isolation of LAB inhibiting the biofilm formation

The employed test system was a modification of the technique described by McCabe *et al.*[35]. We named this test system 'Beaker-wire test'. The 0.016-inch stainless steel wires (Ormco, Glendora, CA, USA) were inserted in cork stoppers and suspended in beakers containing 40 ml of medium A containing 5 % sucrose. Equal amount of *S. mutans* Ingbritt strain and LAB selected from agar spot test were inoculated into medium A without 5% sucrose. *S mutans* Ingbritt strain without LAB was inoculated for control. After incubation at 37℃ for 24 h, the wires were examined for selecting the LAB which inhibited the biofilm formation. To confirm the inhibitory effects of the selected LAB, the same experiments were repeated three times and the biofilm on the wires were weighed. Only one LAB completely inhibited the biofilm formation by *S. mutans* Ingbritt. We named this isolated LAB 'K1270'.

2. 5. Inhibition test for the formation of insoluble glucan and the replication of *S. mutans* Ingbritt

The synthesis of insoluble glucan by *S. mutans* Ingbritt was measured by determining the optical density at 550 nm, as described previously[36,37]. The isolated K1270 and *S. mutans* Ingbritt which were grown in MRS and M17 broth, respectively, were inoculated into 3 ml of medium A containing 5 % sucrose in disposable cuvettes and placed at 30 ℃ for 24 h, the culture supernatant was discarded and the pellet was washed twice gently with phosphate buffered saline (PBS). For a quantitative analysis, the optical density of insoluble glucan was measured at 550 nm by spectrophotometry (Pharmacia Co., Peapack, N.J., USA) after resuspended with 3 ml of PBS. Under the same condition, the number of viable *S. mutans* Ingbritt mixed with or without K1270 were counted on Mitis-Salivarius agar containing 0.2 U/ml of bacitracin (MSB).

To find the effect of culture supernatant of K1270 on the biofilm formation and the replication of S. mutans Ingbritt, the culture supernatant of K1270 grown in MRS broth at 37℃ for 24 h was centrifuged at 4,000 rpm for 10 minutes, adjusted to pH 6.5 by adding of 5 N NaOH, and sterilized by filtration with 0.45 um pore-sized filter. The supernatant with or without treatment of heat at 65℃ for 30 minutes or trypsin at 37℃ for 5 minutes 0.25mg/ml) was added into the equal amount of 2 X M17 broth containing 5 % sucrose and 0.1 M of MES in cuvettes which would be placed at 30 ℃ for 24 h, insoluble glucan was measured at 550nm just as described above. To find the effect of supernatant on bacterial replication under the same condition, the number of viable colonies of *S. mutans* Ingbritt was also counted on MSB agar plates.

2. 6. pH adjustment

Despite the addition of 0.1 M of MES into medium A to exclude the effect of organic acid, the pH of the culture fluid was maintained at pH 6.5 by adding 5 N NaOH every an hour. After 9 h incubation with or without pH adjustment, the weight of biofilm on orthodontic wires was measured. The series of above experiments were repeated three times.

2. 7. H_2O_2 production test

K1270 was inoculated onto MRS agar supplemented with 0.25㎎/㎖ of TMB (3, 3, 5, 5-tetramethylbenzidine, Sigma, St Louis, MO, USA.) and 0.01㎎/㎖ of peroxidase (Sigma), incubated at 37℃ for 24 h under anaerobic condition. H_2O_2 production was determined by observing the color change of K1270 strain into blue. To find whether inhibiting substance against biofilm formation was H_2O_2, catalase(10,000 U or 20,000 U) was added into culture mixture of *S. mutans* Ingbritt and K1270, and then beaker-wire test was carried out. The biofilm on wires were weighed after 24 h incubation at 37℃. The viable cells of *S. mutans* Ingbritt in culture mixture were also counted on MSB agar after serially diluted.

2. 8. The characterization and identification of K1270 strain

The isolated K1270 was identified based on Bergey's Manual of Systematic Bacteriology by Sneath et al.[38], carbohydrate fermentation pattern using the API-50 CHL system(BioM's instructions). Changes in color from violet were monitored after 24 h and 48 h. The results of carbohydrates fermentation were analyzed by APILAB PLUS software version 3.2.2.

2. 9. Chromosomal DNA extraction

Chromosomal DNA of K1270 was extracted as described previously[39]. Two-hundred ml of the culture was harvested, resuspended in 0.3ml of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5), incubated at 65 ℃ for 20 min, and added to a mixture of lysozyme-mutanolysin (40 ㎎/㎖ lysozyme, 200 U/㎖ mutanolysin) for 1 hour at 37℃ prior to the addition of 10% sodium dodecyl sulfate and proteinase K (20㎎/㎖). The mixture was added to a CTAB (hexadecyltrimethyl ammonium bromide)/NaCl solution, incubated for 10 min at 65℃, and extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) mixture. The crude DNA fraction was recovered using a o.6-fold volume of isopropanol. The

precipitated DNA was washed, dried, and resuspended in the TE buffer. Purity was determined by calculating *A260/A280* ratios, and DNA concentration was obtained from the *A260* values by spectrophotometry (Pharmacia).

2. 10. PCR amplification of 16S rDNA and Partial sequencing

The 16S rDNA was amplified from the chromosomal DNA, as prepared above, using a PCR. The oligonucleotide primers for PCR amplification were designed to include sequences of a region known to be highly conserved between species[40,41]. The sequences of the PCR primers used were 5' -AGAGTTTGATCMTGGCTCAG-3' (nucleotide positions 8 to 27 of the *Escherichia coli* 16S rRNA gene) and 5' -AAGGAGGTGWTCCARCC-3' (nucleotide positions 1,506 to 1,522 of the *Escherichia coli* 16S rRNA gene). The PCR reaction was performed using a thermal cycler (Perkin-Elmer 9700) for 30 cycles. The amplification program was as follows: denaturation at 94℃ for 30 sec, primer annealing at 50℃ for 30 sec, and extension at 72℃ for 5 min. The amplified PCR products were recovered and purified using a Wizard PCR Preps DNA Purification System (Promega). The purified PCR products were sequenced with an automatic DNA sequencer (ABI 310, Perkin-Elmer) using the primer 5' -AGAGTTTGATCMTGGCTCAG-3' (nucleotide positions 8 to 27 of the Escherichia coli 16S rRNA gene).

Ⅲ. Results

3. 1. Isolation of K1270 inhibiting the biofilm formation

From *kimchi*, 1,300 strains were isolated. Several strains including K1270 of them showed growth inhibitory effect on *S. mutans* Ingbritt. As shown in Figure 1, K1270 inhibited the growth of *S. mutans* Ingbritt and displayed clear zone around them when spotted on BHI agar previously overlaid with *S. mutans* Ingbritt. Of several strains inhibiting the growth of *S. mutans* Ingbritt, K1270 was proved to have strong inhibitory effect on the biofilm formation by Beaker-wire test. When the amount of biofilm produced on wires both in the control and in the experimental group was compared, K1270 inhibited the biofilm formation up to 94.6 % (Fig. 2). This experiment suggests that K1270 isolated from *kimchi* has the remarkable capability to inhibit the biofilm formation by *S. mutans* Ingbritt.

3. 2. Inhibition of the formation of insoluble glucan and the replication of *S. mutans* Ingbritt by K1270

The amount of insoluble glucan in the experimental group was decreased when compared to the control group. In this experiment, as shown in Figure 3, K1270 inhibited the production of insoluble glucan to 89.6 %. It was shown that K1270 inhibited the replication of S. mutans Ingbritt. By the effect of K1270, viable cells of *S. mutans* Ingbritt were decreased by about 20 folds after incubation for 9 h at 37℃ and 100 folds for 24 h (Table 1). These results indicated that the inhibitory effect of K1270 resulted from killing *S. mutans* Ingbritt in some degree.

3. 3. Acid effect on the biofilm formation

To rule out the effect of organic acid, pH of culture in which *S. mutans* Ingbritt and K1270 were growing was maintained at pH 6.5 by adding 5 N NaOH. Despite of neutralization of culture supernatant, K1270 still inhibited the biofilm formation by *S. mu-*

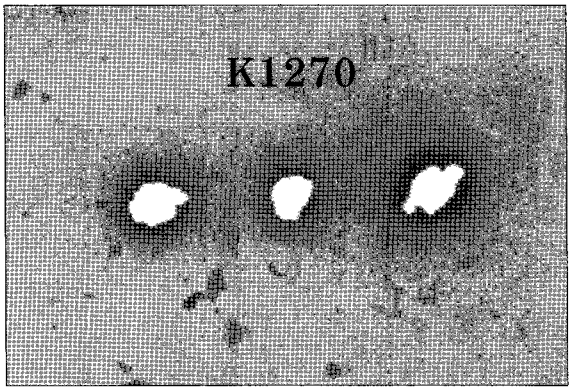

Fig. 1. The effect of K1270 isolated from *kimchi* on the growth of *Streptococcus mutans* Ingbritt. Clear zone was shown around the spot of K1270 after 24 h-incubation at 37℃ by the result of growth inhibition of *S. mutans* Ingbritt.

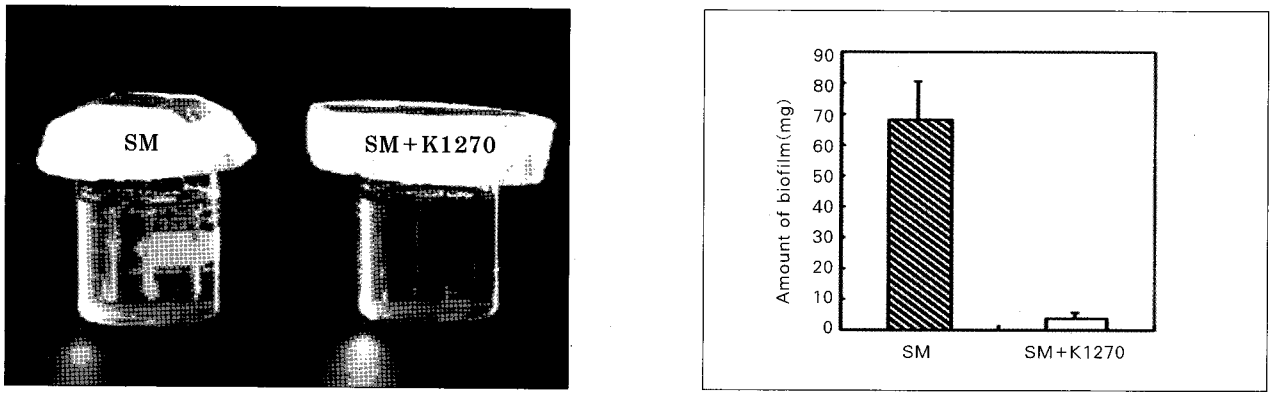

Fig. 2. The effect of K1270 on the biofilm formation by *S. mutans* Ingbritt. The biofilm formation in the experimental group in which *S. mutans* Ingbritt was cocultured with K1270 was completely inhibited when compared to the control group cultured without K1270. This experiment was repeated three times.

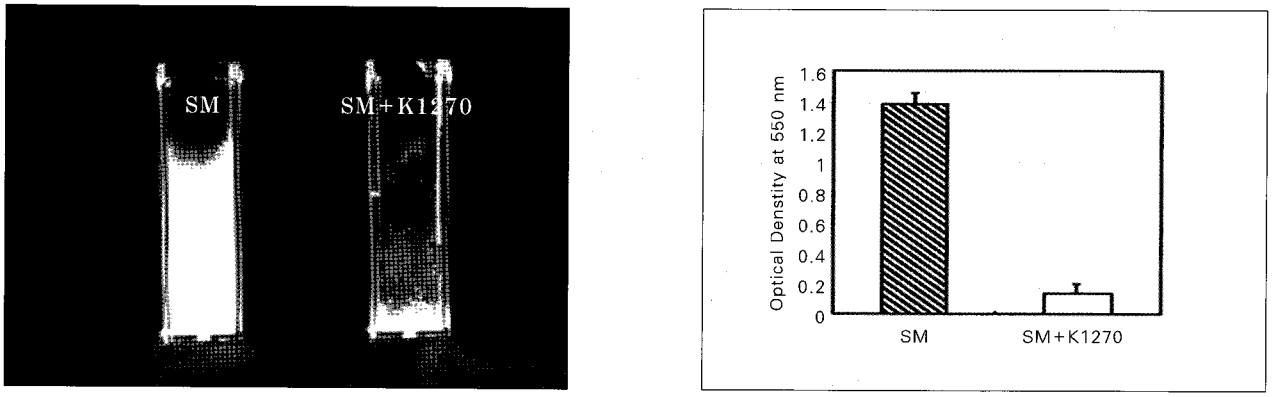

Fig. 3. The effect of K1270 on the production of insoluble glucan by *S. mutans* Ingbritt. The medium A was inoculated with *S. mutans* Ingbritt with or without K1270, and incubated at 37 ℃ for 24 h, placing 30 angle to horizontal plane. The biofilm formation was decreased in the mixed culture of *S. mutans* Ingbritt and K1270 than in *S. mutans* Ingbritt alone. This experiment was repeated three times.

tans Ingbritt (Fig. 4). The viability of *S. mutans* Ingbritt cocultured with K1270 in medium A was not affected by the pH adjustment (data not shown). This suggests that the inhibitory substance of K1270 is not organic acid.

3. 4. H_2O_2 production by K1270 and the effect of catalase

It was found that K1270 produced H_2O_2 by observing blue color of colony on MRS agar supplemented with TMB and peroxidase after incubation for 24 h at 37℃ under anaerobic condition. When 10,000 U or 20,000 U of catalase was added into the culture mixture including *S. mutans* Ingbritt and K1270, the amount of biofilm was increased by 11 % or 15 % when compared to the group without catalase. Namely, the inhibitory effect of K1270 on the biofilm formation was reversed by the addition of catalase in some extent. This experiment indicated that K1270 produced H_2O_2 to kill *S. mutans* Ingbritt, and in turn, it inhibited the biofilm formation.

3. 5. The effect of culture supernatant

As shown in Figure 5, the supernatant of K1270 inhibited the biofilm formation. The biofilm was decreased by about 50 % in experimental group than in control group. This effect was not affected by the addition of catalase but disappeared completely by trypsin treatment (0.25 mg/ml) at 37℃ for 5 minutes or heat treatment at 65℃ for 30 minutes. The

Table 1. Effect on the replication of *S. mutans* Ingbritt by K1270

Tested bacterial strains	Viable cells (CFU/ml)	
	9h	24h
S. mutans (MSB agar)	2.1×10^{8}	1.5×10^{9}
S. mutans + K127		
S. mutans (MSB agar)	1.8×10^{7}	2.0×10^{7}
K1270 (MRS agar)	1.1×10^{9}	3.2×10^{8}
K1270 (MRS agar)	6.3×10^{9}	5.5×10^{8}

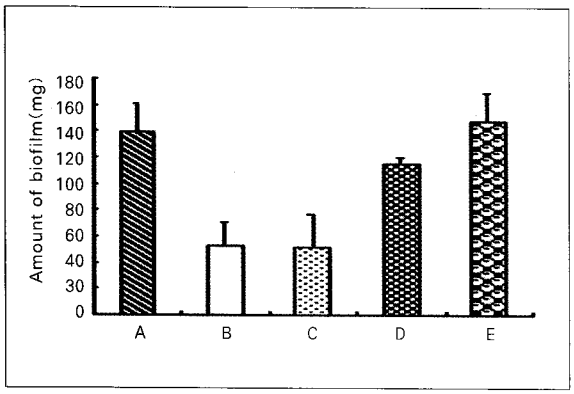

Fig. 5. The effect of culture supernatant of K1270. The supernatant of K1270 incubated in MRS broth at 37℃ for 24 h was prepared and mixed with the equal amount of 2 X M17 broth containing 5% sucrose and 0.1 M of MES. Beaker-wire test was employed for 24 h at 37℃ to measure the biofilm formation after the addition of *S. mutans* Ingbritt with or without heat or trypsin treatment. (A) *S. mutans* Ingbritt (SM) (B) SM + culture supernatant of K1270 (Sup) (C) SM + Sup + catalase (20,000U) (D) SM + Sup treated at 65℃ for 30 minutes (E) SM + Sup treated with 0.25mg/ml of trypsin at 37℃ for 5 minutes. This experiment was repeated three times.

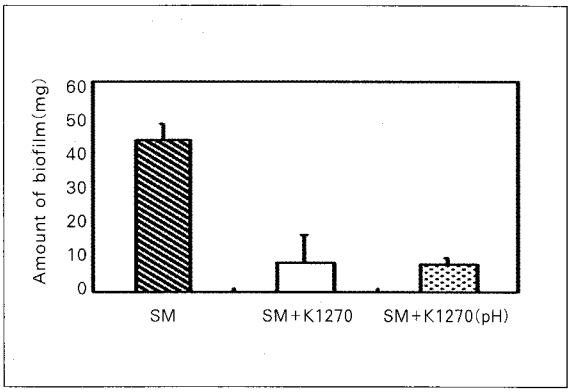

Fig. 4. The effect of pH adjustment on the biofilm formation. The pH of the culture fluid was maintained at pH 6.5 by adding 5 N NaOH every an hour. After 9 h, the weight of the biofillm on orthodontic wires was compared in each group. This experiment was repeated three times.

Table 2. Carbohydrate fermentation of isolated K1270

Fermented	D-Arabinose, L-Arabinose, Ribose, bMethyl-xyloside Galactose, D-Glucose, D-Fructose, N Acetyl glucosamine, Amygdaline, Arbutine, Esculine, Cellobiose, Maltose, Melibiose, Saccharose, Trehalose, D-Raffinose, D-Tagatose
Not fermented	Glycerol, Etythritol, D-Xylose, L-Xylose, Adonitol, D-Mannose, L-Sorbose Rhamnose, Dulcitol, Inositol, Mannitol, Sorbitol, a Methyl-D-mannoside, a Methyl-D-glucoside, Lactose, Inuline, Melezitose, Amidon, Glycogen, Xylitol, b Gentiobiose, D-Turanos, D-Lyxose, D-Fucose, L-Fucose, D-Arabitol, L-Arabitol, Gluconate, 2 ceto-gluconate, 5 ce-to-gluconate

supernatant also showed the inhibitory effect on the multiplication of *S. mutans* Ingbritt (data not shown). These results suggested that K1270 excrete bacteriocin as well as hydrogen peroxide into culture medium, thus leading to inhibition of the biofilm formation by killing *S. mutans* Ingbritt.

3. 6. The characterization and identification of K1270

The isolated K1270 was nonmotile, non-spore forming, Gram-positive, and catalase-negative cocci. Optimal temperature for growth was at 37℃. But it also grew at 50℃ not at 15℃ at which most LAB replicate well. In addition, it grew in the media containing 6.5% NaCl. It produced hydrogen peroxide. The pattern of carbohydrate fermentation of K1270 was summarized in Table 2. The 16S rDNA sequence containing

764 nucleotides is shown in Table 3. This sequence exhibited a high level of similarity (98.42%) to the *Pediococcus pentosaceus* sequence. Based on the 16S

Table 3. Partial sequence of 16S rDNA of isolated K1270

GCTGGCGGGCGTGCCTAATACATGGCAGTCGAACGAACTTCCGTTAATTGATTATG
ACGTACTTGTACTGATTGAGATTTTAACACGAAGTGAGTGGCGAACGGGGTGAGT
AACACGTGGGTAACCTGCCCAGAAGTAGGGGATAACACCTGGAAACAGATGCTAAT
ACCGTATAACAGAGAAAACCGCATGGTTTTCTTTTAAAAGATGGCTCTGCTATCAC
TTCTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGC
AGTGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGC
CCAGACTCCTACGGGAGGCAGCAGTAGGGGAATCTTCCACAATGGGACGCAAGTCT
GATGGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGT
TAAAGAAGAACGTGGGTAAGAGTAACTGTTTACCCAGTGACGGTATTTAACCAGA
AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTA
TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTCTTTTAAGTCTAATGTGAAA
GCCTTCGGCTCAACCGAAGAAGTGCATTGGAAACTGGGAGACTTGAGTGCAGAAGA
GGACAGTGGAACTCCATGTGTAACGGTGAAATGCGTAAATATTTGGAAGAACACC
AGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTC

rDNA sequence as well as cultural and biochemical characteristics, the isolated K1270 was identified as Pediococcus pentosaceus and named *Pediococcus pentosaceus* K1270.

Ⅳ. Discussion

LAB is commonly found in dairy products, plants, and fermented vegetables such as kimchi as well as in the gastrointestinal tract of humans and animals. *Kimchi* is a major traditional Korean vegetable food and has been reported to have many beneficial effects, including the prevention of colon cancer, the improvement of bowl movement, antimutagenic, and anticancer affects[42,43]. More than 30 species belonging to different genera of LAB have been reported in *kimchi*[44-46]. The LAB counts of optimally ripened *kimchi* (pH 4.3) are about 108 CFU/ml. Thus, we selected *kimchi* as LAB source. In our study, among 1,300 colonies grown on MRS and Rogosa SL media, which were isolated from 497 *kimchi* samples, one LAB excellently inhibiting the biofilm formation was isolated and identified.

Among the oral microorganisms, *S. mutans* is considered to be causative agent of dental caries. In this study, *S. mutans* Ingbritt classified as serotype c and highly associated with caries prevalence in worldwide was used for biofilm formation. Our experiment showed that the isolated K1270 inhibited the biofilm formation by *S. mutans* Ingbritt completely (Fig. 2). Moreover, the formation of insoluble glucan was inhibited up to 89.6 % by K1270 when compared to the control group (Fig. 3). Insoluble glucan is highly adherent extracellular carbohydrate, which promotes the firm adherence and accumulation of oral bacteria on tooth surface[47]. Thus, it is considered that the ability of K1270 to inhibit the formation of insoluble glucan acts a great role in the prevention of dental plaque-induced oral diseases, such as dental caries and periodontal disease.

LAB is known to have antimicrobial activity by producing some organic acid[24,25,48], hydrogen peroxide[31-33], and bacteriocins[26-30,49,50]. We find out that the inhibitory effect on biofilm formation resulted from not organic acid but hydrogen peroxide and bacteriocin produced by K1270. It was known that many LAB produced significant amounts of hydrogen peroxide during aerobic growth[51]. In fact, several species of lactobacilli and oral streptococci have been shown to produce 1.0 mM to 10 mM of H_2O_2 in liquid culture[51]. Among oral bacteria, it has been well known that H_2O_2-producing oral streptococci, such as S. oralis, *Streptococcus mitis*, and *Streptococcus sanguis*, inhibited the growth of periodontopathogens

such as *Porphyromonas gingivalis*[52]. In this study, it was observed that K1270 produced H_2O_2. Moreover, K1270 inhibited the growth of *S. mutans* Ingbritt not producing hydrogen peroxide, while K1270 had no effect on the replication of *S. oralis* that excreted hydrogen peroxide. *S. oralis* did not inhibit the multiplication of K1270 either (data not shown). Therefore, the inhibitory effect of K1270 on the biofilm formation resulted from the killing effect on *S. mutans* Ingbritt, partly.

Pediococci are lactic acid bacteria often found living in association with dairy products, plant material, and foods fermented by LAB[53-55]. It has been reported as the dominant microbial population on forage crops and silage. In this study, we isolated and identified Pediococci from *kimchi*. Following examination by cultural and biochemical, and analysis of 16S rDNA sequence, this isolated Pediococci was identified as *P. pentosaceus* K1270. *P. pentosaceus* has been reported that it produced bacteriocin like pediocin and exhibited inhibitory effect against species of Lactobacillus, Lactococcus, Enterococcus, and Listeria, etc[56,57]. In this study, the supernatant of *P. pentosaceus* K1270 showed the inhibitory effect on the multiplication of *S. mutans* Ingbritt (data not shown) and also inhibited biofilm formation by *S. mutans* Ingbritt. Furthermore, this effect was slightly reduced by the addition of catalase and completely abolished with heat or trypsin treatment (Fig. 5). This indicates that the inhibitory effect of K1270 resulted from bacteriocin as well as hydrogen peroxide.

Ⅴ. Conclusion

One LAB inhibiting the biofilm formation by *S. mutans* Ingbritt was isolated from *kimchi* and identified based on the cultural, physiological, and biochemical characteristics as *P. pentosaceus* K1270. This strain was examined to have strong inhibitory activity on biofilm formation by *S. mutans* Ingbritt through the production of both hydrogen peroxide and bacteriocin. Therefore, *P. pentosaceus* K1270 could be proposed as bioprotective agents for the control of the formation of oral biofilm if the ability to colonize oral cavity and its safety for application are approved.

References

1. Fitzgerald RJ, Jordan HV : Polysaccharide-producing bacteria and caries. In HR Harriso(ed) Art and science of dental caries research. Academic Press Inc, New York, 79-86, 1968.
2. Gibbons RJ, Banghart SB : Synthesis of extracellular dextran by cariogenic bacteria and its presence in human dental plaque. Arch Oral Biol, 12:11-24, 1967.
3. Hamada S, Slade HM : Biology, immunology, and carogenicity of *Streptococcus mutans*. Microbiol Rev, 44:331-384, 1980.
4. Loesche WJ : Role of *Streptococcus mutans* in dental decay. Microbiol Rev, 50: 353-80, 1986.
5. Hamada S : Role of glucosyltransferase and glucan in bacterial aggregation and adherence to smooth surface. In: Doyle RJ, Ciarde JE, editors. Glucosyltransferase, Glucans, Sucrose and Dental Caries. IRL Press, Washington, DC, 1983.
6. Kuramitsu HK : Characterization of extracellular glucosyltransferase activity of *Steptococcus mutans*. Infect Immun, 12:738-749, 1975.
7. Germaine GR, Harlander SK, Leung WLS, et al. : *Streptococcus mutans* dextransucrase: functioning of primer dextran and endogenous dextranase in water-soluble and water-insoluble glucan synthesis. Infect Immun, 16:637-648, 1977.
8. Gibbons RJ, Nygaard M : Synthesis of insoluble dextran and its significance in the formation of gelatinous deposits by plaque-forming streptococci. Arch Oral Biol, 13:1249-1262, 1968.
9. Mukasa H, Slade HD : Mechanism of adherence of *Streptococcus mutans* to smooth surfaces. Ⅰ. Roles of insoluble dextran-levan synthetase enzymes and cell wall polysaccharide antigen in plaque formation. Infect Immun, 8:555-562, 1973.
10. Schaeken MJM, de Haan P : Effect of sustained-release chlorhexidine acetate on the human dental plaque flora. J Dent Res, 68:119-123, 1989.
11. Nuuja TT, Murtoma HT, Meurman JH, et al. : The effect of an experimental chewable antiplaque preparation containing chlorhexidine on plaque and gingival index scores. J Dent Res, 71:1156-1158, 1992.

12. Maltz M, Zickert I : Effect of penicillin on *Streptococcus mutans, Streptococcus sanguis* and *lactobacilli* in hamsters and in man. Scand J Dent Res, 90:193-199, 1982.
13. Jordan HV, Depaola PF : Effect of a prolonged application of vancomycin on human oral *Streptococcus mutans* populations. Arch Oral Biol, 22:187-191, 1977.
14. Imazato S, Torii M, Tsuchitani Y : Antibacterial effect of composite incorporating Triclosan against *Streptococcus mutans*. J Osaka Univ Dent Sch, 35:5-11, 1995.
15. Grossman E, Meckel AH, Isaacs RL, et al. : A clinical comparison of antibacterial mouthrinses: effects of chlorhexidine, phenolics, and sanguinarine on dental plaque and gingivitis. J Periodontol, 60:435-440, 1989.
16. Koga T, Inoue M : Effects of dextranases on cell adherence, glucan-film formation and glucan systhesis by *Streptococcus mutans* glucosyltransferase. Archs Oral Biol, 24:191-198, 1979.
17. Fitzgerald RJ, Keyes PH, Sroudt TH, et al. : The effects of a dextranase preparation on plaque and caries in Hamsters, a preliminary report. JADA, 76:301-304, 1968.
18. Inoue M, Yakushiji T, Katsuki M, et al. : Reduction of the adherence of *Streptococcus sobrinus* insoluble alpha-D-glucan by endo-(1----3)-alpha-D-glucanase. Carbohydr Res, 182:277-286, 1988.
19. Inoue M, Yakushiji T, Mizuno J, et al. : Inhibition of dental plaque formation by mouthwash containing an endo-alpha-1, 3 glucanase. Clin Prev Dent, 12:10-14, 1990.
20. Kilara A, Treki N : Uses of lactobacilli in foods; Unique benefits. Devel Indust Microbiol, 25:125-138, 1984.
21. Gildin BR, Gorbach SL : The effect of oral administration of Lactobacillus and antibiotics on intestinal bacterial activity and chemical induction of large bowel tumors. Devel Indust Microbiol, 25:139-150, 1984.
22. Gilliland SE, Nelson CR, Maxwell C : Assimilation of cholesterol by Lactobacillus acidophilus. Appl Environ Microbiol, 49:377-381, 1985.
23. Perdigon G, Nader de Macias ME, Alvarez S, et al. : Effect of perorally administered lactobacilli on macrophage activation in mice. Infect Immun, 53:404-410, 1986.
24. Hill GB, Eschenbach DA, Hotmes KK : Bacteriology of the vagina. Scand J Urol Nephrol Suppl, 86:23-39, 1985.
25. Larsen B, Galask RP : Vaginal microbial flora: composition and influence of host physiology. Ann Intern Med, 96:926-930, 1982.
26. Barefoot SF, Klaenhammer TR : Detection and activity of lactacin B. A bacteriocin produced by *Lactobacillus acidophilus*. Appl Environ Microbiol, 45:1808-1815, 1983.
27. Barefoot SF, Klaenhammer TR : Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. Antimicrob Agents Chemother, 26:328-334, 1984.
28. Klaenhammer TR : Bacteriocins of lactic bactera. Bilchimie, 70:337-349, 1988.
29. McGroarty JA, Reid G : Detection of *Lactobacillus* substance that inhibits *Escherichia coli*. Can J Microbiol, 34:974-978, 1988.
30. Muriana PM, Klaenhammer TR : Purification and partial characterization of lactacin F. a bacteriocin produced by *Lactobacillus acidophilus* 11088. Appl Environ Microbiol, 57:114-121, 1991.
31. Hiller SL, Krohn MA, Rabe LK, et al. : The normal vaginal flora. H_2O_2-producing lactobacilli, and bacterial vaginosis in pregnant women. Clin Infect Dis, 16:273-281, 1993.
32. Hillier SL, Krohn MA, Klebanoff SJ, et al. : The relathionship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant women. Obstet Gynecol, 79:369-373, 1992.
33. Hillier SL, Krohn MA, Nugent RP, et al. : Characteristics of three vaginal flora patterns assessed by Gram stain among pregnant women. Am J Obstet Gynecol, 166:938-944, 1992.
34. Mechnikoff E : The prolongation of Life. G.P. Putnams & Sons, New York. 1988.
35. McCabe RM, Keyes PH, Howell A Jr. : An *in vitro* method for assessing the plaque forming ability of oral bacteria. Archs Oral Biol, 12:1653-1656, 1967.
36. Hamada S, Torii M, Kotani S, et al. : Adherence

of *Streptococcus sanguis* clinical isolates to smooth surfaces and interactions of the isolates with *Streptococcus mutans* glucosyltransferase. Infect Immun, 32:364-372, 1981.
37. Nakahara K, Kawabata S, Ono H, et al. : Inhibitory effect of oolong tea polyphenols on glycosyltransferases of mutans Streptococci. Appl Environ Microbiol, 59:968-973, 1993.
38. Sneath PHA, Mair NS, Sharpe ME, et al. : Bergeys Manual of Systematic Bacteriology Vol.2. Williams & Wilkins, Baltimore, MD. 1986.
39. Chung J, Kim HH, Shin JH, et al. : Identification of Mutanase - Producing *Microbis- pora rosea* from the Soil of Chonnam Province. J Microbiol Biotechnol, 11: 677-684, 2001.
40. Lane DJ, Pace B, Olsen GJ, et al. : Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci *USA*, 82:6955-6959, 1985.
41. Seo WT, Kahng GG, Nam SH, et al. : Isolation and characterization of a novel exopolysaccharide-producing *Paenibacillus* sp. WN9 KCTC 8951P. J Microbiol Biotechnol, 9:820-825, 1999.
42. Jung KO, Park KY : The inhibitory effects of Leek (Buchu) *kimchi* extracts on MCA-induced cytotoxicity and transformaiton in c3H/10T1/2 cells. J Food Sci Nutr, 4:255-259, 1999.
43. Park KY : The nutritional evaluation and antimutagenic and anticancer effects of *kimchi*. J Korean Soc Food Nutr, 24:169, 1995.
44. Lim CR, Park HK, Han HU : Reevaluation of isolation and identification of Gram-positive bacteria in *kimchi*. Korean Journal of Microbiology, 27:404-414, 1989.
45. Lee CW, Ko CY, Ha DH : Microfloral changes of the lactic acid bacteria during *kimchi* fermentation and identification of the isolates. Korean Journal of applied Microbiology and Biotechnology, 20:102-109, 1992.
46. Cheigh HS, Park KY : Biochemical, microbiological, and nutritional aspects of *kimchi* (Korean fermented vegetable products). Critical Reviews in Food Science and Nutrition, 34:175-203, 1994.
47. Yakushiji T, Inoue M, Koga T : Inter-serotype comparison of polysaccharides produced by extracellular enzymes from Streptococcus mutans. Carbohydr Res, 127: 253-266, 1984.
48. Taniguchi M, Nakazawa H, Takeda O, et al. : Production of a mixture of antimicrobial organic acids from lactose by co-culture of Bifidobacterium longum and propionibacterium freudenreichii. Bioscience Biotechnology and Biochemistry, 62:1522-1527, 1998.
49. Ahrne S, Nobaek S, Jeppsson B, et al. : The normal Lactobacillus flora of healthy humal rectal and oral mucosa, J Appl Microbiol, 85:88-94, 1998.
50. Van reenen CA, Dicks LMT, Chikindas ML : Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by Lactobacillus plantarum. J Appl Microbiol, 84:1131-1137, 1988.
51. Wilcox MDP, Drucker DB : Partial characterisation of the inhibitory substances produced by *Streptococcus oralis* and related species. Microbios, 55:135-145, 1988.
52. Leke N, Grenier D, Goldner M, et al. : Effects of hydrogen peroxide on growth and selected properties of *Porphyromonas gingivalis*. FEMS Microbiol Lett, 174:347-353, 1999.
53. Cai Y, Kumai S, Ogawa M, et al. : Characterization and identification of Pediococcus species isolated from forage crops and their application for silage preparation. Appl Environ Microbiol, 65:2901-2906. 1999.
54. Cai Y, Benno Y, Ogawa M, et al. : Influence of *Lactobacillus* spp. from an inoculant and of *Weissella* and *Leu-conostoc* spp. from forage crops on silage fermentation. Appl Environ Microbiol, 64:2982-2987, 1998.
55. Gashe BA : Involvement of lactic acid bacteria in the fermentation of Tef (*Eragrostis tef*), an Ethiopian fermented food. J Food Sci, 50:800-801, 1985.
56. Piva A, Headon DR : Pediocin A, a bacteriocin produced by *Pediococcus pentosaceus* FBB61. Microbiology, 140:697-702, 1994.
57. Strasser de Saad AM, Manca de Nadra MC : Characterization of bacteriocin produced by from wine. J Appl Bacteriol, 74:406-410, 1993.

Abstract

INHIBITION OF BIOFILM FORMATION BY *PEDIOCOCCUS PENTOSACEUS* K1270 ISOLATED FROM *KIMCHI*

Woi-Im Choi, Su-Ji Han*, Shin Kim

Department of Pediatric dentistry, College of Dentistry, Pusan National University
Center of Pharmacokinetics Department of Pharamceutical sciences,
*School of Pharmacy University of Pittsburgh USA **

Pediococcus pentosaceus K1270 was isolated from naturally fermented *kimchi* and identified based on the 16S rDNA sequence as well as cultural and biochemical characteristics. This strain strongly inhibited the formation of biofilm by *Streptococcus mutans* Ingbritt. K1270 also showed antibacterial activity against *S. mutans* Ingbritt. It was observed that K1270 strain produced hydrogen peroxide on MRS agar supplemented with 3, 3, 5, 5-tetramethylbenzidine (TMB) and peroxidase and the inhibitory effect of K1270 strain on the biofilm formation was reversed by the addition of catalase in part. Culture supernatant of K1270 inhibited the biofilm formation and the multiplication of *S. mutans* Ingbritt. This inhibitory effect of culture supernatant was decreased slightly by the addition of catalase and abolished by heat or trypsin treatment. Thus, this study suggests that *P. pentosaceus* K1270 inhibit the biofilm formation through the inhibition of the replication of *S. mutans* Ingbritt by producing hydrogen peroxide and bacteriocin.

Key words : Biofilm, *Kimchi*, Inhibition of dental plaque, *S. mutans*, *P. pentosaceus*