in vivo *36*: 2134-2142 (2022)
doi:10.21873/invivo.12939

Effect of Systemic Administration of Amitriptyline on Oral Microbes in Rats

KAZUNORI NINOMIYA[1#], DURGA PAUDEL[2#], OSAMU UEHARA[3], TETSURO MORIKAWA[4], SYED TAUFIQUL ISLAM[5], ARIUNTSETSEG KHURELCHULUUN[4], DEDY ARIWANSA[4], KOKI YOSHIDA[4], HIROFUMI MATSUOKA[3], HARA HAJIME[1], FUKUI KAYOKO[1], KENJIROU NAKAMURA[1] and YOSHIHIRO ABIKO[4]

[1]*Department of Pharmacology, The Nippon Dental University School of Life Dentistry at Niigata, Niigata, Japan;*
[2]*Advanced Research Promotion Center, Health Sciences University of Hokkaido, Tōbetsu, Japan;*
[3]*Division of Disease Control and Molecular Epidemiology, Department of Oral Growth and Development, School of Dentistry, Health Sciences University of Hokkaido, Tōbetsu, Japan;*
[4]*Division of Oral Medicine and Pathology, Department of Human Biology and Pathophysiology, School of Dentistry, Health Sciences University of Hokkaido, Tōbetsu, Japan;*
[5]*Division of Pediatric Dentistry, Department of Oral Growth and Development, Health Sciences University of Hokkaido, Tōbetsu, Japan*

Abstract. *Background/Aim: Amitriptyline is a major tricyclic antidepressant that is also used to relieve chronic orofacial pain. Recently, alterations in gut flora due to various antidepressants have been demonstrated. However, it remains unknown how antidepressants affect the oral environment, including microbiota and innate immunity. The aim of this study was to investigate the effects of amitriptyline on oral microflora and antimicrobial peptides. Materials and Methods: Sprague-Dawley rats were intraperitoneally injected with amitriptyline for 2 weeks. The DNA extracted from the oral swabs were used to perform 16SrRNA sequencing to evaluate the oral microbiome. Quantitative RT-PCR was performed to evaluate the mRNA levels of antimicrobial peptides in the buccal tissues. Results: No significant differences in salivary flow rates were observed between the amitriptyline and control groups. Taxonomic analysis showed significant alterations in bacteria such as Corynebacterium, Rothia, and Porphyromonas due to amitriptyline administration. The beta diversity showed significant differences between the amitriptyline and control groups. Additionally, the predicted metagenome functions were significantly different between the two groups. The mRNA expression levels of antimicrobial peptides in the amitriptyline group were significantly higher as compared to controls. Conclusion: Systemic administration of amitriptyline may affect the oral environment, including oral microbes and innate immunity in the oral mucosa.*

[#]These Authors contributed equally to this work.

Correspondence to: Yoshihiro Abiko, Division of Oral Medicine and Pathology, School of Dentistry, Health Sciences University of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido, 061-0293, Japan. Tel: +81 133231390, Fax: +81 133231390, e-mail: yoshi-ab@hoku-iryo-u.ac.jp

Key Words: Amitriptyline, oral environment, oral microbiota, innate immunity.

Amitriptyline (AMI) is a tricyclic antidepressant (TCA) drug that increases monoamine levels in the synaptic region by blocking the reuptake of both serotonin and norepinephrine neurotransmitters (1). AMI relieves neuropathic pain, acts as an antidepressant, and has been applied to treat orofacial chronic pain, such as postherpetic neuralgia, trigeminal neuralgia, and burning mouth syndrome (2, 3). AMI has strong binding affinity for alpha-adrenergic, histamine (H1), and muscarinic (M1) receptors, leading to a wide range of side effects. Owing to AMI's anticholinergic effects, dry mouth is one of the most common side effects (4, 5).

The relationship between the enteric nervous systems of the gut and central nervous system has been recently established as a "gut-brain axis" (6). This concept includes the alteration of gut flora caused by psychological stress, while changes in human behavior and appetite increase the sense of anxiety due to gut inflammation (6, 7). In addition,

Table I. *Primer sequences*.

	Forward	Reverse
Beta defensin-1	GGGAGTCTCACGTCCTCTCT	TGAGAATGCCAGCACCCAG
Beta defensin-2	ATTTCTCCTGGTGCTGCTGTC	AGTCCACAAGTGCCAATCTGT
LL-37	CAGTTGTGATGCGCCTGGTA	AAGGCAGGCCTACTGCTCTA
S100a7a	CTGGTGGAAGTTCCCCTGTT	GACAGTGGAGAGTCTGTTGCT
GAPDH	TGCACCACCAACTGCTTAGC	GGATGCAGGGATGATGTTCTG

another concept of "oral-intestinal bacterial association", in which the bacterial flora in the oral cavity affects the intestinal bacterial flora, has recently been proposed (8).

Recently, alterations in the intestinal flora caused by psychotropic drugs, including fluoxetine, AMI, and buspirone, have been shown in animal models (9, 10). Alterations in the intestinal flora may be beneficial in improving depression and anxiety (10). It has not been shown how the oral flora is altered by the administration of psychotropic drugs. As an *in vitro* study suggested that AMI has a cytotoxic effect on the oral mucosa (11), AMI may affect oral flora. However, the effects of AMI administration on the oral cavity, including the oral microbe and innate immunity, remain unclear. Since the alteration of the oral microbes may affect the intestinal microbes, the understanding of changes in the microbes in both environments is important. The aim of this study was to examine the effect of systemic AMI administration on the oral microbes and expression of antimicrobial peptides (AMPs) of the oral mucosa.

Materials and Methods

Animal and AMI treatment. Six-week-old Sprague-Dawley rats were obtained from the Sankyo Lab (Sapporo, Japan). The rats were housed for 1 week to adapt to the laboratory conditions (12 h light/dark cycle), which were then randomly allocated into two groups: the control group (CONT) and the AMI group (n=10; each group). Rats in the AMI group were injected with amitriptyline hydrochloride (20 mg/kg of body weight; A8404, Sigma-Aldrich, St. Louis, MO, USA), intraperitoneally mixed in phosphate buffered saline (PBS) over a period of 2 weeks. AMI was administered intraperitoneally to the rats to avoid direct effects of the drug on the oral mucosa and environment. The CONT group rats were injected with PBS only. The animal study complied with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and was approved by the ethical committee for animals of the Health Sciences University of Hokkaido (approval no: 21-066).

Salivary flow rate. The flow rate of saliva was determined on a day earlier to sample collection as described previously (12). Briefly, the rats were anesthetized with sodium pentobarbital (30 mg/kg body weight). The saliva was allowed to pool in the floor of the oral cavity and collected with a micropipette for 5 min. The salivary volume was measured and flow per minute was then determined.

Sample collection and DNA isolation. Two weeks after AMI injection, rats in both groups were anesthetized as explained before. The oral cavity was swabbed from the tongue, palate, buccal mucosa, and labial mucosa for 30 s using a swab (Isohelix, Kent, UK). The swab was stored in 200 μl Tris-EDTA buffer and frozen until further processing. Oral microbial DNA was extracted following an established protocol (13) using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The extracted DNA was used for 16S ribosomal RNA (16S rRNA) sequencing.

16S rRNA sequencing. The extracted DNA was used to prepare the sequencing libraries following the manufacturer's protocol (16S metagenomic sequencing library preparation instructions, Illumina, San Diego, CA, USA). In brief, the DNA was subjected to two step PCR: Amplicon PCR and Index PCR. In Amplicon PCR, the V3-V4 regions of the bacterial 16S ribosomal RNA (rRNA) gene were targeted using region-specific primers. Extracted DNA, Amplicon PCR primers, and KAPA HiFi HS ReadyMix (Nippon Genetics, Tokyo, Japan) were used to perform Amplicon PCR. The amplified PCR product was cleaned up using AM Pure XP (Beckman Coulter, Brea, CA, USA) and then used for Index PCR. The clean-up done amplified DNA, KAPA HiFi HS ReadyMix, and the Nextera XT index kit (Illumina) were used to perform Index PCR. The DNA was further purified using AM Pure XP and then quantified using a fluorometer (Qubit 3, Thermo Fisher Scientific, Waltham, MA, USA). The library was normalized, pooled, and then loaded into an Illumina MiSeq system.

Analysis of sequencing data. The sequencing data obtained were further analyzed using the software package Quantitative Insights into Microbial Ecology 2 (QIIME2 v2020.2). 16S rDNA database (Greengenes v13.8) was used to assign the 16S rRNA gene sequences. The analysis of sequencing data was evaluated for their differences in diversity, taxonomic abundance, and predicted metagenome function. The differences in diversity were evaluated as alpha diversity and beta diversity. Alpha diversity was further evaluated as Shannon index, Faith's phylogenetic diversity, and Observed operational taxonomic units (OTUs). Kruskal-Wallis test was used to test the significant differences at uncorrected $p<0.05$. To evaluate the beta diversity, a principal coordinate analysis (PCoA) plot in three dimension was plotted based on the branch length of the phylogenetic tree shared between the groups. Two types of PCoA plots were generated: unweighted UniFrac and weighted UniFrac distance metrics. Permutational multivariate analysis of variance (PERMANOVA) was employed to test the significant differences at uncorrected $p<0.05$. The differences in taxonomic abundances between the two groups were evaluated using the analysis of composition of microbiomes (ANCOM) in the

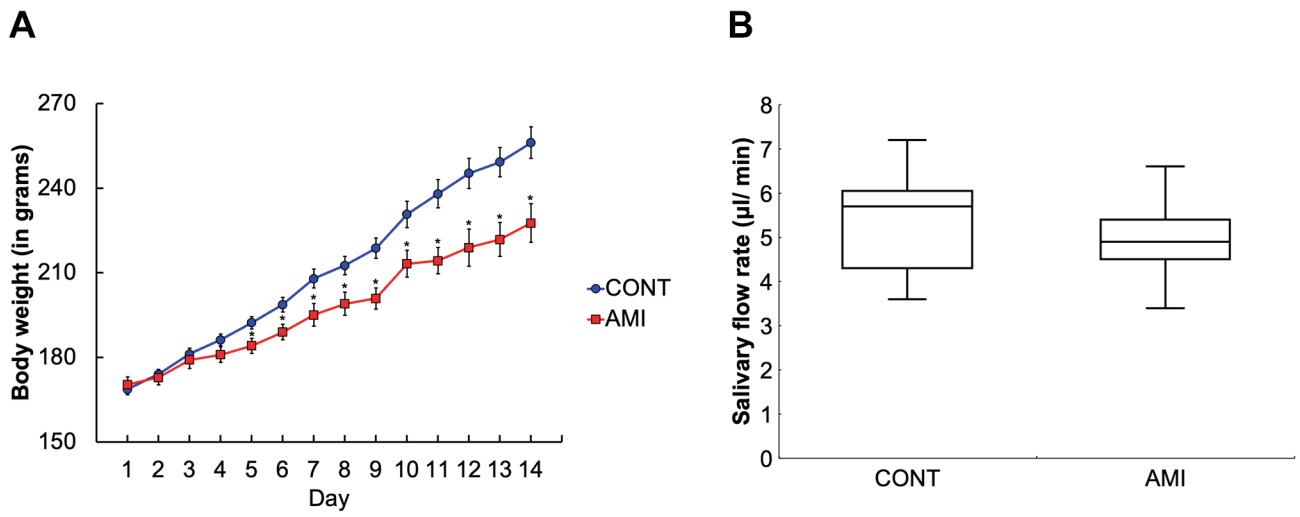

Figure 1. *Body weight and salivary flow rate. (A) The line graph shows the body weight of the rats from day 1-14 of amitriptyline (AMI) administration. The mean body weight was significantly decreased from day 5 of AMI administration until day 14 in AMI rats as compared to controls (CONT) ($p<0.05$; Mann-Whitney U-test). (B) The salivary rate showed no significant differences between the AMI and CONT groups.*

QIIME2 and empirical distribution of W was used to express the final significance. To predict the alteration in metagenome function, Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) software was used and Welch's *t*-test in the statistical analysis of the metagenomic profiles (STAMP) software was used for two-group comparisons with adjusted $p<0.05$ considered as statistically significant.

RNA extraction and reverse transcription. The buccal tissue excised from the rats was used to extract the total RNA. TRIzol Reagent (Thermo Fisher Scientific) was used for RNA isolation, which was then purified using the RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. Reverse transcription of the RNA was done using ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan) following the manufacturer's instructions.

Quantitative polymerase chain reaction (qPCR). The expression of AMPs such as beta defensin-1, beta defensin-2, cathelicidin (LL-37), and S100a7a at the mRNA level was measured using qPCR (LightCycler Nano; Roche Diagnostics, Basel, Switzerland). Real-time PCR was performed using the extracted cDNA, KAPA SYBR FAST qPCR Mix (Kapa Biosystems, Potters Bar, UK), and a primer pair (Table I). ΔΔCq method was used to calculate the relative expression levels. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control gene. All the statistical analyses were performed using SPSS 26.0 (IBM) and Mann-Whitney *U*-test was employed to test the significant differences (uncorrected $p<0.05$ considered as statistically significant).

Results

Changes in body weight. The body weight was recorded daily. The body weight of the AMI group rats was significantly lower from the 5th day of AMI administration until the 14th day when compared with the CONT group (Figure 1A).

Salivary flow rate. The salivary flow rate after 2 weeks of experiment showed no significant differences between the AMI and CONT groups (Figure 1B).

Alpha diversity. A total of 20 samples were used for the sequencing, which generated 664,367 sequences. A mean of 33,218 sequences per sample was generated with a range from 21,743 to 44,253 sequences. The alpha diversity as analyzed using observed OTUs, Faith's phylogenetic diversity, and Shannon index showed no significant differences between the CONT and AMI groups (Figure 2A-C).

Beta diversity. The beta diversity was evaluated as weighted and unweighted UniFrac distance metrics. The weighted UniFrac PCoA plot showed clustering for each group. The weighted UniFrac distance metric was significantly different between the two groups ($p=0.011$; Figure 2D). The unweighted UniFrac distance metric was not significantly different between the two groups ($p=0.137$; Figure 2E).

Taxonomic abundance. The taxonomic analysis showed that the most abundant genus in both groups was *Rothia*, followed by *Facklamia, Streptococcus,* and *Aggregatibacter* (Figure 2F). On analyzing the differential bacteria between the CONT and AMI groups, ANCOM test showed 46 genera to be significantly different. The most significantly altered bacteria at the genus level was *Corynebacterium* (W=7; Table II).

Prediction of metagenome function. The prediction of metagenome function as analyzed using PICRUSt2 software showed 41 MetaCyc pathways to be significantly different

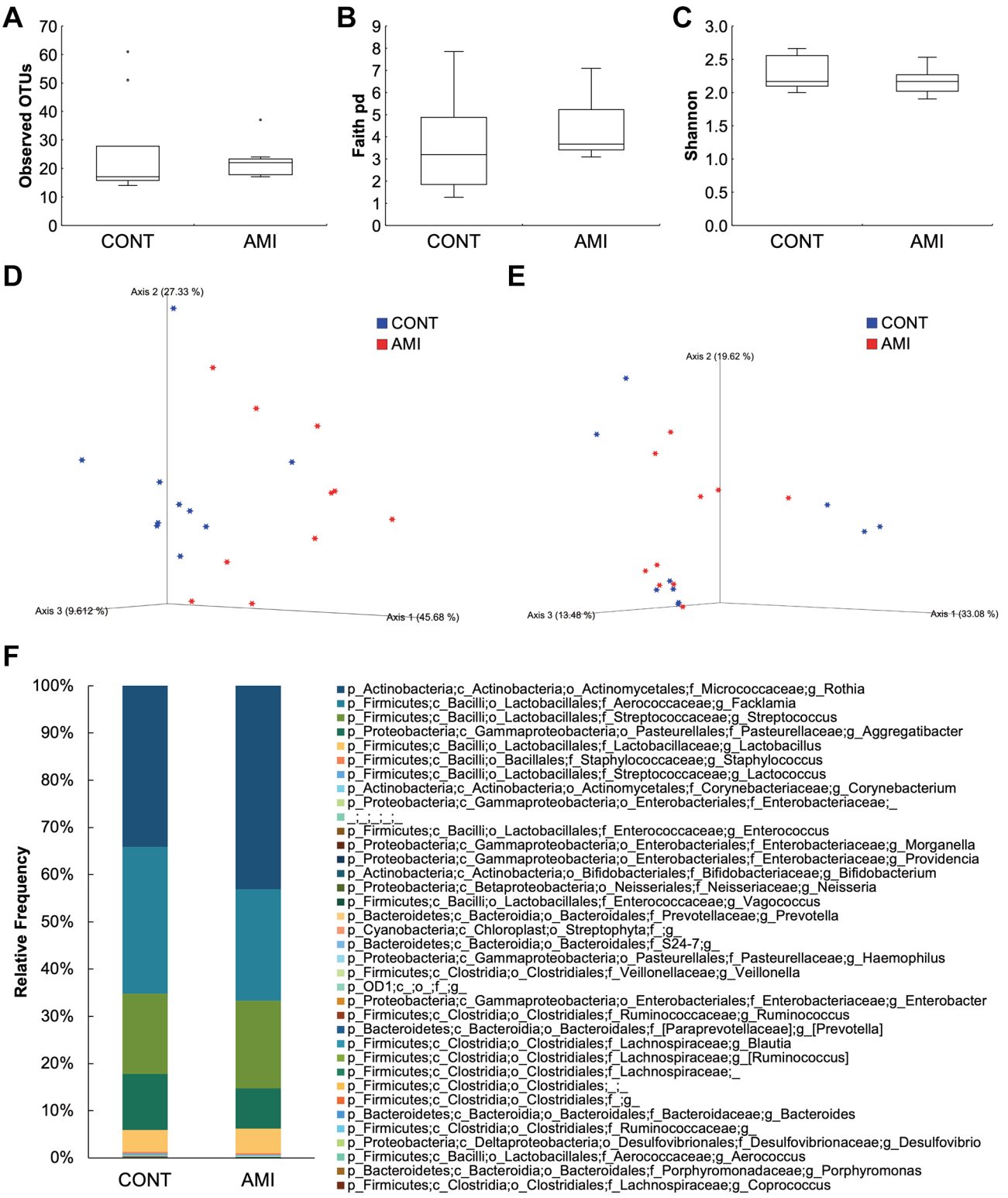

Figure 2. *Metagenomic analysis of the oral cavity. The alpha diversity as evaluated by (A) observed operational taxonomic units, (B) faith phylogenetic diversity, and (C) Shannon index showed no significant differences between the two groups. The PCoA plot to evaluate beta diversity by (D) unweighted UniFrac and (E) weighted UniFrac. The weighted UniFrac was significantly different between the two groups (p=0.011; PERMANOVA). (F) The bar graph shows the abundant bacteria at the genus level. Each group was dominated by genuses, such as Rothia, Facklamia, and Streptococcus.*

Table II. *The ANCOM results and percentile abundances of features in each group.*

Percentile	Median percentile abundance		Max percentile abundance		W
Group	CONT	AMI	CONT	AMI	
p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Corynebacteriaceae;g_Corynebacterium	17	43	50	182	7
p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Micrococcaceae;g_Rothia	9,153	1,5601	1,6018	1,9008	3
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_(Ruminococcus)	1	1	8	11	2
_;_;_;_;_ 6.5	11	24	33	1	
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae;g_Porphyromonas	1	1	1	3	1
p_Firmicutes;c_Clostridia;o_Clostridiales;_;_	1	1	35	5	1
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;_	1	1	38	7	1
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_	1	1	13	1	1
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;_	1	1	3	1	1
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_	1	1	26	3	1
p_Fusobacteria;c_Fusobacteriia;o_Fusobacteriales;f_Leptotrichiaceae;g_Leptotrichia	1	1	3	1	1
p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella	1	1	3	1	1
p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae;g_Akkermansia	1	1	19	1	1
p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium	2	1	82	31	0
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides	1	1	17	4	0
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae;g_Parabacteroides	1	1	8	1	0
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella	1	1	14	22	0
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_	1	1	84	22	0
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_(Paraprevotellaceae);g_(Prevotella)	1	1	35	9	0
p_Cyanobacteria;c_Chloroplast;o_Streptophyta;f_;g_	1	1	8	31	0
p_Firmicutes;c_Bacilli;o_Bacillales;f_Staphylococcaceae;g_Staphylococcus	67.5	123	277	229	0
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Aerococcaceae;g_Aerococcus	1	1	1	3	0
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Aerococcaceae;g_Facklamia	9,914.5	7,447	13,136	14,754	0
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Enterococcus	1	1	9	60	0
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Vagococcus	1	1	7	23	0
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus	183.5	289.5	7,402	14,555	0
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Lactococcus	66	59.5	341	168	0
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Streptococcus	5,246	6,055.5	7,445	12,481	0
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_	1	1	41	4	0
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia	1	1	16	12	0
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Coprococcus	1	1	29	1	0
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira	1	1	10	1	0
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus	1	1	80	18	0
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Veillonella	1	1	8	14	0
p_OD1;c_;o_;f_;g_	1	1	1	18	0
p_Proteobacteria;c_Betaproteobacteria;o_Neisseriales;f_Neisseriaceae;g_Neisseria	1	1	17	15	0
p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae;g_	1	1	8	1	0
p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae;g_Desulfovibrio	1	1	1	3	0
p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;_	17.5	33	116	146	0
p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_Enterobacter	1	1	1	16	0
p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_Morganella	1	5.5	43	26	0
p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_Proteus	1	1	12	1	0
p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_Providencia	1	1	19	46	0
p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;_	1	1	3	1	0
p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Aggregatibacter	3,719	2,676	5,275	7,493	0
p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Haemophilus	1	1	15	10	0

AMI: Amitriptyline; CONT: control.

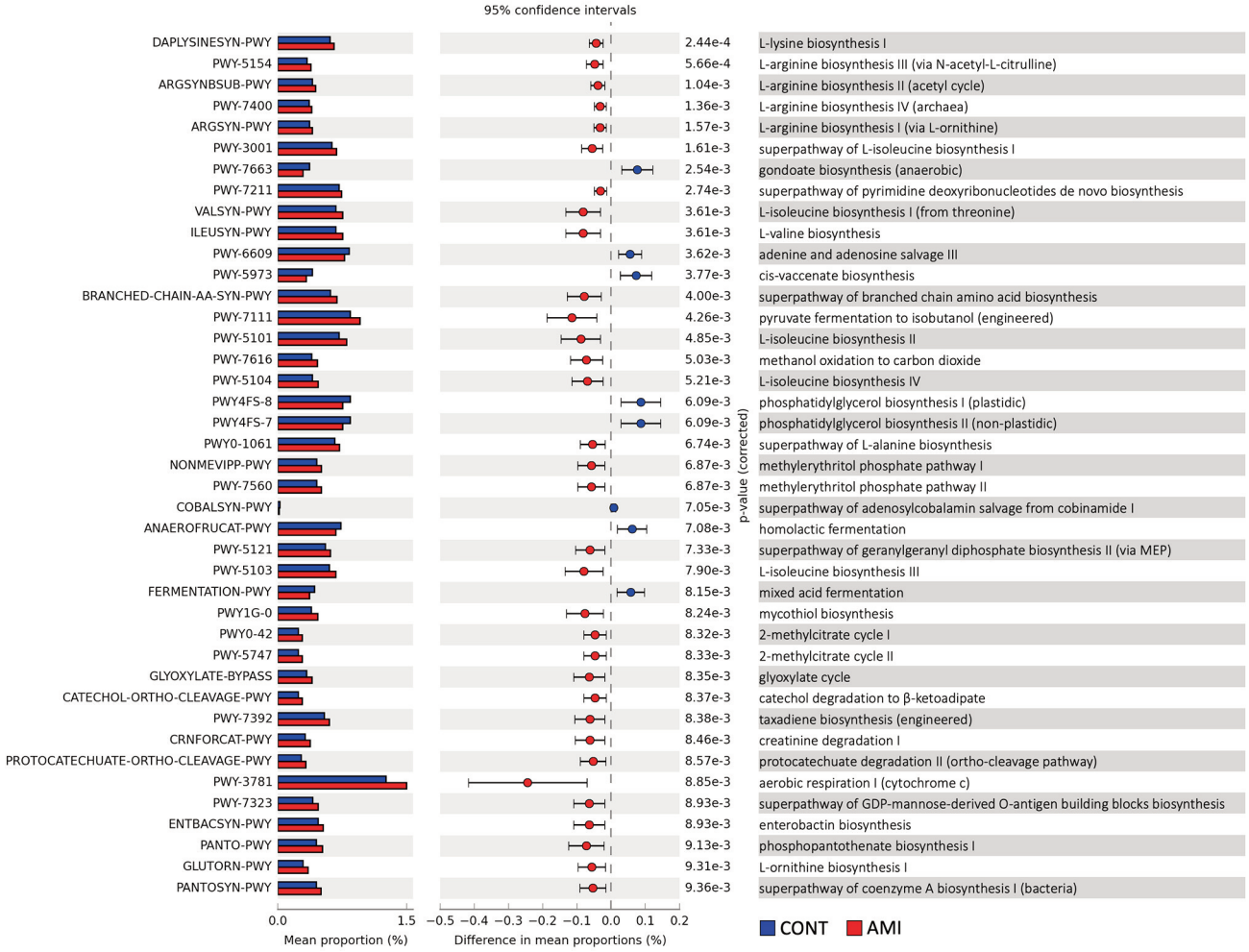

Figure 3. *Predicted metagenome function. The PICRUSt2 and STAMP software predicted 41 MetaCyc pathways to be significantly altered between the two groups. Thirty-three MetaCyc pathways were increased, whereas eight pathways were decreased in the amitriptyline (AMI) group ($p<0.05$; Welch's t-test).*

between the two groups (Figure 3). Thirty-three MetaCyc pathways such as DAPLYSINESYN-PWY and PWY-5154, were increased in the AMI group as compared to the CONT group. Eight pathways such as PWY-7663 and PWY-6609 were decreased in the AMI group as compared to the CONT group (Figure 4). Among the altered pathways, pathways related to amino acid biosynthesis were the highest number of pathways altered between the two groups.

Relative gene expression of AMPs. The mRNA expression levels of AMPs as evaluated using qPCR showed that beta-defensin-2, LL-37, and S100a7a were significantly increased in the oral tissue (buccal mucosa) of rats belonging to the AMI group as compared to the CONT group ($p<0.05$). However, the mRNA level of beta-defensin-1 did not differ significantly between the two groups (Figure 4).

Discussion

To our knowledge, this is the first report demonstrating that intraperitoneal administration of AMI alters the oral microbes, accompanied by altered expression of AMPs in the oral mucosa. In this study, we examined the effect of systemic AMI administration on the oral microbe on mice using next-generation sequencing. Although the alpha diversity did not differ significantly, the beta diversity significantly differed between the AMI and CONT groups. The expression of AMPs, including BD-2, LL-37, and S100a7a, was significantly higher in the oral mucosa of the AMI group than in that of the CONT group. These results indicate that systemic administration of AMI may alter the environment of the oral cavity, including the oral microbes and AMPs produced by the oral mucosa. Although an effect on the gut flora induced by the administration of several

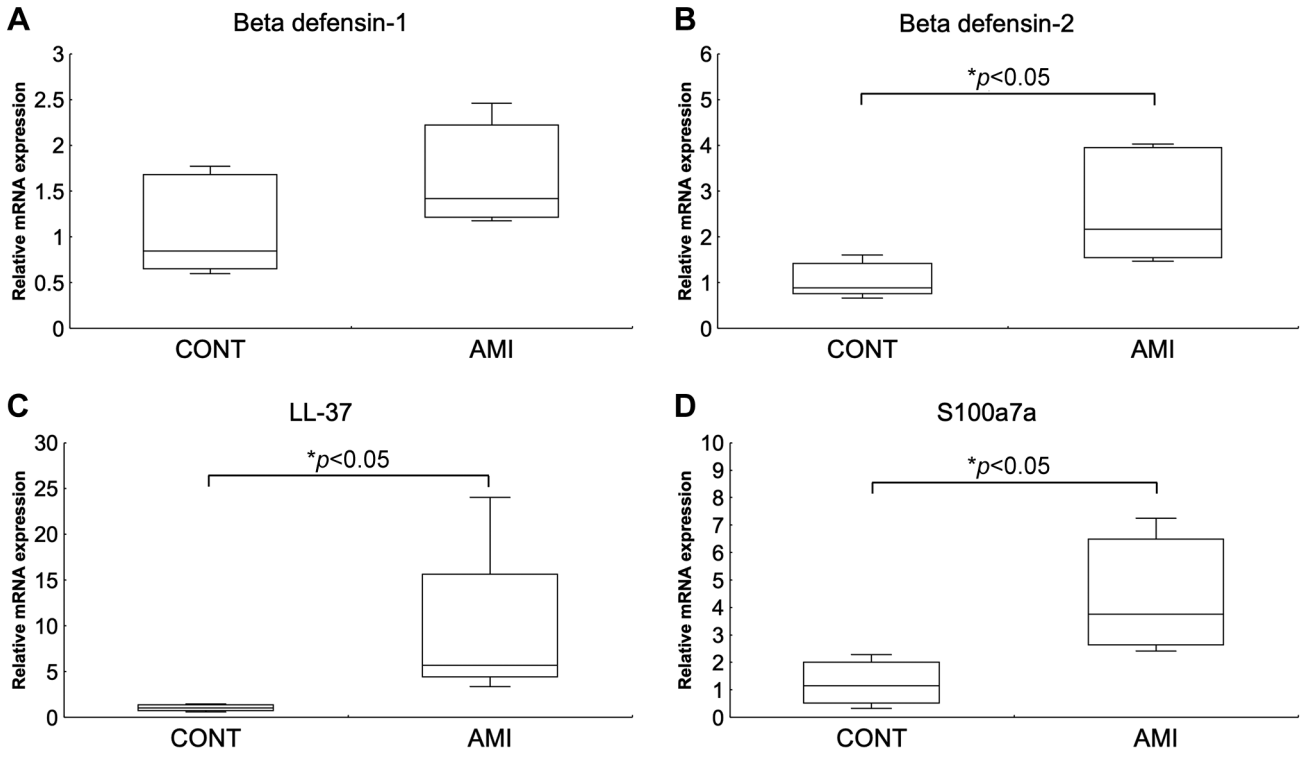

Figure 4. *mRNA expression levels of antimicrobial peptides. The mRNA expression levels of beta-defensin-1 did not alter in the buccal mucosa of rats between two groups. The mRNA expression levels of LL-37 and S100a7a were significantly increased in the oral tissue of rats belonging to the amitriptyline (AMI) group when compared to those in the control (CONT) group (p<0.05; Mann-Whitney U-test).*

antidepressants, including tricyclic antidepressants, SSRI, and SNRI (9, 10, 14), has been observed in mice, the effect on the oral flora has not been observed. A balance in the oral flora is crucial for maintaining oral health, and its imbalance may disrupt the oral environment. Our data may provide important information regarding how antidepressant medications affect the oral environment.

This study did not verify how AMI alters the oral flora. AMI, a tricyclic antidepressant, causes a significant reduction in salivary flow, presumably due to muscarinic receptor blockade (15). The reduced salivary flow rate due to AMI might be the most likely cause of alterations in the oral flora. However, no reduction in the flow rate of saliva was observed in the present study. AMI exhibits action against gram-positive and gram-negative bacteria *in vitro* and provides significant protection from *Salmonella typhimurium in vivo* (16). In addition to antimicrobial actions, the cytotoxic effect of AMI on the oral mucosa has been shown (11). Moreover, systemic toxicity of AMI has been demonstrated in animal models (17, 18). The systemic toxicity may have caused the body weights of the AMI group rats to be significantly lower as compared to those in the CONT group in our study. Since the present study employed intraperitoneal systemic administration of AMI, it is not known how much AMI reached the local area of the oral region. The antimicrobial activities and toxic effects of AMI on the oral mucosa may directly alter the oral flora.

The bacterial taxonomy results obtained by ANCOM showed a significantly higher proportion of *Corynebacterium, Rothia,* and *Porphyromonas* in the oral mucosa of mice in the AMI group than in the CONT group. *Corynebacterium,* a gram-positive bacterium, is a normal inhabitant of the oral and airway mucosa and contributes to biofilm formation (19). *Corynebacterium* has an important role in supragingival biofilm formation and contributes to late colonization (19). *Corynebacterium matruchotii* is involved in dental calculus formation and is a risk factor for periodontitis (20). *Corynebacterium* is significantly enriched in healthy periodontal tissues (21-23). *Rothia,* a gram-positive coccus, is also abundant in healthy periodontal tissue (21, 23). In general, increased levels of both *Corynebacterium* and *Rothia* may not have harmful effects on the periodontal tissues. On the other hand, the abundance of *Porphyromonas* typified by *Porphyromonas (P.) gingivalis* may increase the risk of periodontitis, since it is a crucial periodontal pathogen (24).

Upregulated expression of epithelial AMPs, including BD-2, LL-37, and S100a7a, was observed in the oral mucosa of the AMI group. These epithelial AMPs are upregulated by stimulation with several types of bacterial infections and

inflammatory reactions (25). *P. gingivalis stimulates* the upregulation of BD-2 and LL-37 in the oral epithelium (26, 27). The upregulation of epithelial AMPs might have occurred as a result of increased *P. gingivalis* in the AMI group. Although an increased proportion of several bacteria was observed, increased epithelial AMPs may have a protective role against infectious changes in the oral mucosa.

We also demonstrated the alterations in the predicted metagenome function. Notably, enrichment of several amino acid metabolism-related pathways, such as lysin biosynthesis and arginine biosynthesis, between the AMI and CONT groups were observed. Polyamine, butyric acid, arginine and proline metabolism, and lysine degradation were found to be metabolic signatures of dental plaque in inflamed periodontal surfaces (28). Our functional analysis data may reflect the dental plaque formation. Further studies are required to clarify this speculation.

In conclusion, this study demonstrated that systemic administration of AMI alters the oral flora and expression of AMPs in the oral mucosa. These findings suggest that systemic administration of AMI affects the environment of the oral cavity, including the oral microbe and innate immunity, in the oral mucosa.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization: Yoshihiro Abiko, Kazunori Ninomiya; Methedology: Durga Paudel, Osamu Uehara, Tetsuro Morikawa; Investigation: Kazunori Ninomiya, Durga Paudel, Osamu Uehara, Tetsuro Morikawa, Ariuntsetseg Khurelchuluun, Dedy Ariwansa, Syed Taufiqul Islam, Koki Yoshida; Resources: Durga Paudel, Osamu Uehara, Hara Hajime, Fukui Kayoko, Kenjirou Nakamura, Yoshihiro Abiko; Validation: Syed Taufiqul Islam, Ariuntsetseg Khurelchuluun, Dedy Ariwansa, Koki Yoshida; Formal Analysis: Osamu Uehara, Hirofumi Matsuoka, Durga Paudel, Yoshihiro Abiko; Writing- original draft preparation: Yoshihiro Abiko, Osamu Uehara, Durga Paudel, Kazunori Ninomiya; Writing- review and editing: Hara Hajime, Fukui Kayoko, Kenjirou Nakamura, Hirofumi Matsuoka.

Acknowledgements

This research was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP 21K21094.

References

1 McClure EW and Daniels RN: Classics in chemical neuroscience: Amitriptyline. ACS Chem Neurosci *12(3)*: 354-362, 2021. PMID: 33438398. DOI: 10.1021/acschemneuro.0c00467

2 Lino PA, Martins CC, Miranda G, de Souza E Silva ME and de Abreu M: Use of antidepressants in dentistry: A systematic review. Oral Dis *24(7)*: 1168-1184, 2018. PMID: 28836365. DOI: 10.1111/odi.12747

3 Pettengill CA and Reisner-Keller L: The use of tricyclic antidepressants for the control of chronic orofacial pain. Cranio *15(1)*: 53-56, 1997. PMID: 9586488. DOI: 10.1080/08869634.1997.11745992

4 Bryson HM and Wilde MI: Amitriptyline. A review of its pharmacological properties and therapeutic use in chronic pain states. Drugs Aging *8(6)*: 459-476, 1996. PMID: 8736630. DOI: 10.2165/00002512-199608060-00008

5 Scully C: Drug effects on salivary glands: dry mouth. Oral Dis *9(4)*: 165-176, 2003. PMID: 12974516. DOI: 10.1034/j.1601-0825.2003.03967.x

6 Mayer EA, Tillisch K and Gupta A: Gut/brain axis and the microbiota. J Clin Invest *125(3)*: 926-938, 2015. PMID: 25689247. DOI: 10.1172/JCI76304

7 Clapp M, Aurora N, Herrera L, Bhatia M, Wilen E and Wakefield S: Gut microbiota's effect on mental health: The gut-brain axis. Clin Pract *7(4)*: 987, 2017. PMID: 29071061. DOI: 10.4081/cp.2017.987

8 Schmidt TS, Hayward MR, Coelho LP, Li SS, Costea PI, Voigt AY, Wirbel J, Maistrenko OM, Alves RJ, Bergsten E, de Beaufort C, Sobhani I, Heintz-Buschart A, Sunagawa S, Zeller G, Wilmes P and Bork P: Extensive transmission of microbes along the gastrointestinal tract. Elife *8*: e42693, 2019. PMID: 30747106. DOI: 10.7554/eLife.42693

9 Zhang W, Qu W, Wang H and Yan H: Antidepressants fluoxetine and amitriptyline induce alterations in intestinal microbiota and gut microbiome function in rats exposed to chronic unpredictable mild stress. Transl Psychiatry *11(1)*: 131, 2021. PMID: 33602895. DOI: 10.1038/s41398-021-01254-5

10 Kim JK, Han SK, Joo MK and Kim DH: Buspirone alleviates anxiety, depression, and colitis; and modulates gut microbiota in mice. Sci Rep *11(1)*: 6094, 2021. PMID: 33731795. DOI: 10.1038/s41598-021-85681-w

11 Al-Musawi M, Durham J, Whitworth JM, Stone SJ, Nixdorf DR and Valentine RA: Effect of topical neuromodulatory medications on oral and skin keratinocytes. J Oral Pathol Med *46(2)*: 134-141, 2017. PMID: 26991677. DOI: 10.1111/jop.12437

12 Chen SY, Wang Y, Zhang CL and Yang ZM: Decreased basal and stimulated salivary parameters by histopathological lesions and secretory dysfunction of parotid and submandibular glands in rats with type 2 diabetes. Exp Ther Med *19(4)*: 2707-2719, 2020. PMID: 32256753. DOI: 10.3892/etm.2020.8505

13 Abusleme L, Hong BY, Hoare A, Konkel JE, Diaz PI and Moutsopoulos NM: Oral microbiome characterization in murine models. Bio Protoc *7(24)*: e2655, 2017. PMID: 29333479. DOI: 10.21769/BioProtoc.2655

14 Lukić I, Getselter D, Ziv O, Oron O, Reuveni E, Koren O and Elliott E: Antidepressants affect gut microbiota and Ruminococcus flavefaciens is able to abolish their effects on depressive-like behavior. Transl Psychiatry *9(1)*: 133, 2019. PMID: 30967529. DOI: 10.1038/s41398-019-0466-x

15 Hunter KD and Wilson WS: The effects of antidepressant drugs on salivary flow and content of sodium and potassium ions in human parotid saliva. Arch Oral Biol *40(11)*: 983-989, 1995. PMID: 8670028. DOI: 10.1016/0003-9969(95)00079-5

16 Mandal A, Sinha C, Kumar Jena A, Ghosh S and Samanta A: An investigation on *in vitro* and *in vivo* antimicrobial properties of the antidepressant: Amitriptyline hydrochloride. Braz J Microbiol *41(3)*: 635-645, 2010. PMID: 24031539. DOI: 10.1590/S1517-83822010000300014

17 Liu X, Emery CJ, Laude E, Herget J, Gill G, Cope G and Barer GR: Adverse pulmonary vascular effects of high dose tricyclic antidepressants: acute and chronic animal studies. Eur Respir J *20(2)*: 344-352, 2002. PMID: 12212966. DOI: 10.1183/09031936.02.00225402

18 Hughes IE and Radwan S: The relative toxicity of amitriptyline, imipramine, maprotiline and mianserin in rabbits *in vivo*. Br J Pharmacol *65(2)*: 331-338, 1979. PMID: 760906. DOI: 10.1111/j.1476-5381.1979.tb07834.x

19 Mark Welch JL, Rossetti BJ, Rieken CW, Dewhirst FE and Borisy GG: Biogeography of a human oral microbiome at the micron scale. Proc Natl Acad Sci U.S.A. *113(6)*: E791-E800, 2016. PMID: 26811460. DOI: 10.1073/pnas.1522149113

20 Ooi SW, Smillie AC, Kardos TB and Shepherd MG: Intracellular mineralization of Bacterionema matruchotii. Can J Microbiol *27(3)*: 267-270, 1981. PMID: 7237277. DOI: 10.1139/m81-042

21 Cai Z, Lin S, Hu S and Zhao L: Structure and function of oral microbial community in periodontitis based on integrated data. Front Cell Infect Microbiol *11*: 663756, 2021. PMID: 34222038. DOI: 10.3389/fcimb.2021.663756

22 Tsai CY, Tang CY, Tan TS, Chen KH, Liao KH and Liou ML: Subgingival microbiota in individuals with severe chronic periodontitis. J Microbiol Immunol Infect *51(2)*: 226-234, 2018. PMID: 27262209. DOI: 10.1016/j.jmii.2016.04.007

23 Meuric V, Le Gall-David S, Boyer E, Acuña-Amador L, Martin B, Fong SB, Barloy-Hubler F and Bonnaure-Mallet M: Signature of microbial dysbiosis in periodontitis. Appl Environ Microbiol *83(14)*: e00462-17, 2017. PMID: 28476771. DOI: 10.1128/AEM.00462-17

24 How KY, Song KP and Chan KG: Porphyromonas gingivalis: An overview of periodontopathic pathogen below the gum line. Front Microbiol *7*: 53, 2016. PMID: 26903954. DOI: 10.3389/fmicb.2016.00053

25 Méndez-Samperio P: Recent advances in the field of antimicrobial peptides in inflammatory diseases. Adv Biomed Res *2*: 50, 2013. PMID: 24516850. DOI: 10.4103/2277-9175.114192

26 Tada H, Shimizu T, Matsushita K and Takada H: Porphyromonas gingivalis-induced IL-33 down-regulates hCAP-18/LL-37 production in human gingival epithelial cells. Biomed Res *38(3)*: 167-173, 2017. PMID: 28637951. DOI: 10.2220/biomedres.38.167

27 Taguchi Y and Imai H: Expression of beta-defensin-2 in human gingival epithelial cells in response to challenge with Porphyromonas gingivalis *in vitro*. J Periodontal Res *41(4)*: 334-339, 2006. PMID: 16827729. DOI: 10.1111/j.1600-0765.2006.00879.x

28 Sakanaka A, Kuboniwa M, Hashino E, Bamba T, Fukusaki E and Amano A: Distinct signatures of dental plaque metabolic byproducts dictated by periodontal inflammatory status. Sci Rep *7*: 42818, 2017. PMID: 28220901. DOI: 10.1038/srep42818

Received May 30, 2022
Revised July 5, 2022
Accepted July 6, 2022