INHIBITORY EFFECT OF *LACTOCOCCUS LACTIS 1370* ON THE FORMATION OF DENTAL PLAQUE IN CHILDREN

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국문초록

소아에서 *Lactococcus lactis 1370*에 의한 치태형성 억제 효과

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치태 억제효과가 있는 것으로 알려진 *Lactococcus lactis 1370*으로 제조한 양치용액이 실제 소아들의 구강내에서 치태의 형성을 억제하는지 여부와 이들 균주의 시간경과에 따른 구강내 잔류율을 알아보고자 소아 30명을 대상으로 위양치액(Lactobacillus casei)과 유산균 양치액(*Lactococcus lactis 1370*)으로 양치하도록 한 뒤 시간경과에 따른 치태지수, 치태부착면적율, 그리고 *Lactococcus lactis 1370*의 생균수를 count하여 다음과 같은 결과를 얻었다.

1. 대조군과 실험군에서 24시간후 치태지수는 각각 평균 2.43과 2.06으로 양치에 의해 치태지수는 평균 0.37 감소하였으며 치태형성억제율은 약 15%로 유의한 감소를 보였다 ($P<0.05$).
2. 48시간 후 치태지수는 대조군과 실험군에서 각각 평균 2.95와 2.17로 양치에 의해 치태지수는 평균 0.78 감소하였으며 치태형성억제율은 약 26%로 24시간후에 비해 좀더 많은 감소를 보였다($P<0.05$).
3. 대조군과 실험군에서 24시간후 치태부착면적율은 각각 평균 21.2%와 15.6%로 양치에 의 해 치태부착면적율은 평균 5.63% 감소하였으며 치태형성억제율은 약 26%로 유의한 감소를 보였다($P<0.05$).
4. 48시간후 치태부착면적율은 대조군과 실험군에서 각각 평균 33%와 17.8%로 양치에 의해 치태부착면적율은 평균 15.1% 감소하였으며 치태형성억제율은 약 46%로 24시간 후에 비해 좀 더 많은 감소를 보였다($P<0.05$).
5. 양치후 시간에 따른 구강내 생균수를 count한 결과 1시간 이후까지는 급격한 감소를, 3시 간후에는 약간의 감소를 보였으며 3시간부터 6시간 사이에는 약간의 증가추세를 보였다.

주요어 : 양치, 유산균, 치아우식증, 치태지수

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Ⅰ. Introduction

Despite leaping advancement in modern dental medicine, dental caries and periodontal disease are the most prevalent dental disease in man, and dental caries especially in children is becoming a very important dental disease. Dental caries develops through a series of process in which inorganic materials of a tooth are decalcified followed by destruction of organic materials. Prevention of dental caries is important since it is irreversible at the initial stage[1].

Since dental caries is a disease that develops due to various factors working in a complex way, various ways are being tried to prevent this disease[2-5]. Until now, fluoride has been used for preventing dental caries, and it is true that fluoride has contributed in a great deal in preventing this disease. But when over-supplied, the complications such as discoloration of teeth, disturbance of bone formation, and osteoporosis are reported[6,7].

Recently interests have been increased in the suppression and disintegration of plaque biosynthesis which is the direct cause of decalcifying teeth[8]. Plaque is composed of bacteria and acellular material, and glucan. Acellular material is an extracellular polysaccharide synthesized from sucrose by glucosyltransferase (GTF) of *Streptococcus mutans*(S. mutans) in the mouth which is composed of dextran, mutan, and fructan[8,9]. Non water-soluble mutan is sticky and has a strong adhesive characteristic which promote the adhesion and proliferation of bacteria including *S. mutans* to the tooth surface; and water-soluble dextran and fructan are the energy sources for the bacteria. The acid, produced as the byproduct of these bacteria, are continually destroying the tooth substance, if it is not neutralized from the buffering action of saliva[3,9,10].

The most simple and effective way to remove the plaque is the mechanical method using a toothbrush or other oral hygiene products[11]. However, this method is influenced by individual's brushing habit and anatomical structure of the teeth.

Suppressing the plaque formation is more fundamental way[2,11,12] for prevention of dental caries and periodontal disease. In the mean time, although efforts were made to inhibit *S. mutans* proliferation and to reduce the plaque using antibacterial substances such as mouth rinse, tooth paste, gum, and gel,[13-18] effects were temporary. Although attempts were made to break down plaque using dextranase and mutanase which could affect the substrate of plaque, glucan[19-21], and to develope anticaries vaccine[22-24], these one have not been realized yet clinically.

Thus, recently, among the normally residing bacteria in the mouth, researches are interesting in suppressing the plaque formation using the bacteria of antagonist to *S. mutans*[25-27], *Streptococcus oralis, Streptococcus mitior, Streptococcus sanguis, Enterococcus durans, Lactococcus spp., and Lactobacillus acidophilus*[28].

The purpose of this study is to determine the inhibitory effect of *Lactococcus lactis 1370* on the formation of dental plaque.

Ⅱ. Methods and materials

1. Subjects

The subjects of the present study included 30 children between the ages of 10-13 years who did not have systemic disease and had the completely erupted permanent and healthy central incisors, lateral incisors and 1st molars with no dental caries, damages due to trauma, or restorative material on the buccal and lingual surfaces of these applicable teeth, under the consent from the parents.

2. Methods

1) Culturing of the bacteria and making of the mouthwash

After pressurization and steam pasteurization of milk containing 3% skim milk for 10min, *Lactococcus lactis 1370* was inoculated and the mouthwash was cultured between 26-28 h in an incubator at 5% CO_2 and at 37 ℃. The bacterial concentration of the mouthwash would be $1.8\text{-}3.2\times10^8$CFU/ml. Also, the control mouthwash was made with Lactobacillus casei using the same method, the bacterial concentration of the mouthwash would be about 5×10^8CFU/ml.

2) Measuring the suppression ratio of plaque formation

① Oral prophylaxis

In order to totally remove plaque existing in the mouth before the experiment, we performed oral prophylaxis using a low speed handpiece mounted with a rubber cup

and pumice in all subjects by asking them to come over to the hospital on the first day of using the above lactic acid mouthwash and pseudo-mouthwash.

② Mouthwashing

After performing oral prophylaxis, we divided 30 subjects into two groups of 15, into A and B, and instructed the subjects in group A to rinse the mouth with the *Lactococcus lactis 1370* suspension mouthwash, and those in group B to rinse the mouth with control mouthwash. As for the method of mouthwashing, we instructed each subject to hold and rinse with 10ml of the mouthwash in the mouth for one minute for 4 times a day, after each meal and before bedtime. We instructed them not to drink water for one hour after the use of mouthwash and to eat meal and snack as usual but not to brush teeth. We asked each subject to come to the hospital 24 h and 48 h after the use of the mouthwash and measured Plaque Index and plaque area rate in each subject. The measurement of the plaque index was done by a same individual, and the distribution of mouthwash and the measurement of Plaque Index were done with the double blinded method.

③ Measurement of the plaque index

For each oral examination, the teeth was colored with diluted Trace 28 (The Lovic Co.USA), and detailed examination was made on the buccal surface of the right and left 1st molar of the maxilla, the lingual surface of the right and left 1st molar of the mandible, the labial surface of the right permanent central incisor of the maxilla, and the lingual surface of the left permanent central incisor of the mandible. The measurement of dental plaque was done using the Quigley-Hein's Plaque Index improved by Turesky et al.[29,30] and the main evaluation criteria are as follows.

Plaque Index

0 : No plaque .

1 : Separate flecks of plaque at the cervical margin of the tooth.

2 : A thin continuous band of plaque(up to 1mm) at the cervical margin.

3 : A band of plaque wider than 1mm. But covering less than one third of crown

4 : Plaque covering at least one third but less than two thirds of the crown.

5 : Plaque covering two thirds or more of the crown.

④ Measurement of the rate of plaque area

With the four maxillary incisors, we measured the ratio of the area with plaque attachment and the total labial surface. Using the image analyzing method proposed by Angmar-Manson and Bjelkhagen[31-33], a CCD camera was used to take picture of the pattern of plaque attachment on the four maxillary incisors, and after digitalizing the picture with a computer, we measured the colored area with a computer image analyzing program (Image pro plus®), and calculated the colored area into percentile against the total labial surface area.

3) Measuring of inhibitory effect of *Lactococcus lactis 1370* on the formation of dental plaque with time

① Oral prophylaxis

In order to remove all plaque in the mouth from the study subjects before the study, we asked them to come to the hospital before using mouthwash, and performed oral prophylaxis using a low-speed dental handpiece mounted with a rubber cup and pumice.

② Mouthwashing

After performing oral prophylaxis, we instructed all 30 of the study subjects to rinse mouth with the lactic acid mouthwash. The method of mouthwashing was to rinse the mouth by holding 10ml of the mouthwash in the mouth for a minute and the subjects were instructed not to drink water for one hour after mouthwashing. And they were instructed to eat and snack normally but not to use toothbrush.

③ The assessment of the number of *Lactococcus lactis 1370* in saliva

We measured the number of Lactococcus lactis 1370 in 1ml of saliva taken at 1hr, 3h and 6h after using mouthwash. After diluting 1ml of saliva with Brain heart infusion broth, the resulting solution was inoculated into Brain heart infusion agar, and incubated at 37℃ in a CO_2 incubator for a day; and the number of *Lactococcus lactis 1370* was counted.

4) Statistical analysis

Between the control mouthwash group and *Lactococcus lactis 1370* mouthwash group, the plaque indices validated with the data after each time period were compared using Wilcoxon's signed ranks test, and the validity of the plaque area rate was determined using Mann-Whitney test.

Ⅲ. Results

1. Plaque index

At 24h and 48h after rinsing the mouth with the control mouthwash and *Lactococcus lactis 1370* mouthwash, when the plaque index investigated with the colored condition on the tooth surfaces such as the buccal surface of the left and right 1st. molars of the maxilla, lingual surface of the right and left 1st. molar of the mandible, labial surface of the right permanent central incisor of the maxilla, and lingual surface of the left permanent central incisor of the mandible, the results showed that the average plaque indices at 24 h after rinsing mouth with control mouthwash and *Lactococcus lactis 1370* mouthwash was 2.43 and 2.06, respectively, showing that the indices decreased by an average of 0.37 due to mouthwash, and the inhibition rate of plaque formation due to the lactic acid bacteria mouthwash was about 15%, showing a significant decrease ($p < 0.05$)(Fig. 1, 5, Table 1); and those at 48 h after were 2.95 and 2.17, respectively, showing that the plaque indices had decreased by an average of 0.78 due to the mouth washing; the inhibition rate of plaque formation due to the lactic acid bacteria mouthwash was about 26%, showing larger difference ($p < 0.05$) with the control group at 24 h after mouthwashing(Fig. 2, 5, Table 1).

2. Plaque area rate

At 24 h and 48 h after using the control mouthwash and *Lactococcus lactis 1370* mouthwash, the results of measuring the plaque area rate with the colored condition of the total labial tooth surfaces of the four maxillary incisors showed that in the control mouthwash group and *Lactococcus lactis 1370* mouthwash group at

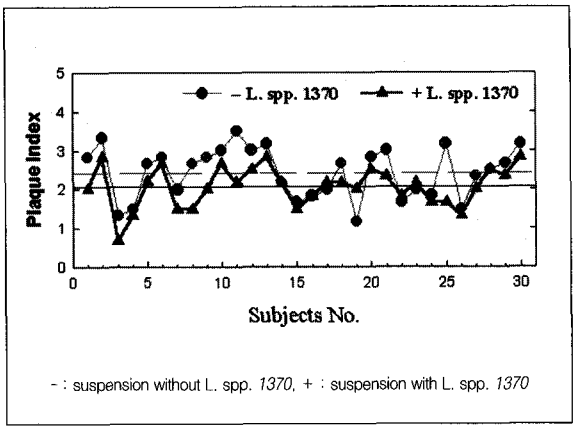


Fig. 1. Distribution of plaque index measured in each of subject at 24 hours.

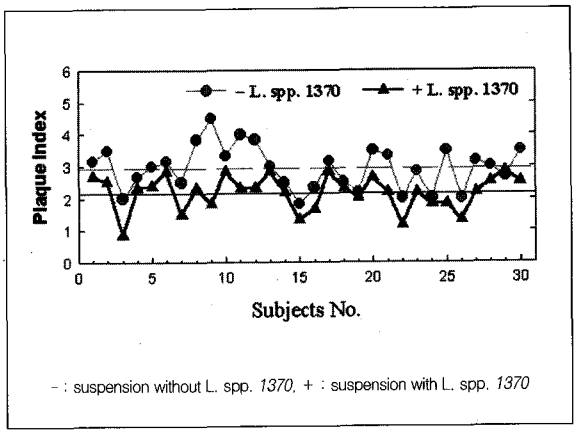


Fig. 2. Distribution of plaque index measured in each of subject at 48 hours.

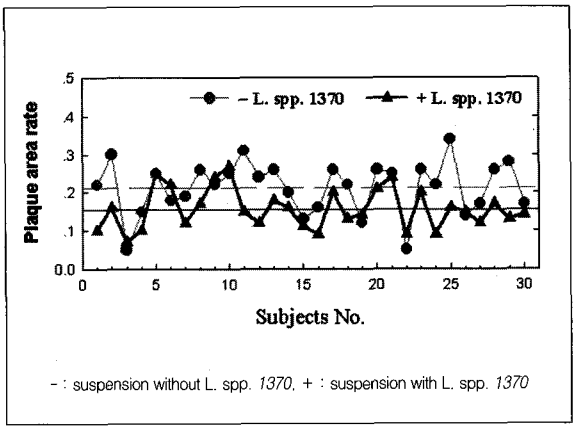


Fig. 3. Distribution of plaque area rate measured in each of subject at 24 hours.

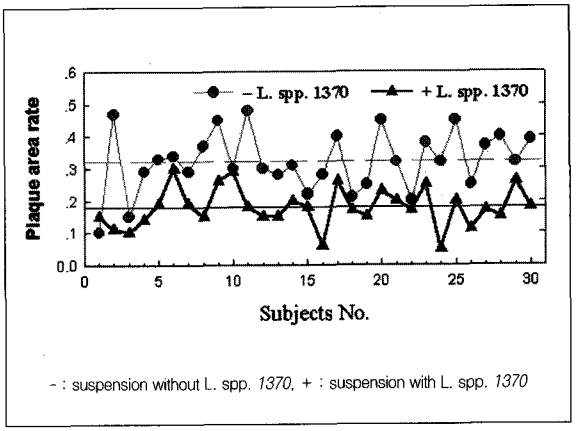
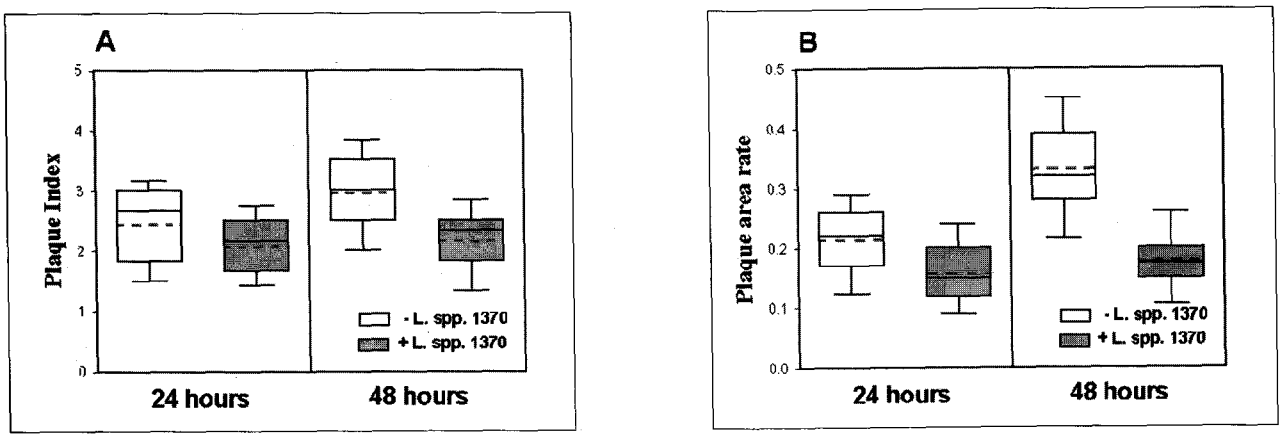


Fig. 4. Distribution of plaque area rate measured in each of subject at 48 hours.

Table 1. Plaque Index and plaque area rate, as function of rinsing suspension

Measurements	Suspension phase	*L. casei*	*L. lactis 1370*	⊿P
Plaque Index	24 hours	2.43±0.66	2.06±0.52	0.37±0.48*
	48 hours	2.95±0.68	2.17±0.54	0.78±0.59*
Plaque area rate	24 hours	21.2±7.00	15.6±5.33	5.63±6.26#
	48 hours	33.0±8.39	17.8±6.14	15.1±8.72#

L. casei : Suspension with *Lactobacillus casei*
L. lactis 1370 : Suspension with *Lactococcus lactis 1370*
⊿P = Difference between L. casei and L. lactis *1370*
*p<0.05, by Mann-Whitney test, # P<0.05 by Wilcoxon's test



- : suspension without L. spp. *1370*, + : suspension with L. spp. *1370*

Fig. 5. Inhibitory effect of *Lactococcus lactis 1370* on the formation of dental plaque represented by the schematic box plot: Plaque Index(A) and plaque area rate(B). Open boxes represent the suspension with Lactobacillus casei and shaded boxes represent the suspension with *Lactococcus lactis 1370*. Horizontal solid bars in the boxes indicate the median values and dashed bars indicate mean values. Error bars indicate the standard deviations.

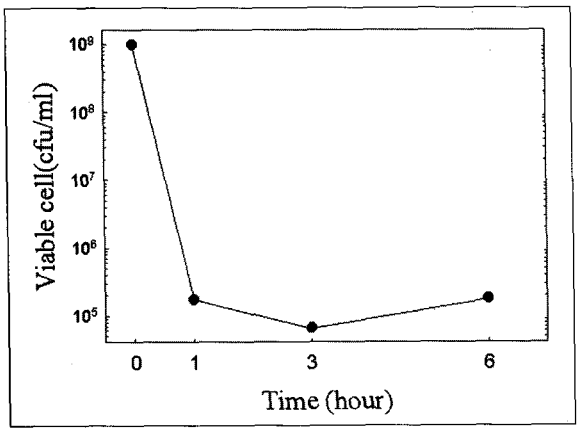


Fig. 6. Time course of changes in Lactococcus lactis 1370 count (CFU/ml).

24h after mouthwashing, the rates were 21.2% and 15.6%, respectively, showing an average decrease of 5.63%, and the decreased plaque area rate due to mouthwashing was about 26%, showing a significant decrease ($p < 0.05$) (Fig. 3, 5, Table 1); at 48 h after mouthwashing, the rates were 33.0% and 17.8%, and the decreased plaque area rate due to mouthwashing was about 46%, showing larger difference ($p < 0.05$) compared with the control group than at 24 h after mouthwashing.(Fig. 4, 5, Table 1)

3. Inhibitory effect of *Lactococcus lactis 1370* on the formation of dental plaque with time

The results of counting the number of bacteria within 1ml saliva taken at 1 h, 3 h, and 6 h after using mouthwash showed that the number decreased drastically at 1 h after using mouthwash, decreased slightly between 1 h and 3 h after, and increased slightly between 3 h and 6 h, recovering up to the number at 1 h after using mouthwash(Fig. 6). Considering these results, most of *Lactococcus lactis 1370* after using mouthwash were washed away due to eating and the self-cleaning effect

of saliva, but some remain in the mouth at retention sites, proliferating later.

Ⅳ. Discussion

Deciduous teeth play important roles in not only for intake of nutrients but also inducing the alveolar bone development and securing of the eruption spaces for permanent teeth. As the most frequently occurring oral disease in children, dental caries induces various difficulties including the developmental disorder and the difficulties in the eruption of permanent teeth, and malocclusion. Among many microorganism in the mouth, the main cause of dental caries is known to be *S. mutan*. The effect of S. mutans on inducing dental caries has a close relationship with the characteristics of the bacteria attaching strongly on tooth surfaces and of producing acid as a byproduct[3].

S. mutans group was divided into eight serotypes of a through h, and the sera c, e, and f are called S. mutans, and the sera d and g are called S. sobrinus[34] ; *S. mutans* goes through the process of 2 steps to cover teeth surfaces. The first reversible step is the interaction between the tooth surface covered by saliva and bacteria, and the second irreversible step is the synthesis of non-water soluble glucan from sucrose by to glucosyltranferase(GTF). *S. mutans* attached to tooth surfaces takes various dietary sugars and produces the acid. A acid produced by oral bacteria decalcifies enamel and dentin of teeth, and this phenomenon is called dental caries[3].

Although attempts were made to prevent the proliferation of bacteria by topical application of anti-bacterial solution such as chlorhexidine, iodine, and antibiotics such as oral penicillin including vancomycin and kanamycin[13-18], most of these methods do not have a lasting effect and the risk of the complication due to the destruction of normal oral flora in the mouth. In order to decompose the synthesized plaque, the method of using dextranase or mutanase was designed. Ebius et al[35] reported that suppressing biosynthesis mutan or decomposing mutan are effective ways to control plaque. In order to decompose non-water soluble mutan, a study was attempted on the separation of the bacteria secreting mutanase and purification of the enzyme[36]. Although plaque is basically made with glucan, it is difficult to remove plaque by the enzyme which can decompose glucan since plaque contains many substances and various proliferating bacteria. Thus, it would be more effective to prevent the formation of glucan rather than decomposing glucan from plaque by the enzymes such as destranase and mutanase.

In order to obtain lasting effect without destroying the equilibrium of the oral environment, studies are currently done in preventing the formation of plaque using normally existing bacteria in the mouth[25-28]. It was proven that the bacterium, as a mutant form of *S. mutans* that can not produce acid due to difficulty in carbohydrate metabolism within cells or lack of lactate dehydrogenase activation, can not induce dental caries[37]. Streptococcus salivarius TOVE-R, which forms colony and plaque on tooth surfaces but does not induce dental caries, were already separated[38]. Also, Enterococcus faecalis existing in the mouth is reported to be a possible substitute bacterium since it produces bacteriocin which has the antibacterial effect by suppressing the proliferation of streptococci[39]. Substitute bacteria need to be non-pathogenic, to proliferate continuously in the mouth and to be able to suppress the proliferation of pathogens. Theoretically, this substitution method can expect lasting effect with simple inoculation of substitute bacteria[5].

Among acid producing bacteria, *Lactococcus* is the most safe ingest that normally exists in the mouth. Water-soluble glucan synthesized by *Lactococcus lactis 1370* is reported not to suppress the proliferation of oral bacteria including *S. mutans*, but to suppress the formation of plaque[40].

The present study was conducted to determine the suppressing effect of *Lactococcus lactis* 1370 on the formation of plaque and to confirm whether this bacterium remains in the mouth for a certain period of time to show a lasting effect in the mouth in children. The results showed that the plaque index of the *Lactococcus lactis 1370* mouthwash group at 24 h compared to the control group was decreased in an average of 0.52, showing about 15% decrease. The plaque index at 48h compared to the control group was decreased in an average of 0.78, showing about 26% decrease($p<0.05$). Also, the plaque area rate at 24 h was decreased in an average of 5.63%, showing about 26% decrease, and was decreased in an average of 15% at 48 h, showing about 46% decrease. These data suggested that the effect of inhibiting plaque formation was getting better with time. The plaque area rate showed a higher rate of decreased than the plaque index, due to the fact that different lo-

cations of teeth were used to determine.

The results of the experiment for determining the inhibitory effect of *Lactococcus lactis 1370* on the formation of dental plaque with time showed that the number of the bacteria showed a drastic decrease until 1 h, slight decrease until 3 h, and slight increase between 3 h to 6 h. We think that these results are due to self-cleaning effect of saliva immediately after mouth washing. But the number of the bacteria remained in the mouth was increased after a certain period of time resulted from proliferation. Further studies are needed to assess the long time effect of mouth washing.

Most of the microbes included in diary products are Streptococcus thermophillus and *Lactobacillus bulgaricus*, and under the claim by Metchnikoff in 1907 when he said ingesting these microbes not only promotes lactose tolerance but also has many beneficial effect to health including the immune effect and the anti-cancer effect[41,42]. Various types of diary products containing activated lactobacilli are sold. However, lactobacilli was pointed out to have a close relationship with dental caries[43], and although taking diary products containing these activated lactobacilli is good for overall health of individuals, they do not have a good effect on oral health. However, *Lactococcus lactis 1370* used in the present study does not inhibit the proliferation of *S. mutans*, not destroying the normal bacterial flora. We think that follow-up studies are needed on the commercialization of this bacterium into diary drinks.

Ⅴ. Conclusions

This study was undertaken to evaluate the clinical effect of inhibiting plaque formation of *Lactococcus lactis 1370*, a acid producing bacterium residing in the mouth. 30 children were asked to use 10ml of control mouthwash and mouthwash containing *Lactococcus lactis* 1370. The plaque index and plaque area rate at 24 h and 48 h after the use of the mouthwashes were measured. And the number of *Lactococcus lactis 1370* was counted at 1 h, 3 h, and 6 h in the mouth. The results are as follow.

1. The mean plaque index at 24 h after the use of the control mouthwash and the mouthwash containing *Lactococcus lactis 1370* were 2.43 and 2.06, respectively. The inhibiting rate of plaque formation was 15%($P<0.05$).
2. The mean plaque index at 48 h after the use of the control mouthwash and the mouthwash containing *Lactococcus lactis 1370* were 2.95 and 2.17, respectively. The inhibiting rate of plaque formation was 26%, showing more decrease than at 24 h($P<0.05$).
3. The mean plaque area rate at 24 h after the use of the control mouthwash and the mouthwash containing *Lactococcus lactis 1370* were 21.2% and 15.6%, respectively. The inhibiting rate of plaque formation was 26%($P<0.05$).
4. The mean plaque area rate at 48h after the use of the control mouthwash and the mouthwash containing *Lactococcus lactis 1370* were 33.0% and 17.8%, respectively. The inhibiting rate of plaque formation was 46%($P<0.05$).
5. The number of *Lactococcus lactis 1370* in the mouth decreased significantly from mouthwashing to 3h, but increased slightly between 3 h and 6 h.

As seen with the above results, we think that using the mouth wash with *Lactococcus lactis 1370* would prevent the formation of plaque in the mouth and can be an effective method to prevent dental caries and periodontal disease.

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Abstract

INHIBITORY EFFECT OF LACTOCOCCUS LACTIS 1370 ON THE FORMATION OF DENTAL PLAQUE IN CHILDREN

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This study was undertaken to evaluate the clinical effect of inhibiting plaque formation of Lactococcus lactis 1370, a acid producing bacterium residing in the mouth. 30 children were asked to use 10ml of control mouthwash and mouthwash containing Lactococcus lactis 1370. The plaque index and plaque area rate at 24h and 48h after the use of the mouthwashes were measured. And the number of Lactococcus lactis 1370 was counted at 1h, 3h, and 6h in the mouth. The results are as follow.

1. The mean plaque index at 24h after the use of the control mouthwash and the mouthwash containing Lactococcus lactis 1370 were 2.43 and 2.06, respectively. The inhibiting rate of plaque formation was 15%($P<0.05$).
2. The mean plaque index at 48h after the use of the control mouthwash and the mouthwash containing Lactococcus lactis 1370 were 2.95 and 2.17, respectively. The inhibiting rate of plaque formation was 26%, showing more decrease than at 24h($P<0.05$).
3. The mean plaque area rate at 24h after the use of the control mouthwash and the mouthwash containing Lactococcus lactis 1370 were 21.2% and 15.6%, respectively. The inhibiting rate of plaque formation was 26%($P<0.05$).
4. The mean plaque area rate at 48h after the use of the control mouthwash and the mouthwash containing Lactococcus lactis 1370 were 33.0% and 17.8%, respectively. The inhibiting rate of plaque formation was 46%($P<0.05$).
5. The number of Lactococcus lactis 1370 in the mouth decreased significantly from mouthwashing to 3h, but increased slightly between 3h and 6h.

As seen with the above results, we think that using the mouth wash with Lactococcus lactis 1370 would prevent the formation of plaque in the mouth and can be an effective method to prevent dental caries and periodontal disease.

Key words : Mouthwash, Lactococcus lactis, Dental caries, Plaque index.