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Original Article

Comparison of the antimicrobial effects of probiotics on bacteria associated with peri-implantitis

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Purpose: Peri-implantitis is associated with an increased abundance of Gram-negative anaerobic bacteria around dental implants. This study aimed to investigate the effects of probiotics on bacteria associated with peri-implantitis through in vitro experiments and to propose probiotics suitable for the control of peri-implantitis-associated bacteria. **Materials and Methods:** The spent culture medium (SCM) was collected from 18 species of probiotics. The antimicrobial activity of SCM collected from probiotic bacteria was evaluated using susceptibility assay. **Results:** The genus *Lacticaseibacillus* exhibited the strongest antimicrobial activity against peri-implantitis-associated bacteria. In *Limosilactobacillus* genus. *Limosilactobacillus reuteri* showed strong antimicrobial activity, and in *Bifidobacterium* genus, *Bifidobacterium longum* showed strong antimicrobial activity against peri-implantitis related bacteria. The genus *Lactobacillus* and *Ligilactobacillus* exhibited weak antimicrobial activity compared to other probiotics. *Streptococcus thermophilus* showed weaker antimicrobial activity against peri-implantitis-associated bacteria. **Conclusion:** *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, and *Lacticaseibacillus rhamnosus* were found to exhibit the strongest antimicrobial activity against peri-implantitis-associated bacteria, and these probiotics may be effective in inhibiting the growth of bacteria associated with peri-implantitis. **(J Dent Rehabil Appl Sci 2026;42(2):133-42)**

Key words: peri-implantitis; probiotics; antimicrobial activity; prevention effect

Introduction

Dental implant therapy has become a common treatment option for tooth loss. However, as with all medical procedures, complications can occur. One such complication is peri-implantitis affecting dental implants.[1] Peri-implantitis is a condition that requires careful handling in dentistry because it can lead to implant failure and serious oral health problems.[2] Previous studies analyzing the microbial ecosystem of implanted patients showed that stable implants in healthy periodontal tissue are colonized primarily by cocci-type bacteria, and fusiform, motile bacilli, and spirochetes were rarely detected.[3] Furthermore, another group investigating plaque bacteria on implant placements in healthy implants and implants with peri-implantitis reported that the proportion of *Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* was higher around implants with peri-implantitis compared to the control group.[3,4]

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host".[5] Research is actively being conducted on maintaining human health using probiotics and on the role of probiotics. Among

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probiotics, Ligilactobacillus (formerly Lactobacillus) plays an important role in maintaining health by protecting humans from pathogenic bacteria and stimulating innate immunity. Representative probiotic genera include *Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus* spp.[6,7] Microbial dysbiosis is associated with various diseases of the body. Furthermore, gut microbial dysbiosis results in the induction of disease related to immune deregulation such as allergies, autoimmune diseases, and inflammatory diseases, while dysbiosis of oral microbiome leads to dental caries, periodontitis, and other immune dysregulation-related conditions. Bacteriocins produced by probiotics inhibit pathogens from attaching to epithelial cells and hard tissues in the gut and oral cavity through competition, clearing niche, and spatial segregation. Also, the characteristics of bacteriocin vary depending on probiotics, and their efficacies also vary.

Recently, numerous studies have been conducted to apply probiotics for the treatment and prevention of oral diseases.[8-10] Research is currently being conducted on each individual probiotic to determine its applicability for oral health. Consequently, studies comparing the effects of various probiotics on oral diseases are rare. Therefore, this study aims to investigate the effects of probiotics on bacteria associated with peri-implantitis through in vitro experiments and to propose probiotics suitable for the control of peri-implantitis-associated bacteria.

Materials and Methods

Porphyromonas gingivalis ATCC 33277, *Fusobacterium nucleatum* ATCC 25586 and *Prevotella intermedia* ATCC 25611 was cultured using tryptic soy broth (TSB) (BD biosciences, Franklin Lakes, USA) supplemented with hemin (1 μg/ml) (Sigma-Aldrich Co., San jose, USA) and vitamin K (0.2 μg/ml) (Sigma-Aldrich Co.). *Tannerella forsythia* ATCC 43037 was cultured in modified NOS medium, Peri-implantitis-associated bacteria were managed at 37°C under anaerobic condition (5% H_2, 10% CO_2, and 85% N_2). Seventeen probiotic strains were used in this study (Table 1). *Lactobacillus, Lacticaseibacillus, Limosilactobacillus, Lactiplantibacillus, Ligilactobacillus, Lactococcus* and *Bifidobacterium* spp. were cultured using Rogosa and sharpe (MRS; BD biosciences). *Streptococcus* spp was cultured using TSB (BD bioscience). Probiotics was cultivating at 37°C under anaerobic condition.

The antimicrobial activity of probiotics against

Table 1. The list of probiotic bacteria used in this study

Genus	Species
Lactobacillus	*Lactobacillus acidophilus ATCC 4355* *Lactobacillus gasseri ATCC 1992* *Lactobacillus delbrueckii ATCC 12315* *Lactobacillus helveticus ATCC 15009*
Lacticaseibacillus	*Lacticaseibacillus casei ATCC 334* *Lacticaseibacillus rhamnosus ATCC 7469* *Lacticaseibacillus paracasei ATCC 335*
Limosilactobacillus	*Limosilactobacillus fermentum ATCC 14931* *Limosilactobacillus reuteri ATCC 23272*
Lactiplantibacillus	*Lactiplantibacillus plantarum ATCC 10012*
Ligilactobacillus	*Ligilactobacillus salivarius ATCC 11742*
Lactococcus	*Lactococcus lactis ATCC 29071*
Bifidobacterium	*Bifidobacterium bifidum ATCC 35914* *Bifidobacterium breve ATCC 15700* *Bifidobacterium longum ATCC 15702* *Bifidobacterium animalis ATCC 27673*
Streptococcus	*Streptococcus thermophilus ATCC BAA-250*

peri-implantitis-associated bacteria was investigated using susceptibility assay. The susceptibility assay was conducted according to the methods of Clinical Laboratory Standard Institute (CLSI) and used spent conditioned media (SCM) derived from probiotics after filtrating 0.22 μm of a polyvinylidene fluoride (PVDF) filter (Millipore, Billerica, USA). The prepared SCM were added to the 12th row, and the appropriate medium for each bacterium was dispensed from 1st to 11th row of 96-well plate (SPL life Sciences, Pocheon, Korea). Two-fold serial dilutions of the prepared SCM were performed to the 2nd row. After counting bacteria using bacterial counting chamber (Marienfeld Superior, Lauda-Königshofen, Germany), the concentration of peri-implantitis-associated bacteria was adjusted to 1.5×10^7 cells/ml. The bacteria were inoculated into the prepared 96-well plate. The plates were incubated at 37°C in anaerobic condition for 36 h, and the optical density was measured at a wavelength of 660 nm with microplate reader (BioTek, Winooski, USA).

All experiments were conducted triplicate in three times. Data distribution was assessed using Kolmogorov-Smirnov test and analyzed by Kruskal Wallis test. As a post-hoc test, Mann-Whitney U test was performed using IBM SPSS statistics Ver. 22 (IBM, Armonk, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Since it was not possible to test all probiotics at once, the probiotic bacteria were divided into groups A through F in alphabetical order and the test was conducted. In the investigation into the antimicrobial effects of probiotic bacteria on peri-implantitis-associated bacteria, *F. nucleatum* was first investigated. In group A, B. *breve* significantly inhibited the growth of *F. nucleatum* in SCM at concentrations of 12.5% or higher ($P < 0.05$), and growth of *S. thermophilus* and *B. bifidum* significantly reduced in SCM at concentrations of 25% or higher ($P < 0.05$) (Fig. 1A). In group B, *B. longum* and *B. animalis* significantly decreased the growth of *F. nucleatum* in SCM at a concentration of 12.5% and 25%, respectively ($P < 0.05$)(Fig. 1B). In case of group C, *L. acidophilus, L. casei*, and *L. gasseri* significantly reduced the growth of *F. nucleatum* at a SCM concentration of 12.5%, 3.2%, and 25%, respectively (Fig. 1C). *L. delbrueckii, L. helveticus*, and *L. fermentum* showed inhibitory effect on the growth of *F. nucleatum* at SCM concentrations of 25% and above ($P < 0.05$)(Fig. 1D). In group E, at SCM concentrations exceeding 6.3%, *L. paracasei* exhibited significant inhibition of *F. nucleatum* growth ($P < 0.05$)(Fig. 1E). In group F, *L. rhamnosus* significantly inhibited the growth of *F. nucleatum* at SCM concentrations of 3.2% or higher ($P < 0.05$)(Fig. 1F).

Next, conducting susceptibility assays for *P. intermedia*, in group A, probiotic bacteria significantly inhibited the growth of *P. intermedia* in SCM at concentrations of 25% or higher ($P < 0.05$) (Fig. 2A). In group B, *B. longum* and *B. animalis* significantly decreased the growth of *P. intermedia* in SCM at concentrations of 25% or higher ($P < 0.05$)(Fig. 2B). In case of group C, *L. casei* significantly reduced the growth of *P. intermedia* at a SCM concentration of 6.3%, and *L. acidophilus* and *L. gasseri* significantly inhibited the bacterial growth at a SCM concentration of 25% ($P < 0.05$)(Fig. 2C). *L. delbrueckii*, *L. helveticus*, and *L. fermentum* showed inhibitory effect on the growth of *P. intermedia* at SCM concentrations of 25% and above ($P < 0.05$)(Fig. 2D). In group E, *L. paracasei* significantly showed inhibition of *P. intermedia* growth at SCM concentrations of 12.5% or higher ($P < 0.05$) (Fig. 2E). In group F, at SCM concentrations exceeding 6.3%, *L. rhamnosus* significantly showed inhibition of *P. intermedia* growth ($P < 0.05$)(Fig. 2F).

In experiment using *P. gingivalis*, in group A, probiotic bacteria significantly inhibited the growth of *P. gingivalis* at SCM concentrations of 25% and above ($P < 0.05$)(Fig. 3A). In group B, *B. longum* and *B. animalis* significantly decreased the growth of *P. gingivalis* at SCM concentration of 12.5% and 25%, respectively ($P < 0.05$)(Fig. 3B). In case of group C, *L. casei* significantly reduced the growth of *P. gingivalis* at SCM concentration of 3.2% or higher, and *L. acidophilus* and *L. gasseri* significantly inhibited the bacterial growth at a SCM concentration of 25% ($P < 0.05$) (Fig. 3C). In group C, *L. delbrueckii*, *L. helveticus*, and *L. fermentum* showed inhibitory effect on the growth

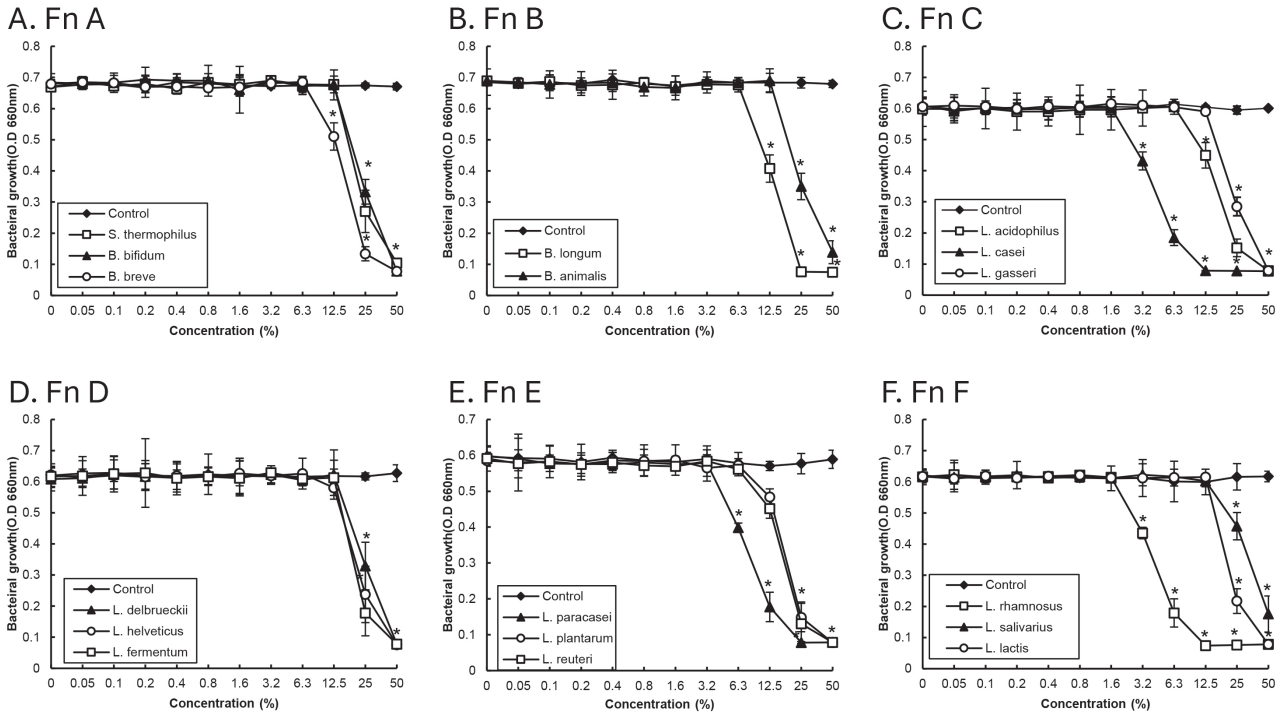

Fig. 1. Antimicrobial activity of the SCM of probiotic bacteria against *F. nucleatum*. The SCM of probiotics was collected. After preparing various concentrations of the SCM in 96-well plate, *F. nucleatum* was inoculated into the prepared 96-well plate. The plates were incubated, and the bacterial growth was measured by spectrophotometer.
Asterisk (*) indicates significant difference compared to control group ($P < 0.05$).

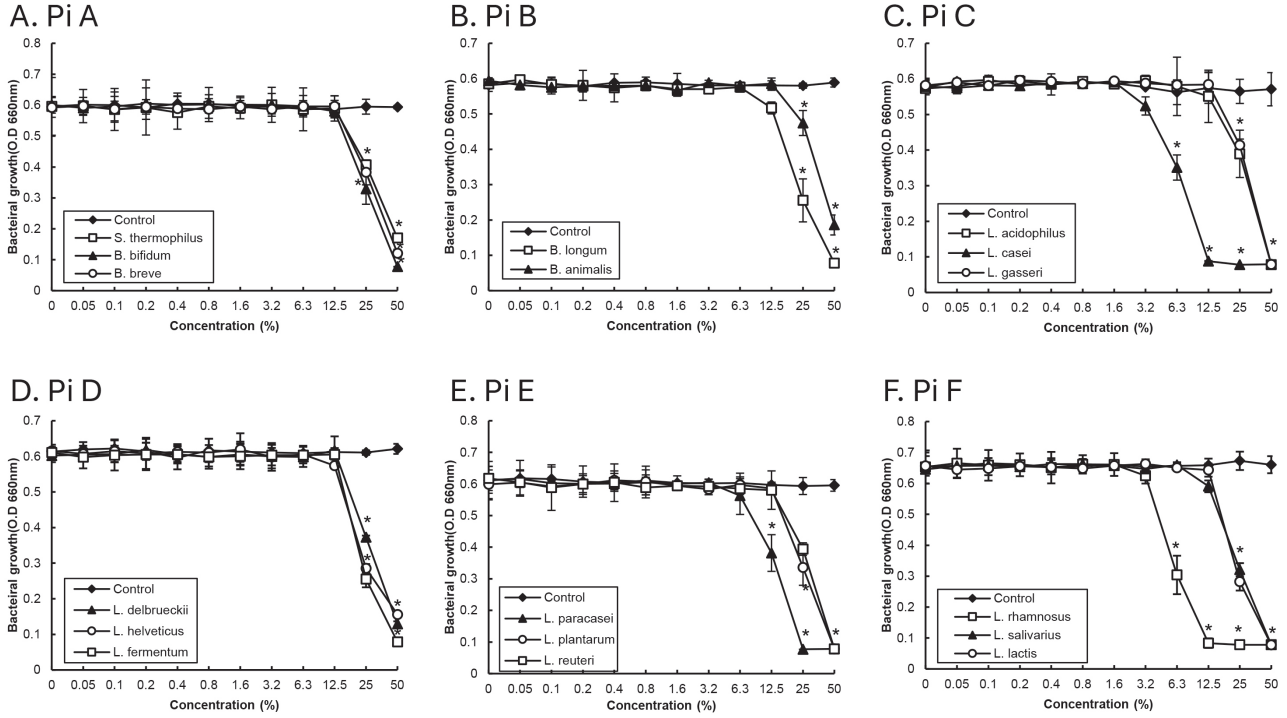

Fig. 2. Antimicrobial activity of the SCM of probiotic bacteria against *P. intermedia*. The SCM of probiotic bacteria was collected. After preparing various concentrations of the SCM in 96-well plate, *P. intermedia* was inoculated into the prepared 96-well plate. The plates were incubated, and the bacterial growth was measured by spectrophotometer.
Asterisk (*) indicates significant difference compared to control group ($P < 0.05$).

of *P. gingivalis* at SCM concentrations of 25% and above ($P < 0.05$)(Fig. 3D), *L. plantarum* and *L. reuteri* significantly reduced the *P. gingivalis* growth at SCM concentration of 25% ($P < 0.05$)(Fig. 3E). In group E, *L. rhamnosus* significantly inhibited the growth of *P. gingivalis* at SCM concentrations of 6.3% or higher ($P < 0.05$). *L. salivarius* and *L. lactis* significantly decreased the bacterial growth at a SCM concentration of 25% ($P < 0.05$)(Fig. 3F).

Finally, conducting susceptibility assay for *T. forsythia*, in group A, probiotic bacteria significantly inhibited the growth of *T. forsythia* in SCM at concentrations of 25% or higher ($P < 0.05$) (Fig. 4A). In group B, *B. longum* and *B. animalis* significantly decreased the growth of *T. forsythia* in SCM at a concentration of 12.5% and 25%, respectively ($P < 0.05$)(Fig. 4B). In group C, *L. casei* significantly reduced the growth of *T. forsythia* at a SCM concentration of 6.3%, and *L. acidophilus* and *L. gasseri* significantly inhibited the bacterial growth at a SCM concentration of 25% ($P < 0.05$)(Fig. 4C). *L. delbrueckii*, *L. helveticus*, and *L. fermentum* showed inhibitory effect on the growth of *T. forsythia* at SCM concentrations of 25% and above ($P < 0.05$)(Fig. 4D). In group E, *L. paracasei* significantly showed inhibition of *T. forsythia* growth at SCM concentrations of 6.3% and above($P < 0.05$)(Fig. 4E). In group F, at SCM concentrations exceeding 3.2%, *L. rhamnosus* significantly showed inhibition of *T. forsythia* growth ($P < 0.05$). *L. salivarius* and *L. lactis* significantly decreased the bacterial growth at SCM concentrations of 25% and above ($P < 0.05$)(Fig. 4F).

The antimicrobial activity of probiotic bacteria was set to 100% for each control group, and the growth rate of peri-implantitis-associated bacteria was converted and compared. As shown in Table 2, the inhibition of the growth of peri-implantitis-associated bacteria by probiotics was expressed as percentages at a SCM concentration of 25%. Among probiotics, *L. casei*, *L. paracasei* and *L. rhamnosus* showed the strongest antimicrobial activity against peri-implantitis-associated bacteria. Next, *B. longum* exhibited stronger antimicrobial activity compared to other probiotic bacteria. In addition, other probiotic bacteria showed slightly different antimicrobial effects depending on the peri-implantitis-associated bacteria.

Discussion

Dental implants are increasingly being used as a solution to replace missing teeth due to their many advantages over dentures.[11] However, as with all medical procedures, complications can occur, and one such complication is peri-implantitis. This condition is not limited to inflammation around the implant but can lead to implant failure and serious oral health problems. Although the occurrence of peri-implantitis is associated with various factors, fundamentally, microbiological factors play a pivotal role.[12] Bacterial biofilms form on the surface of implants similar to plaque around natural teeth, and, as with periodontitis, anaerobic Gram-negative bacteria such as *F. nucleatum*, *P. intermedia*, *P. gingivalis*, *T. forsythia*, and *T. denticola* are reported to increase within the biofilm.[13]

Since specific bacteria play a significant role in causing peri-implantitis, this study compared the antimicrobial effects of probiotic bacteria against periodontitis-related bacteria. Among probiotic bacteria, bacteria belonging to the genus Lacticaseibacillus showed strong antibacterial activity against peri-implantitis-associated bacteria. These bacteria were formerly classified as Lactobacillus, but recently the name was changed to Lacticaseibacillus.[14] *L. casei*, *L. paracasei* and *L. rhamnosus* belong to this group, and these probiotics showed strong antibacterial activity compared to other species. These bacteria produce five class II bacteriocins as small peptide molecules which inhibit the growth of Gram-positive and Gram-negative bacteria.[15] The probiotic bacteria belonging to the genus Limosilactobacillus were found to exhibit relatively strong antibacterial activity. *L. fermentum* and *L. reuteri* belong to this group, and produce six class II bacteriocins.[16] Among the genus Bifidobacterium, *B. longum* appeared to exhibit strong antibacterial activity against peri-implantitis-associated bacteria.[17] This bacterium also produces class II bacteriocin.

Bacteriocins act on susceptible cells by forming pores in the cytoplasmic membrane, thereby inducing the leakage of small intracellular substances.

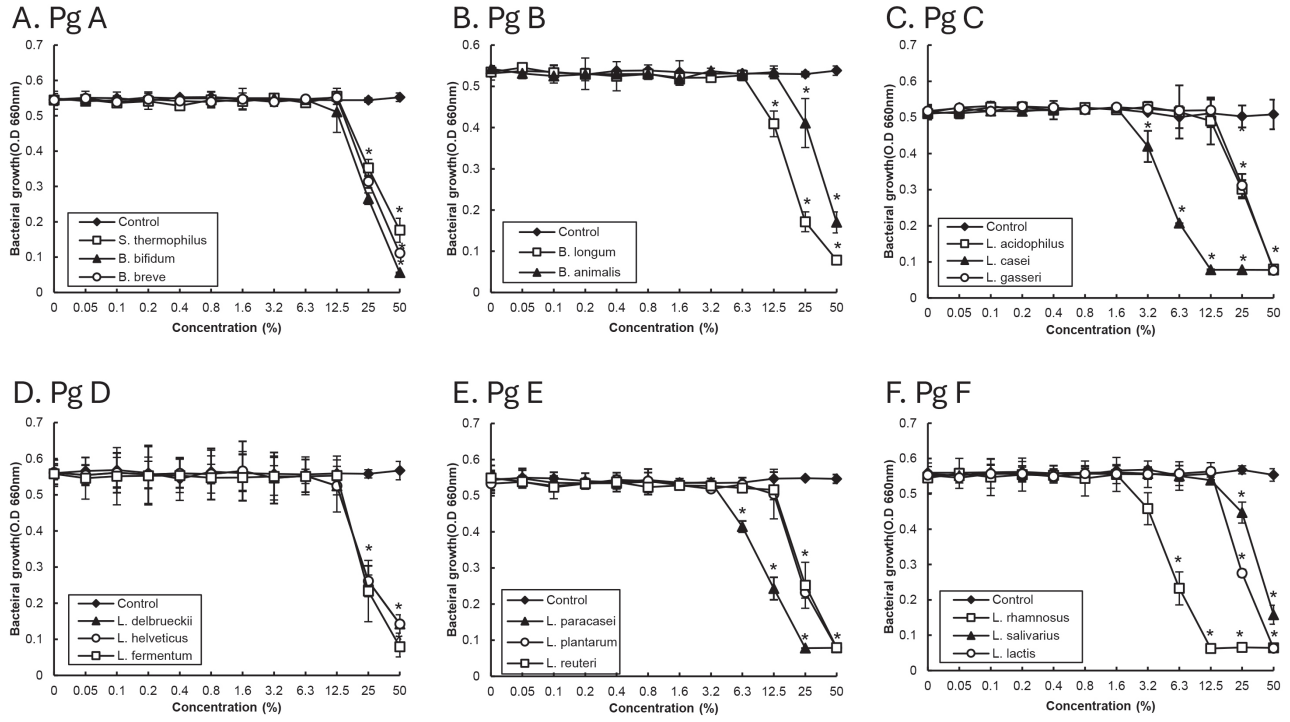

Fig. 3. Susceptibility of *P. gingivalis* for the SCM of probiotics. The SCM of probiotic bacteria was collected. After preparing various concentrations of the SCM in 96-well plate, *P. gingivalis* was inoculated into the prepared 96-well plate. The plates were incubated, and the bacterial growth was measured by spectrophotometer.
Asterisk (*) indicates significant difference compared to control group ($P < 0.05$).

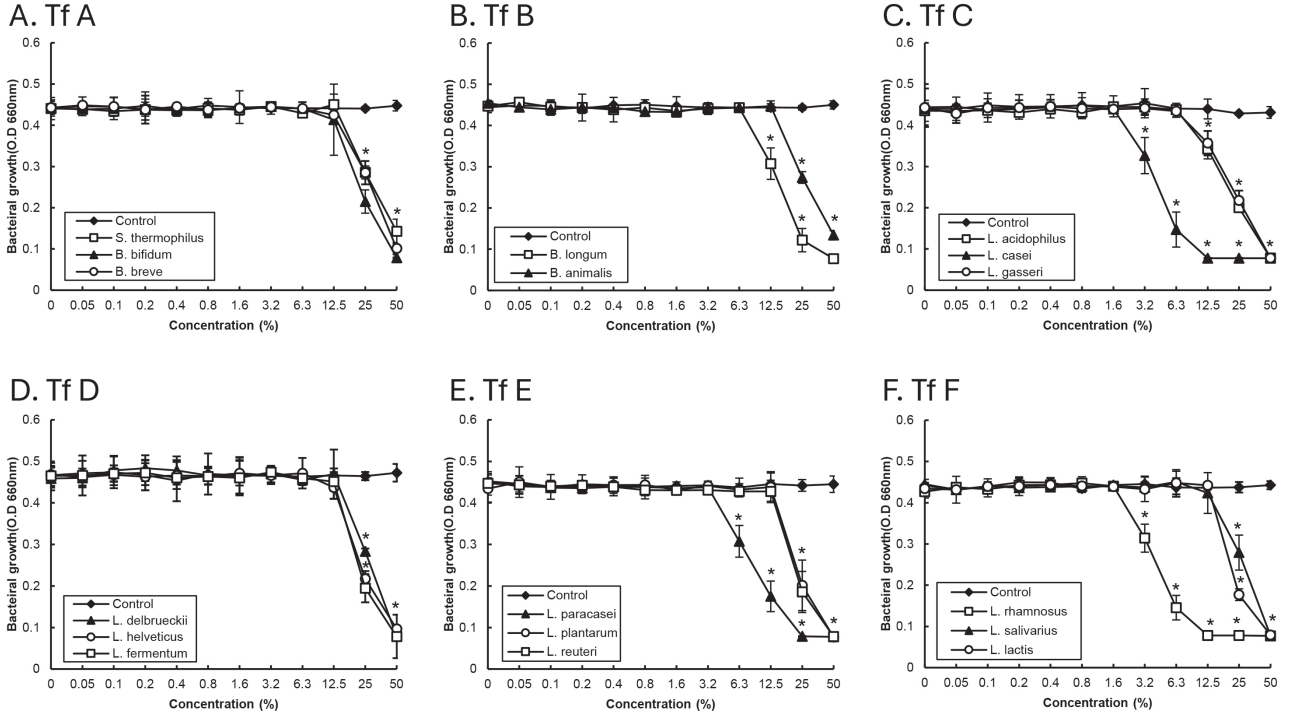

Fig. 4. Antimicrobial activity of the SCM of probiotic bacteria against *T. forsythia.* The SCM of probiotics was collected. After preparing various concentrations of the SCM in 96-well plate, *T. forsythia* was inoculated into the prepared 96-well plate. The plates were incubated, and the bacterial growth was measured by spectrophotometer.
Asterisk (*) indicates significant difference compared to control group ($P < 0.05$).

Table 2. Percent of growth inhibition at a SCM concentration of 25%

Strains	*F. nucleatum*	*P. intermedia*	*P. gingivalis*	*T. forsythia*
B. animalis	55.1 ± 4.14	21.03 ± 4.64	26.25 ± 7.63	46.39 ± 4.72
B. bifidum	57.36 ± 6.14	51.72 ± 14.69	59.91 ± 8.08	62.37 ± 8.27
B. breve	90.77 ± 3.69	40.94 ± 21.13	49.65 ± 6.22	43.03 ± 7.16
B. longum	100 ± 0	64.72 ± 13.13	79.45 ± 5.92	88.16 ± 8.14
L. acidophilus	85.88 ± 4.78	36.19 ± 4.76	47.34 ± 13.57	65.38 ± 2.29
L. casei	100 ± 0	100.21 ± 0.03	100 ± 0	100 ± 0
L. delbrueckii	53.55 ± 5.86	42.16 ± 7.46	45.53 ± 7.57	47.11 ± 7.45
L. fermentum	81.58 ± 8.8	68.66 ± 5.32	67.73 ± 5.19	70.02 ± 5.54
L. gasseri	59.99 ± 13.48	30.78 ± 16.65	45.23 ± 7.16	60.38 ± 6.72
L. helveticus	70.52 ± 7.72	60.82 ± 0.93	62.01 ± 0.87	64.08 ± 0.91
L. lactis	74.62 ± 6.1	63.87 ± 18.03	58.9 ± 3.71	73.64 ± 5.7
L. paracasei	100 ± 0	100 ± 0	100 ± 0	100 ± 0
L. plantarum	86.08 ± 8.74	49.96 ± 13.61	67.41 ± 6.72	66.17 ± 18.07
L. reuteri	89.57 ± 12.8	38.73 ± 7.05	63.14 ± 27.22	70.59 ± 14.78
L. rhamnosus	100 ± 0	99.15 ± 2.42	100 ± 0	100 ± 0
L. salivarius	30.32 ± 4.81	45.5 ± 62.16	23.04 ± 11.06	46.17 ± 15.27
S. thermophilus	67.94 ± 11.02	36.43 ± 8.39	41.26 ± 3.56	42.88 ± 3.63

Numerical data was expressed as mean ± SD and qualitative data was expressed in percentage.

This leads to depolarization of the cell membrane and consequently causes apoptosis.[18] Gram-negative bacteria generally exhibit resistance to various bacteriocins produced by Gram-positive bacteria due to their outer cell membranes. This outer membrane acts as an effective barrier.[19] Bacteriocins can be roughly divided into three categories according to their genetic and biochemical characteristics. There is class I, consisting of peptides less than 5 kDa; class II, consisting of heat-stable peptides less than 10 kDa; and finally, class III, consisting of heat-stable peptides greater than 30 kDa.[20] Class II bacteriocins are divided into three subclasses. Subclass IIa exhibit a common N-terminal domain (Tyr-GlyAsn-Gly-Val-X-Cys). Subclass IIb is composed of bacteriocins that are formed by two peptides called 705α and 705 β. Subclass IIc includes circular peptides that are dependent on the Sec system.[18] Carnobacteriocin BM1 and piscicolin 126, are class IIa bacteriocin, are anchored to outer membrane via specific receptors utilizing the mannose phosphotransferase system, allowing them to act more efficiently on Gram-negative bacteria than other bacteriocins.[21] In this context, the amino acid sequences of the receptors may vary depending on the bacterial species, which can lead to differences in the sensitivity of target cells. All probiotic bacteria used in this study produce class II bacteriocins.[15] Therefore, there appears to be no correlation between the bacteriocin type and the antimicrobial activity against peri-implantitis-associated bacteria, and it is expected to be caused by differences in subclass bacteriocins.[22] To verify this, further studies investigating the types of bacteriocins produced by probiotics are required through molecular experiments and genetic analysis.

Conclusion

In this study, the antimicrobial activity of probiotic bacteria against peri-implantitis-associated bacteria was investigated, and *L. casei*, *L. paracasei*, and *L. rhamnosus* belonging to the genus Lacticaseibacillus were found to exhibit the strongest antimicrobial activity against peri-implantitis-associated bacteria. This

is presumed to be because these bacterial species produce specific bacteriocins that act on Gram-negative bacteria, and it is speculated that peri-implantitis-associated bacteria can be controlled using these probiotics.

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임플란트 주위염 관련 세균에 대한 프로바이오틱스의 항미생물 효과 비교

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단국대학교 치과대학 구강미생물학교실

목적: 치주염은 치과 임플란트 주변에 그람 음성 혐기성 세균이 증식하는 것과 관련이 있다. 본 연구의 목적은 체외 실험을 통해 치주염 관련 세균에 대한 프로바이오틱스의 효과를 조사하고, 치주염의 치료 및 예방에 적합한 프로바이오틱스를 제시하는 것이다.
연구 재료 및 방법: 18종의 프로바이오틱스 균주에서 배양액을 수집하였다. 수집된 프로바이오틱스 균주의 배양액에 대한 항미생물 활성은 감수성 검사를 통해 평가하였다.
결과: 락토티세이바실러스(Lacticaseibacillus) 속이 치주염 관련 세균에 대해 가장 강력한 항미생물 활성을 나타냈다. 리모실락토바실러스(Limosilactobacillus) 속에서는 *Limosilactobacillus reuteri*가, 비피도박테리움(Bifidobacterium) 속에서는 *Bifidobacterium longum*이 임플란트 주위염 관련 세균에 대해 강력한 항균 활성을 보였다. 락토바실러스(Lactobaillus) 속과 리길락토바실러스(Ligilactobacillus) 속은 다른 프로바이오틱스에 비해 약한 항미생물 활성을 보였다. *Streptococcus thermophilus*는 치주염 관련 세균에 대해 약한 항균 활성을 나타냈다.
결론: *Lacticaseibacillus casei, Lacticaseibacillus paracasei, Lacticaseibacillus rhamnosus*는 임플란트 주위염 관련 세균에 대해 가장 강력한 항균 활성을 나타내는 것으로 확인되었고, 이 프로바이오틱 세균들은 임플란트 주위염 관련 세균에 대한 성장 억제에 효과적일 것이다.

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주요어: 임플란트 주위염; 프로바이오틱스; 항미생물 활성; 예방효과

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