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Antimicrobial Activity of P-113 against Bacterial Flora of the Human Oral Cavity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Periodontitis is a chronic disease associated with dental biofilm formation by bacteria. The primary clinical signs include plaque formation, gingivitis, gingival bleeding, and attachment loss. P-113, a 12-amino acid antimicrobial peptide, is derived from Histatin 5 (Hst5) secreted by the human parotid

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and submandibular salivary glands and has been reported to reduce plaque formation, gingivitis, and gingival bleeding. This study aimed to investigate the effect of P-113 on periodontal disease-associated bacteria and the relative abundance of bacteria using real-time polymerase chain reaction (real-time PCR) and Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Saliva samples were collected from participants for analyses. Real-time PCR results showed significant changes in the abundance of the periodontal disease-associated bacteria, *Fusobacterium nucleatum* and *Tannerella forsythia*, after the use of the P-113 mouthwash. After culturing in an anaerobic environment, MALDI-TOF MS analysis was performed. The results showed that the relative abundance of *Streptococcus mitis* decreased and that of beneficial *Streptococcus salivarius* increased after the use of the P-113 mouthwash. These results indicate the potential of P-113 mouthwash as a treatment that can influence the proportion of pathogens and probiotics to establish equilibrium in the salivary environment.

Keywords: Antimicrobial peptide; MALDI-TOF MS; Real-time PCR.

1. INTRODUCTION

Periodontal disease has a negative impact on the overall quality of life. Periodontal diseases are highly prevalent, affecting up to 90% of the global population. In Taiwan, 98.6% of the population has oral-cavity problems [1]. Quality of life is affected by disabilities resulting from periodontal disease, such as tooth loss, periodontal ligament loss, and destruction of the surrounding alveolar bone [2]. Furthermore, periodontal disease not only affects the oral cavity, but is also correlated with multiple systemic diseases, such as diabetes, cardiovascular diseases, premature birth, and rheumatoid arthritis [3]. Therefore, its high prevalence, related disabilities, and association with systemic diseases have made the periodontal disease a public health concern [1]. Active bacterial infections and microbial dysbiosis are causes of periodontal disease [4]. A high abundance of gram-negative anaerobic organisms has been reported in specimens from participants with periodontal disease [5]. In Scoransky's studies, the complexes are ranked first to fifth, with the first being most closely associated with severe periodontitis, including *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, and the fifth being the least associated [6]. Therefore, bacterial control and pathogen removal are critical in the treatment of periodontitis. The primary treatment approach includes maintenance of proper oral hygiene and nonsurgical mechanical debridement, whereas aggressive treatment involves surgical flaps and alternative anti-infective approaches [7]. However, the inability to effectively control microorganisms in the long term may be the reason for the short-term effects of treatment [8]. Furthermore, treatment failure may result from pathogen suppression during the active phase of therapy and re-colonization after therapy [9]. Further research efforts are needed to improve treatment efficacy [7]. P-113 (AKRHHGYKRKFH), a 12-amino acid antimicrobial peptide, is derived from Histatin5 (Hst5) secreted by the human parotid and submandibular salivary glands and has been reported to reduce plaque formation, gingivitis, and gingival bleeding in phase II clinical trials [10]. Using mouthwash is a common and effective way of oral hygiene to control oral bacteria.

Real-time PCR is commonly used to identify target genes [11]. Previous studies have utilized this technique to quantify the load of viruses and other microorganisms [12]. It provides high specificity and sensitivity for detecting target viruses and bacteria. In addition, real-time PCR requires lesser time and workforce than the traditional method [13]. MALDI-TOF MS is another tool that has been widely utilized in a variety of studies for identifying microbes, such as gut microbiota, urinary tract and blood pathogens [14,15]. Because of its advantages, such as high accuracy, less turnaround time, lower cost, lower sample volume, and faster identification speed, in comparison to conventional laboratory procedures for microbial identification, such as phenotypic tests involving gram-staining, biochemical profiling, DNA–DNA hybridization, and whole-genome DNA probes, MALDI-TOF MS has revolutionized clinical laboratory practice [15,16]. Bacteria can be identified using MALDI-TOF MS without any knowledge of microbial taxonomic affiliation [17, 18]. The spectra of proteins extracted from microorganisms generated by MALDI-TOF MS are matched with reference spectra in databases, based on scoring algorithms [19].

In summary, effective control of microorganisms is urgently required for improving the quality of life. Treatment for suppressing pathogens during the active phase of therapy and avoiding recolonization after therapy requires further research. Hence, this study focused on the antimicrobial activity of P-113 against the bacterial flora of the human oral cavity. Changes in specific target strains and in bacterial distribution were analyzed using real-time PCR and MALDI-TOF MS.

2. MATERIALS AND METHODS

2.1 Settings and Participants

Participants were enrolled from Taipei Medical University in Taiwan. The participants were selected from among those older than 20 years (n = 37) that 10.8% were male and 98.2% were female, with no history of any serious oral disease such as periodontal disease or oral cancer, systemic disease, or usage of medications such as antibiotics, anti-inflammatory drugs, and immune inhibitors, and exclude who were smoking, betal nut chewing, and pregnant. The P-113 mouthwash was supplied by General Biologicals Corporation, Hsinchu, Taiwan. Saliva samples were collected without treatment (Collection I). Afterward, saliva samples were collected after the use of a mouthwash containing P-113 three times a day for 4 days (Collection II) and 14 days (Collection III) for short and long period treatments, respectively. All saliva samples were collected after lunch and rinsing the mouth. Hence, samples from the participants were collected after using a mouthwash containing P-113 to investigate the antimicrobial activity of P-113 against bacterial flora in the human oral cavity.

2.2 Saliva Sample Collection

Saliva samples were collected from all participants and followed previous study [20]; they were asked to spit saliva into a 15-mL sterilized tube. Next, 2 mL of saliva sample was transferred into a collection tube containing DNA storage buffer (1 mM EDTA and 10 mM Tris-HCL) for real-time PCR analysis. After sampling, the samples were centrifuged at 8,000 rpm for 10 min, and the supernatant was removed. DNA was purified using a LabTurbo DNA Extraction Kit. Each sample for the MALDI-TOF Biotyper process was preserved in a CMP^{TM} anaerobic TranSwab container before sample preparation for bacterial culture. After removal from the CMP^{TM} Anaerobic TranSwab, all samples were transferred into 0.5 mL of tryptic soy broth (TSB), mixed with 0.5 mL of glycerol, and stored at −80°C for 10-20 minutes. After the sample collection, several samples were pooled. Next, 10,000 x to 200,000 x dilutions (beginning from 10−4 to 2 × 10−5) of the saliva samples were prepared and 100 µL of diluted samples was transferred onto prereduced anaerobic blood agar (TSB with 5% sheep blood, 0.5 mg/ml hemin, 2 µg/ml vitamin K_1, 0.5% yeast extract, and 0.05% cysteine HCl-H_2O)and chocolate agar (CAP) for anaerobic incubation (anaerobic gas mixture, 80% N_2, 10% CO_2, 10% H_2, 37°C) for 4 days' duration in a Whitley DG250 Workstation.

2.3 Real-time RT-PCR

The samples were analyzed to quantify six target strains: *Actinobacillus actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Treponema denticola* (Td), and *Tannerella forsythia* (Tf). The counts of the target strains were evaluated by real-time PCR analysis. A total of 25 µL of the extracted DNA was amplified using Master Mix (General Biologicals Corporation, GBC) and analyzed on a Real-Time PCR System from Roter gene Q (QIAGEN, Inc., Hilden, Germany). Absolute quantification of target strains was performed using a standard curve prepared by plasmids with reference genes. DNA quantity was determined based on the genomic size of each bacterium and the mean weight of one nucleotide pair [19]. The real-time PCR conditions were as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 5 s, and 58°C for 30 s. The samples were analyzed to quantify the reference genes of six target strains and the PCR primers used for the quantification are shown as the following sequences: lktA of Aa: 5'-CAGCATCTGCGATC CCTGTA-3' (forward), and 5'-TCAGC CCTTTGT CTTTCCTAGGT-3' (reverse) [21]; 16S rRNA of Fn: 5'-CGCA GAAGGTGAAAG TCCTGTAT-3' (forward), and 5'-TGGTCCTCACTGATTCACA CAGA-3' (reverse) [22]; 16S rRNA of Pg: 5'-TACCCATCG TCGCCTTGGT-3' (forward), and 5'-CGGA CTAAAA CCGCATACACTTG-3' (reverse) [23]; phoC of Pi: 5'-TGTCGGTTTA CTGGCTA TGTTCTC -3' (forward), and 5'-CTTGTCTG TTGGCCATCTTGAAG-3' (reverse) [24]; 16S rRNA of Td: 5'-AGAGCAAGCTCT CCCTTA CCGT-3′ (forward), and 5'-TAAGGGCGG CTTGAA ATAATGA-3′ (reverse) [25]; 16S rRNA of Tf: 5'-ATCCTGGCTCAGG

ATGAACG-3′ (forward), and 5'-TACGCATAC CCATCCGCAA-3′ (reverse) [22]. The results of real-time PCR were analyzed by the Ct value, which was converted into the number of bacteria according to the standard curve of the target strains. The number of bacterial counts in the standard curve was calculated using the genomic DNA size from each bacterium.

2.4 Microbial Identification and Quantification by MALDI-TOF MS Analysis

Bacterial colonies grown on the agar plates were transferred onto a target polished steel plate (MBT 384, Bruker DaltonicsInc). Then, 1 µL of 70% formic acid (Sigma-Aldrich, Missouri, USA) was used to extract proteins from bacterial colonies, and 1 µL of HCCA solution (10 mg/ml α-cyano-4-hydroxycinnamic acid in a mixture of 50% acetonitrile (ACN), 47.5% ultra-pure water, and 2.5% trifluoroacetic acid (TFA)) was overlaid on the samples after air-drying. The dried samples were analyzed on an Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) after repeating the air-drying process. Spectra ranging from 2,000 Da to 20,000 Da were recorded at the maximal laser frequency. Raw spectra were analyzed using the MALDI BioTyper 3.1 software package (default settings; BrukerDaltonik GmbH, Bremen, Germany, BioTyper® database renewed at 2014/9/8). There were approximately 5989 spectra in this version of the database. Log scores, according to the criteria recommended by the manufacturer, ranged from 0 to 3.00. Scores ≥ 1.7 were considered as confidence identification, and scores <1.7 as no reliable identification [19].

2.5 Data Sources/Measurement and Statistical Analysis

Bacterial counts were performed using real-time PCR data, and significance was established using Mann-Whitney statistical analysis [26]. The relative abundance (RA) of bacteria identified by MALDI Biotyper was calculated by determining the proportion of the detectable bacterial colony count of each species among the total bacterial colony count and is shown as percentages. Statistical analysis of the data from Biotyper was performed using GraphPad Software. The results are shown as the means and standard errors of the means (SEM), and the level of significance was determined using the Student's t-test.

3. RESULTS

3.1 Antimicrobial Activity of the P-113 Mouthwash against the periodontal Disease-Associated Bacteria in the Saliva of Participants by Real-time PCR Analysis

To investigate the antimicrobial activity of the P-113 mouthwash in the saliva of participants, saliva samples were collected at three time points, and the periodontal disease-associated strains *Actinobacillus actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Treponema denticola* (Td), and *Tannerella forsythia* (Tf), were identified in saliva samples via real-time PCR analysis.

Saliva was collected from participants before the use of the P-113 mouthwash (Collection I), 4 days after use (Collection II), and 14 days later (Collection III). Periodontal disease-associated bacteria were affected after using the P-113 mouthwash, but only two species, *F. nucleatum* and *T. forsythia*, showed significant differences in number of bacteria, according to the Mann-Whitney statistical test. The abundance of *F. nucleatum* decreased after the use of the P-113 mouthwash in samples from collection III ($P = 0.0358$; Fig. 1), and the abundance of *T. forsythia* decreased in saliva samples from collection II ($P = 0.0144$; Fig. 1) and collection III ($P = 0.009$; Fig. 1).

3.2 Antimicrobial Activity of the P-113 Mouthwash against Bacterial Flora in the Saliva of Participants by MALDI-TOF MS Analysis

To assess the effect of the P-113 mouthwash on bacterial distribution in the saliva of participants, samples were collected at three time points. Saliva was collected from participants before the use of the P-113 mouthwash (Collection I), 4 days after use (Collection II), and 14 days later (Collection III). Eighty species were identified by MALDI-TOF analysis (Fig. 2), and two species, *Streptococcus mitis* and *S. salivarius*, showed significant differences in relative abundance compared to collection I, according to Student's t-test. The relative abundance of *S. mitis* decreased by 1.6-fold after the use of the P-113 mouthwash in samples from collection III ($P = 0.0473$; Fig. 3a), and the relative abundance of *S. salivarius* increased by 1.9-fold in collection II ($P = 0.0448$; Fig. 3b).

4. DISCUSSION

The quality of life is affected by periodontal disease. Therefore, bacterial control and pathogen removal are critical. However, the current treatment failure results from the suppression of pathogens during the active phase of therapy and recolonization after therapy [9]. Further research is needed to improve treatment efficacy [7]. Using mouthwash is a common and effective oral hygiene method for controlling bacteria. In this study, we assessed the antimicrobial activity of P-113 against bacterial flora in the human oral cavity by using a mouthwash containing P-113. This peptide was used in mouthwash and phase I/II human clinical trial. The result showed that P-113 can decrease gingivitis and dental plaque in oral cavity [27]. In addition, P-113 has evidenced effective therapy on oral candidiasis in HIV patients [28]. In previous studies indicated that the P-113 peptide was modified; Nal-P-113, replaced histidine residues with large amount of *β*-naphthylalanine, and effectively improved periodontal disease-associated bacteria [29].

We used real-time PCR and MALDI-TOF MS to identify periodontal disease-associated bacteria. Real-time PCR was used to analyze oral saliva from the participants before and after using the P-113 mouthwash. The results showed that the abundance of bacteria did not dramatically change after the application of the P-113 mouthwash. This finding may be explained by the fact that the oral commensal microflora of humans can maintain the equilibrium of the oral cavity [30] however, two species, *Fusobacterium nucleatum* and *Tannerella forsythia*, showed significant differences in relative abundance after the use of the P-113 mouthwash compared to collection I and III. The abundance of *Fusobacterium nucleatum* decreased after the use of the P-113 mouthwash. *Fusobacterium nucleatum and Tannerella forsythiae* are common species in the oral cavity and can act as periodontal pathogens [31]. Moreover, recent research has shown that there is a correlation between *Fusobacterium nucleatum*, oral cancer, and uncontrolled type-2 diabetes [32]. Previous studies have indicated that *Tannerella forsythia* may be a risk factor for atherosclerosis [33]. Our results indicate that the risk of oral disease with *Fusobacterium nucleatum* and *Tannerella forsythia* may be reduced after the application of the P-113 mouthwash.

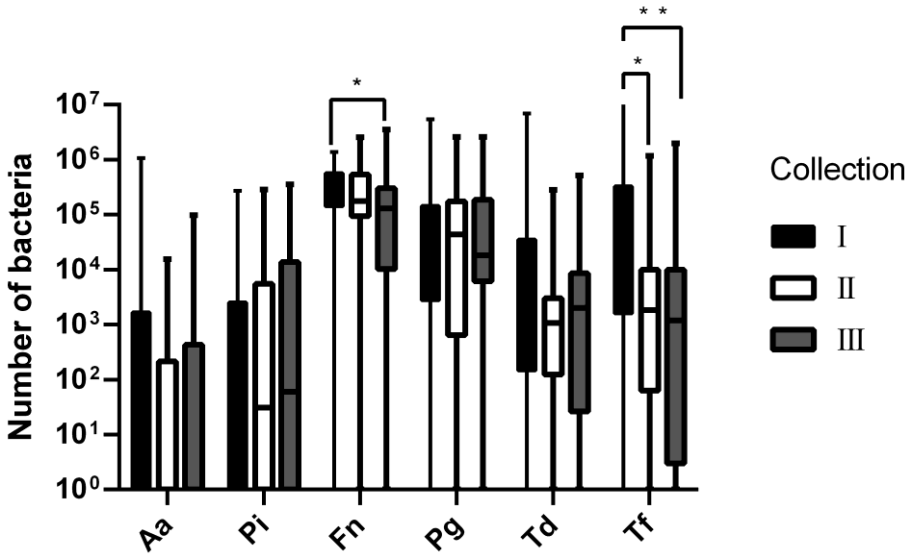

Fig. 1. Real-time PCR analysis of periodontal disease-associated bacteria in participants before and after using the P-113 mouthwash

*Real-time PCR analysis was performed to evaluate the differences in the abundance of strains Actinobacillusactinomycetemcomitans (Aa), Fusobacteriumnucleatum (Fn), Porphyromonasgingivalis (Pg), Prevotellaintermedia(Pi), Treponemadenticola (Td), and Tannerella forsythia (Tf), in the saliva samples of participants. The samples from participants were collected at three time points: before using the P113-mouthwash (Collection I), 4 days after use (Collection II), and 14 days after use (Collection III). *P < 0.05, **P < 0.01 Statistical test performed with Mann-Whitney Statistical test*

In this study, 80 species were identified by MALDI-TOF analysis, and the bacterial distribution in the saliva of the participants in this study did not dramatically change after the application of the P-113 mouthwash. This finding may be explained as follows: salivary properties vary greatly among individual participants, making it more difficult to determine changes. On the other hand, the results may simply mean that the P-113 mouthwash does not dynamically influence the bacterial distribution in the saliva of participants. However, two species, *Streptococcus mitis* and *S. salivarius*, showed significant differences in relative abundance after

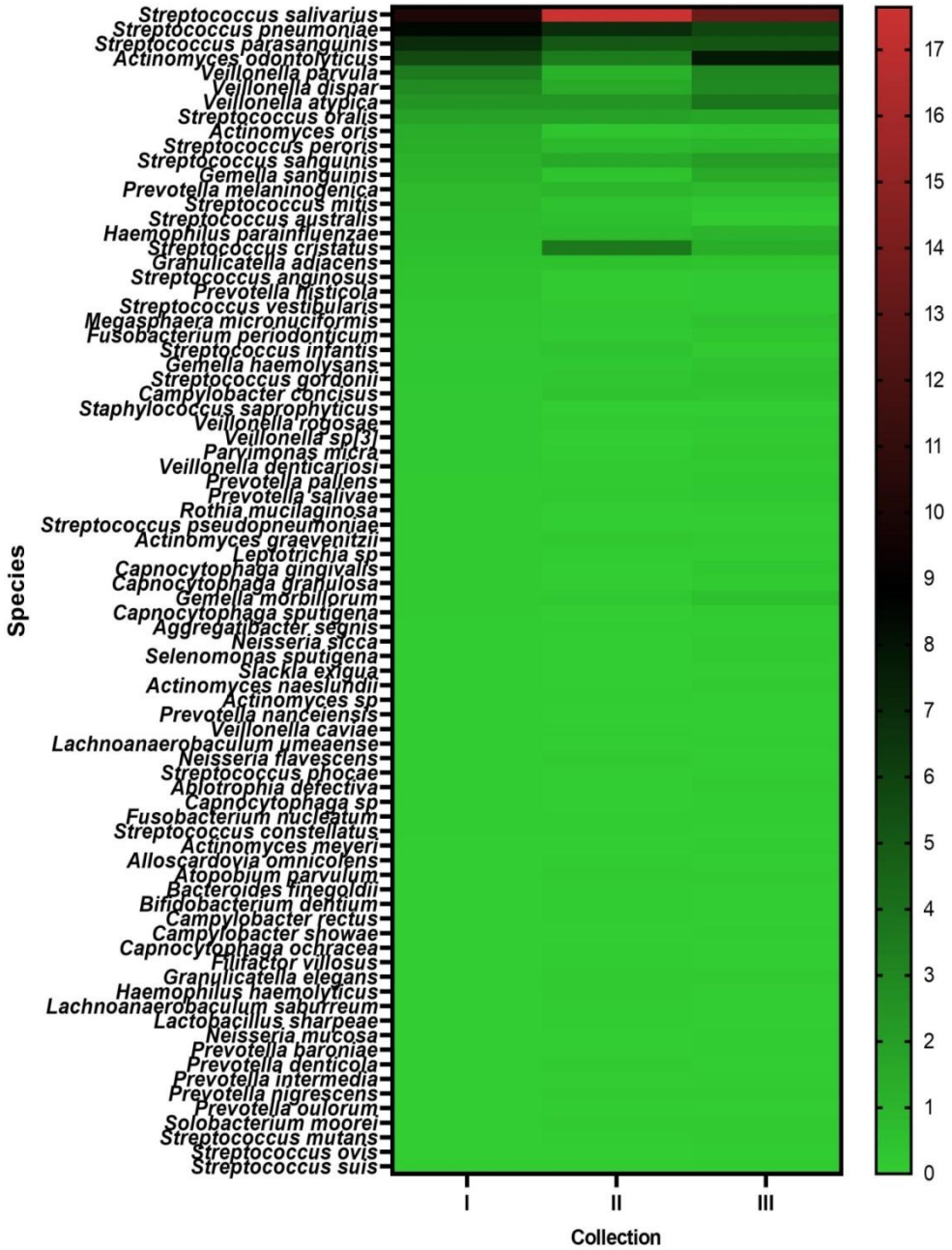

Fig. 2 Bacterial abundances in saliva samples collected from participants analyzed by MALDI Biotyper

The relative abundance of bacteria in the saliva cultured in an anaerobic environment and identified as MALDI Biotyper in this study represented in a heatmap. The samples were collected at three time points: before the application of the P-113 mouthwash (Collection I), 4 days after the use of the P-113 mouthwash (Collection II), and 14 days after the use of the P-113 mouthwash (Collection III)

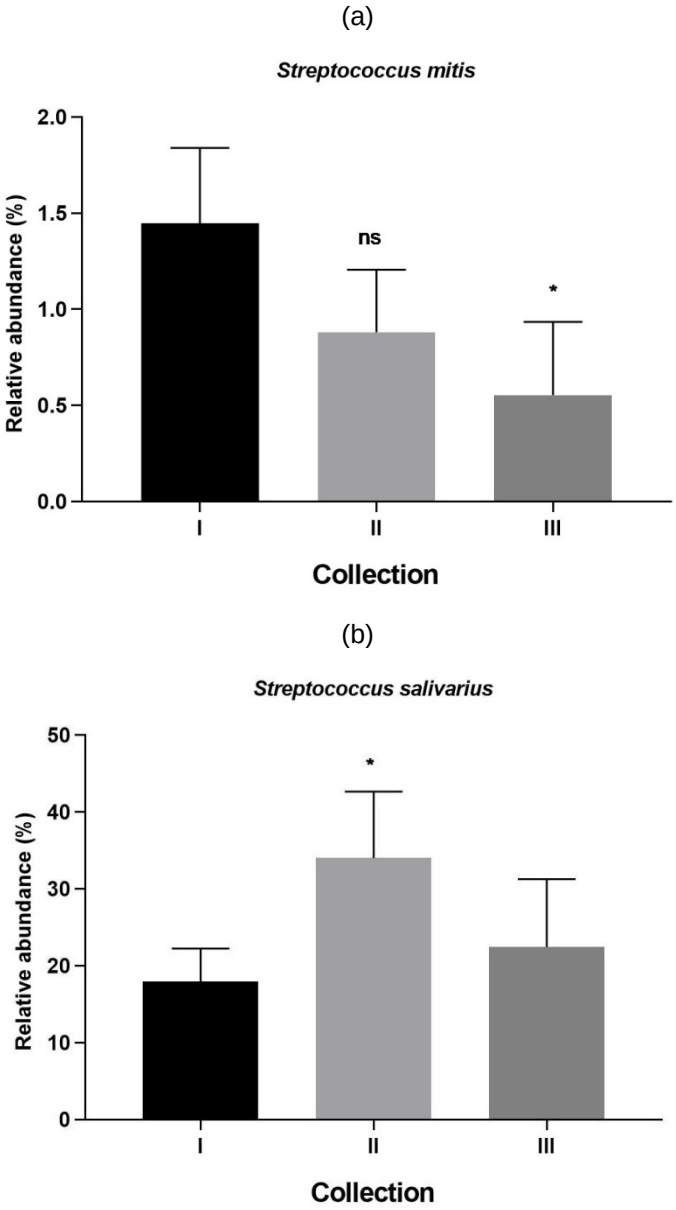

Fig. 3. Bacterial strains in the saliva samples of the participants before and after the use of the P-113 mouthwash

*(a) Streptococcus mitis and (b) Streptococcus salivarius. Distribution of bacterial relative abundance in the saliva collected before and after application of the mouthwash is shown in a bar plot. The samples were collected at three time-points and more details are shown in the materials and methods section. (ns means no statistical significance, *P < 0.05 according to the unpaired t test)*

the use of the P-113 mouthwash compared to collection I. The relative abundance of *S. mitis* decreased after the use of the P-113 mouthwash. *S. mitis* is a common species in the oral cavity and can act as an etiological agent of endocarditis and odontogenic infection [34]. Our results indicate that the risk of infection with *S. mitis* may be reduced by the application of P-113 mouthwash. Moreover, the relative abundance of *S. salivarius*, which secretes an antibiotic peptide that supports the immune system by combating *streptococcal* pathogenic strains, increases in saliva after the use of the P-113 mouthwash [35]. In our study, the results imply that although the P-113 mouthwash does not significantly affect bacterial distribution in saliva, it can decrease the relative abundance of odontogenic-infection-inducing *S. mitis* and increase that of beneficial

S. salivarius. These results indicate the potential of P-113 mouthwash as a treatment that can influence the proportion of pathogens and probiotics to establish a new equilibrium in the salivary environment.

Two limitations of this study were the diversity of sample types and bacterial culture conditions. Real-time PCR has high specificity and sensitivity for target species, but detects one species at a time [11]. Therefore, multiplex PCR can be considered a detection method for multiple species and may enable the quantification of bacteria in the gingival sulcus in future studies.

Only saliva samples were collected in this study. The oral cavity is a diverse habitat for microbes. Hence, samples from different parts of the oral cavity could provide us important information regarding the change in bacterial distribution after using the mouthwash containing P-113. For example, initially, the gingival sulcus is a specific site for bacteria to aggregate. Therefore, bacterial samples from the gingival sulcus should also be collected during the test to determine whether bacterial distribution at an infection site is affected by the use of the P-113 mouthwash. Furthermore, this study was conducted under anaerobic conditions only. Multiple gas environments, such as aerobic, 5% CO_2, and 10% CO_2 conditions, can also be considered in experiments to increase the diversity of bacterial culture conditions.

5. CONCLUSION

In conclusion, real-time PCR results showed significant changes in the abundance of periodontal disease-associated bacteria, *Fusobacterium nucleatum* and *Tannerella forsythia*, after the use of the P-113 mouthwash. In addition, 80 species were cultured in an anaerobic environment and identified by MALDI-TOF MS. The relative abundance of odontogenic-infection-inducing *S. mitis* decreased and that of beneficial *S. salivarius* increased after the use of the P-113 mouthwash. This study explored the oral condition of healthy subjects after using P-113 mouthwash. Further studies we will evaluate the oral condition of patients who have periodontal disease. These results indicate the potential of P-113 mouthwash as a treatment that can influence the proportion of pathogens and probiotics to establish an equilibrium in the salivary environment.

ETHICAL APPROVAL AND CONSENT

The study was approved by the Taipei Medical University-Joint Institutional Review Board on August 24th, 2015 (clinical trial number: NCT3351530, November 17, 2017). Informed consent was obtained from all participants before the specimens were collected.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Van der Velden U. The onset age of periodontal destruction. J Clin Periodontol. 1991;(0303-6979 (Print)).
2. Benjamin RM. Oral health: The silent epidemic. Public Health Rep. 2010;125(2): 158-9.
3. Darveau RP. Periodontitis: A polymicrobial disruption of host homeostasis. Nat Rev Microbiol. 2010;1740-1534(Electronic)).
4. RP D. Periodontitis: A polymicrobial disruption of host homeostasis. Nat Rev Microbiol. 2010;8(7):481-90.
5. Slots J, Listgarten MA. Bacteroides gingivalis, Bacteroides intermedius and Actinobacillus actinomycetemcomitans in human periodontal diseases. J Clin Periodontol. 1988;15(2):85-93.
6. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontol. 2000;2002(28):12-55.
7. Lasserre JF, Brecx MC, Toma S. Oral Microbes, Biofilms and Their Role in Periodontal and Peri-Implant Diseases. 2018;11(10).
8. Corrêa MG, et al., Short-term microbiological effects of photodynamic therapy in non-surgical periodontal treatment of residual pockets: A split-mouth RCT. Lasers in surgery and medicine. 2016;48(10): 944-950.
9. Socransky SS, et al. Associations between

microbial species in subgingival plaque samples. Oral Microbiology and Immunology. 1988. 3(1):1-7.
10. Van Dyke T, et al. Clinical and microbial evaluation of a histatin-containing mouthrinse in humans with experimental gingivitis: a phase-2 multi-center study. J Clin Periodontol. 2002;29(2):168-76.
11. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol. 2000;2005. 38:135-87.
12. Zheng HH, et al. Simultaneous detection of classical swine fever virus and porcine circovirus 3 by SYBR green I-based duplex real-time fluorescence quantitative PCR. Molecular and Cellular Probes. 2020;50.
13. Rydzak P, et al. Combining multiplex PCR and high-resolution melting for the detection and discrimination of arthropod transmitted viruses of cereals. J Virol Methods. 2020;278:113823.
14. Haiko J, et al. Identification of urinary tract pathogens after 3-hours urine culture by MALDI-TOF mass spectrometry. J Microbiol Methods. 2016;129:81-4.
15. Jang KS, Kim YH. Rapid and robust MALDI-TOF MS techniques for microbial identification: A brief overview of their diverse applications. J Microbiol. 2018; 56(4):209-216.
16. Singhal N, et al. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol. 2015;6:791.
17. Patel R. Matrix-assisted laser desorption ionization-time of flight mass spectrometry in clinical microbiology. Clin Infect Dis., 2013(1537-6591 (Electronic)).
18. Cherkaoui A, et al. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J Clin Microbiol. 2010;48(4): 1169-75.
19. Bhatti MM, et al. Rapid identification of positive blood cultures by matrix-assisted laser desorption ionization-time of flight mass spectrometry using prewarmed agar plates. J Clin Microbiol. 2014;52(12): 4334-8.
20. Costa MM, et al. Optimization and Standardization of Human Saliva Collection for MALDI-TOF MS. Diagnostics (Basel). 2021;11(8).
21. Yoshida A, et al. Development of a 5' nuclease-based real-time PCR assay for quantitative detection of cariogenic dental pathogens Streptococcus mutans and Streptococcus sobrinus. J Clin Microbiol, 2003;41(9):4438-41.
22. Suzuki N, et al. Quantitative microbiological study of subgingival plaque by real-time PCR shows correlation between levels of Tannerella forsythensis and Fusobacterium spp. J Clin Microbiol, 2004;42(5):2255-7.
23. Yoshida A, et al. Development of a 5' fluorogenic nuclease-based real-time PCR assay for quantitative detection of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. J Clin Microbiol, 2003;41(2):863-6.
24. Nagashima S, et al. Use of the genomic subtractive hybridization technique to develop a real-time PCR assay for quantitative detection of Prevotella spp. in oral biofilm samples. J Clin Microbiol, 2005;43(6):2948-51.
25. Yoshida A, et al. TaqMan real-time polymerase chain reaction assay for the correlation of Treponema denticola numbers with the severity of periodontal disease. Oral Microbiol Immunol. 2004. 19(3):196-200.
26. Duque C, et al. Microbiological, lipid and immunological profiles in children with gingivitis and type 1 diabetes mellitus. J Appl Oral Sci. 2017;25(2):217-226.
27. Zaiou M. Multifunctional antimicrobial peptides: therapeutic targets in several human diseases. J Mol Med (Berl). 2007;85(4):317-29.
28. Helmerhorst EJ, OFG, Choi L, Cheng JW, Reiner NE. Evaluation of a new host-derived synthetic antifungal peptide (PAC-113) in the treatment of oral candidiasis. . Int. Meet. Antimicrob. Chemother. Clin. Pract. Italy, poster 9; 2007.
29. Wang H, et al. The Effects of Antimicrobial Peptide Nal-P-113 on Inhibiting Periodontal Pathogens and Improving Periodontal Status. Biomed Res Int. 2018. 2018:1805793.
30. Choi H, et al. Real-time PCR quantification of 9 periodontal pathogens in saliva samples from periodontally healthy Korean young adults. J Periodontal Implant Sci, 2018;48(4):261-271.
31. Loozen G, et al. Inter-bacterial correlations in subgingival biofilms: a large-scale survey. J Clin Periodontol. 2014;41(1):1-10.

32. Didilescu AC, et al. Investigation of six selected bacterial species in endo-periodontal lesions. Int Endod J. 2012. 45(3):282-93.
33. Staletovic D, et al. Presence of Tannerella forsythia in patients with chronic periodontal disease and atherosclerosis. Vojnosanitetski Pregled. 2020;77(6):614-619.
34. Kutlu SS, et al. Community-acquired Streptococcus mitis meningitis: A case report. Int J Infect Dis. 2008;12(6): e107-9.
35. Wescombe PA, et al. Streptococcal bacteriocins and the case for *Streptococcus salivarius* as model oral probiotics. Future Microbiol. 2009;4(7):819-35.

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