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研究題目：Soybean peptide inhibits the biofilm formation of periodontopathic bacteria

目　的：

Antimicrobial agents are effective approach in biofilm control for the prevention and treatment of periodontal disease. Since the increased development of antimicrobial resistance, reducing the use of existing antimicrobial drugs is an urgent issue, and the great attention has been emphasized to explore alternative and adjuvant therapies for antibacterial drugs. The purpose of the present study is to clarify the effect to biofilm of soybean-derived peptide.

対象および方法：

Soybean peptide, BCBS-11（RIRLLQRFNKR）was synthesized at Eurofins Genomics（Tokyo, Japan）. Chlorhexidine, CHX（FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan）, was used as positive control. *Porphyromonas gingivalis* FDC 381, *Fusobacterium nucleatum* ATCC 25586 and *Streptococcus mitis* ATCC 903 were used in this study. These bacteria（2 x 10^8 CFU/ mL）were cultured with or without BCBS-11. The amount of biofilm was measured by crystal violet stain（Chroma-Gesellschaft Co. Ltd., Münster, Germany）to evaluate the inhibitory effect of BCBS-11 on biofilm formation and the eradication effect of established biofilm. The minimum inhibitory concentration（MIC）and the minimum bactericidal concentration（MBC）was analyzed for the antibacterial mechanism. The data are expressed as the means ± standard errors of the mean（SEMs）. A one-way ANOVA was performed with GraphPad Prism 7.0 graphing and statistical software, and $P < 0.05$ was considered statistically significant.

結果および考察：

The inhibitory effects of BCBS-11 on biofilm formation are shown in Figure1. The minimum biofilm inhibitory concentration of BCBS-11 against *F. nucleatum*（100 μM）（Figure 1B）was lower compared with that against *P. gingivalis*（400 μM）（Figure 1A）, indicating that BCBS-11 inhibits biofilm formation against F. nucleatum more strongly than *P. gingivalis*. BCBS-11 was almost as effective as CHX against *F. nucleatum*（Figure 1B）. However, BCBS-11 did not inhibit the biofilm formation of S. *mitis* although CHX inhibited that of *S. mitis*（Figure 1C）.

The results on the established biofilms are shown in Figure2. BCBS-11 did not eradicate the established biofilm of *P. gingivalis*（Figure 2A）. The amount of *F. nucleatum* biofilm was

reduced significantly by BCBS-11 at 400 μM, although neither BCBS-11 nor CHX completely eradicated the mature biofilm (Figure 2B). Both BCBS-11 and CHX were not able to eradicate established biofilms of *S. mitis* (Figure 2C). In general, antimicrobial peptides with strong cationic properties have been reported to adhere to the surface of biofilms. Thus, BCBS-11 also adhered to the surface of *F. nucleatum* mature biofilm, and its antibacterial activity reduced the amount of biofilm. However, it was considered that it could not penetrate into the biofilm.

To identify the mechanism of the antibacterial effect of BCBS-11, we analyzed MIC and MBC assay (Table 1). The MIC and MBC for BCBS-11 indicated that it has bactericidal activity against *P. gingivalis* and *F. nucleatum*. However, the MIC and MBC for BCBS-11 against *S. mitis* were greater than 400 μM, suggesting that BCBS-11 does not exhibit significant antimicrobial activity against *S. mitis*. CHX exhibited bactericidal activity against these all bacteria.

It was suggested that inhibition of biofilm was induced by the electrostatic interaction between cationic peptide and periodontopathic bacteria. Generally, the outer membrane of Gram-negative bacteria consists of a thin layer of cytoplasmic membrane and negatively-charged surface. BCBS-11 interacts with them electrostatically and leads to membrane disruption. In contrast, Gram-positive bacteria have a cell wall that contains a thick peptidoglycan layer and a strong negatively-charged teichoic acid on the cell wall surface, and thus, they do not have membrane-disruptive manner.

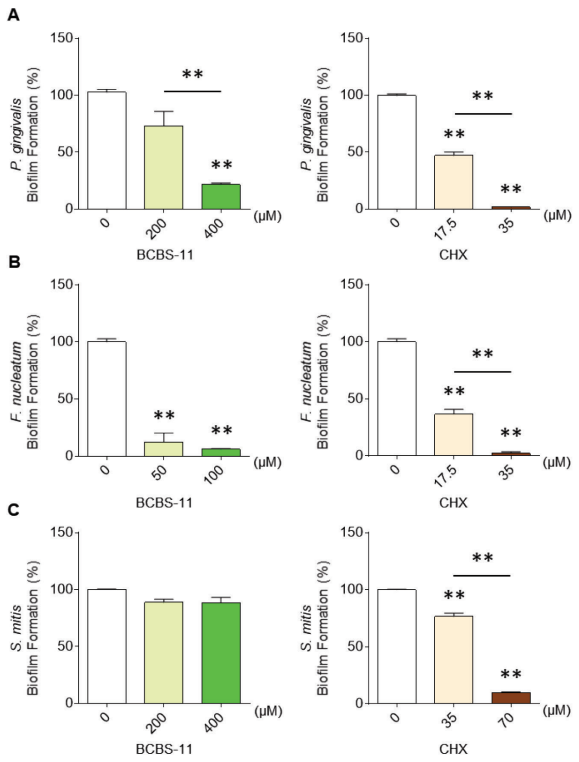

Figure 1. The inhibitory effect on biofilm formation

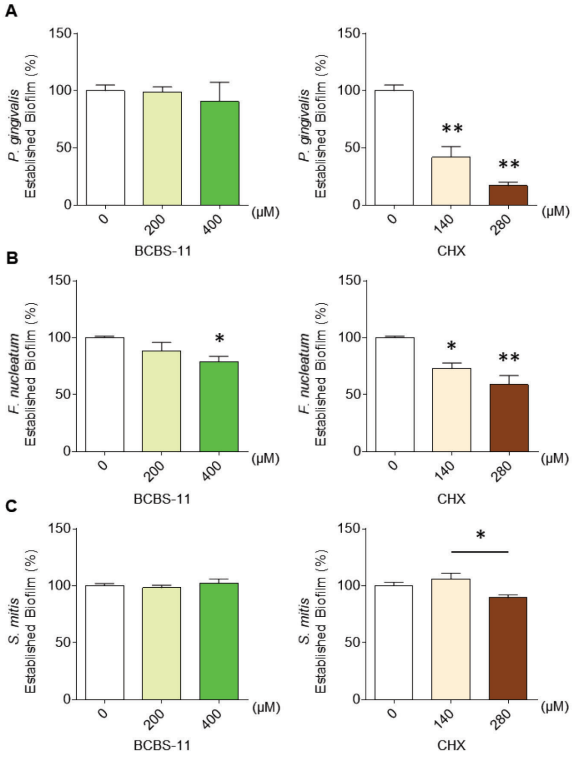

Figure 2. Eradication of established biofilm

Table 1. MIC and MBC values of BCBS-11 with regard to *P. gingivalis*, F. nucleatum and *S. mitis*.

(μM)	*P. gingivalis*		*F. nucleatum*		*S. mitis*	
	MIC	MBC	MIC	MBC	MIC	MBC
BCBS-11	100	100	50	100	＞400	＞400
Chlorhexidine	17.5	17.5	35	35	70	70

The identification of peptides with antimicrobial activity against periodontal pathogens may enable personalized treatment of biofilm-related diseases and provide alternatives to existing antibiotics. Since BCBS-11, soybean peptide, is a food-derived peptide, it could be safely used for prevention or treatment of periodontal diseases in a super-aging society and expected to contribute to the maintenance and improvement of QOL.

We have concluded that BCBS-11 inhibited biofilm formation of periodontopathic bacteria by bactericidal activity. It was suggested that this peptide may be effective at controlling oral biofilm.

成果発表：(予定を含めて口頭発表、学術雑誌など)

Poster Presentation

1. Hnin Yu Lwin、野中由香莉、松岸　葵、多部田康一：大豆ペプチドは *Porphyromonas gingivalis* および *Fusobacterium nucleatum* の バイオフィルム形成を阻害する。特定非営利活動法人　日本歯科保存学会 2021 年度春季学術大会（第 154 回）。Web 開催、2021 年 6 月 10 日～23 日：P76 頁、2021。
2. Hnin Yu Lwin, Yukari Aoki-Nonaka, Aoi Matsugishi, Koichi Tabeta：Soybean peptide inhibits biofilm of periodontopathic bacteria via bactericidal activity. The 99th General Session of the IADR. Virtual meeting, July 21-24, 2021.
3. Hnin Yu Lwin、野中由香莉、松岸　葵、多部田康一：Analysis of anti-biofilm effect of soybean-derived peptide。第 65 回 春季日本歯周病学会学術大会。 2022 年 6 月 3 日～ 4 日。（In the preparation）

Oral Presentation

1. Hnin Yu Lwin、野中由香莉、松岸　葵、高橋直紀、日吉　巧、多部田康一：Analysis of anti-biofilm effect against periodontopathic bacteria by soybean-derived peptide. 令和 4 年度　第 55 回 新潟歯学会 総会。2022 年 4 月 16 日。(In the preparation)

Academic Journal：In the progress.