UNIVERSITAS TRISAKTI
FAKULTAS KEDOKTERAN GIGI
FACULTY OF DENTISTRY – UNIVERSITAS TRISAKTI
KAMPUS B – Jl. Kyai Tapa No. 260 – Grogol – Jakarta Barat 11440 – Indonesia
Telp : +62-21-5672731 (Hunting)
Fax : +62-21-5655787
E-mail : fkg@trisakti.ac.id
Website : https://trisakti.ac.id

Dokumen Uji Kemiripan Jurnal Internasional Ke-2

Bukti lampiran :

1. Protokol dan prosedur
2. Dokumen yang diexclude
3. Hasil uji kemiripan artikel jurnal internasional

Kusnoto, Joko et al., "Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects" J Contemp Dent Pract .2025 Apr 1;26(4):339-347.

Doi : https://doi.org/10.5005/jp-journals-10024-3857.

Link : https://www.thejcdp.com/abstractArticleContentBrowse/JCDP/19/26/4/40476/abstractArticle/Article

UNIVERSITAS TRISAKTI
FAKULTAS KEDOKTERAN GIGI
FACULTY OF DENTISTRY – UNIVERSITAS TRISAKTI
KAMPUS B – Jl. Kyai Tapa No. 260 – Grogol – Jakarta Barat 11440 – Indonesia
Telp : +62-21-5672731 (Hunting)
Fax : +62-21-5655787
E-mail : fkg@trisakti.ac.id
Website : https://trisakti.ac.id

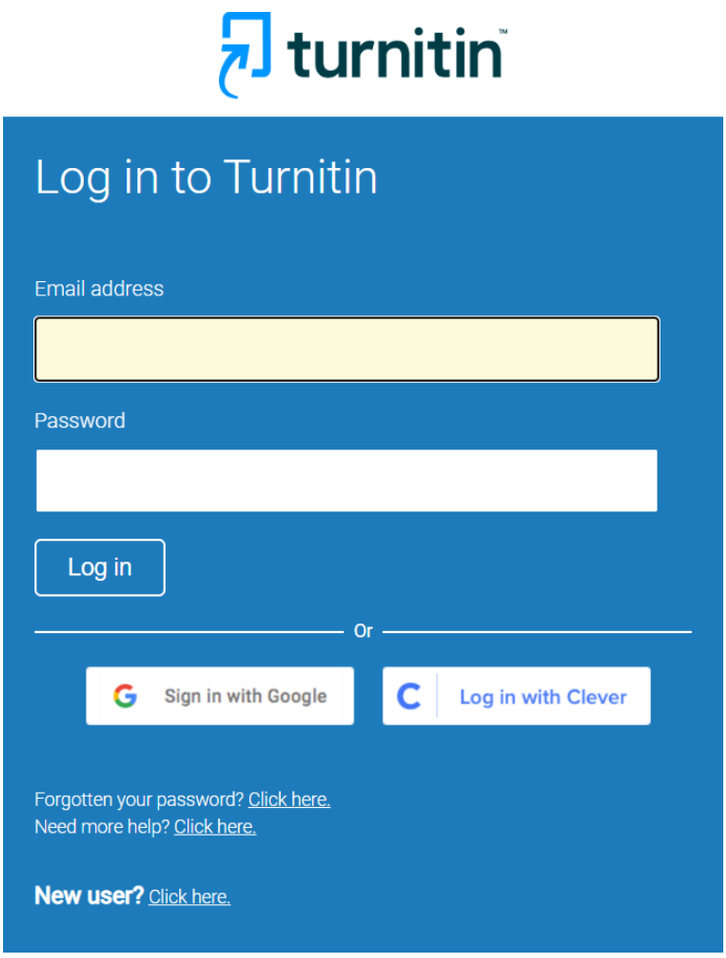
1. **Persiapan dokumen.**

Persiapkan dokumen yang akan diuji similaritasnya :

Kusnoto, Joko et al., "Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects" J Contemp Dent Pract .2025 Apr 1;26(4):339-347.

Doi : https://doi.org/10.5005/jp-journals-10024-3857
Link : https://www.thejcdp.com/abstractArticleContentBrowse/JCDP/19/26/4/40476/abstractArticle/Article

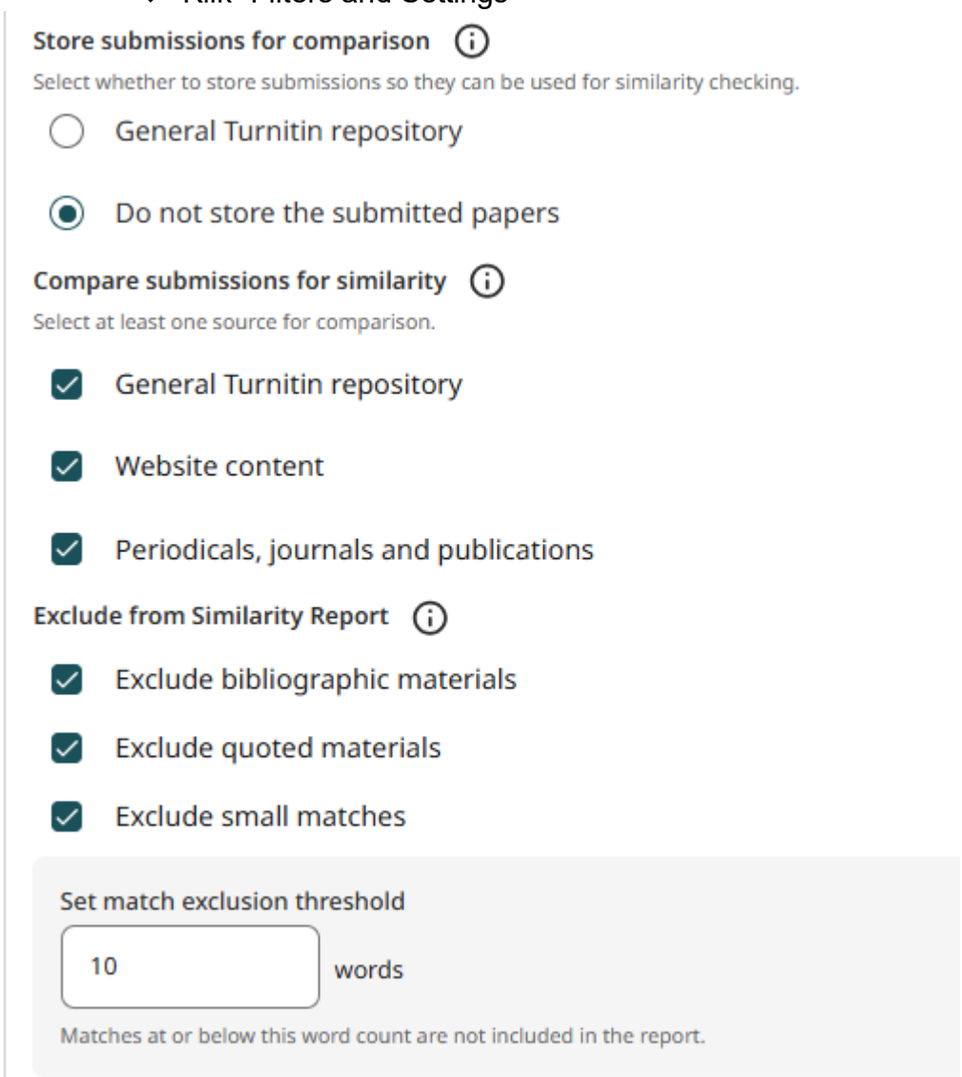
2. **Persiapan Perangkat Lunak Turnitin.**
   - Buka Turnitin melalui https://www.turnitin.com/login_page.asp



- Masukkan alamat email dan password
- Pilih kelas pada daftar "Class Name"
- Pilih salah satu Assignment pada daftar "Assignment title".

3. Pengecekan Similarity

- Pada Assignment title yang dipilih, klik "Submit"
- Pilih email yang sesuai
- Ketik judul pada kolom "Submission title"
- Klik "Choose from this computer" untuk unggah dokumen
- Kemudian klik "Upload"
- Klik "Confirm"
- Klik "Go to Assignment Inbox"
- Klik nilai "Similarity"
- Hasil Similarity ditampilkan sebagai berikut:
- Klik "Filters and Settings"



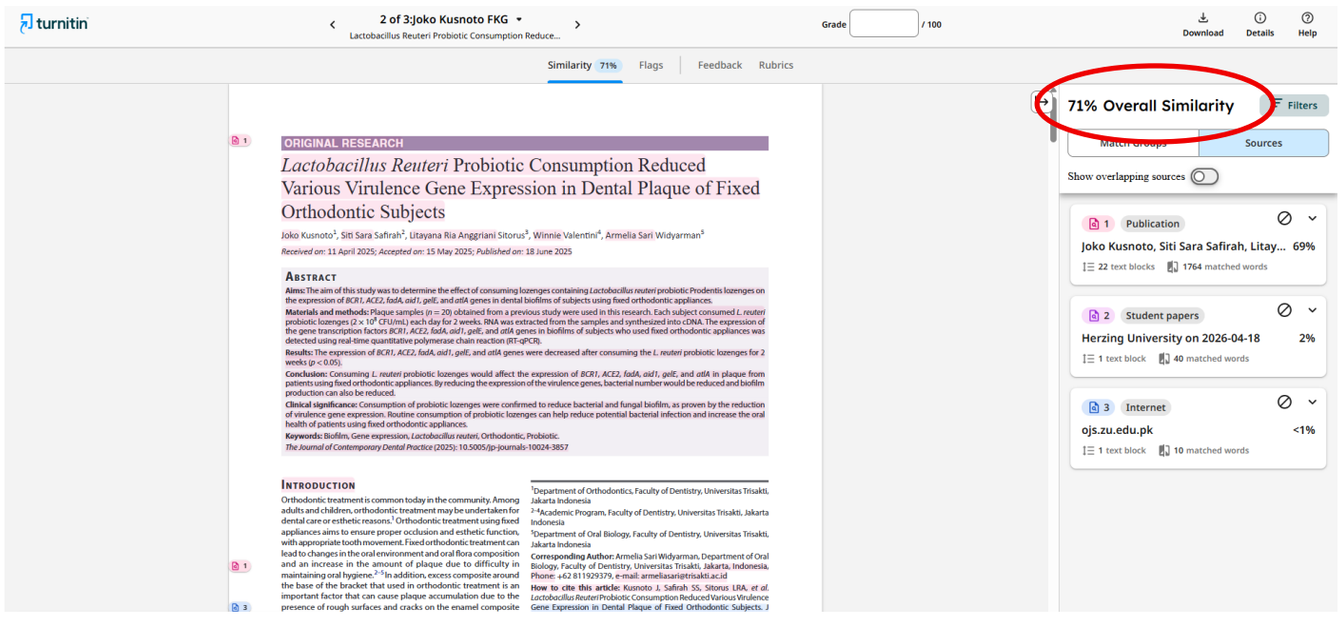
Terdapat filter yaitu:

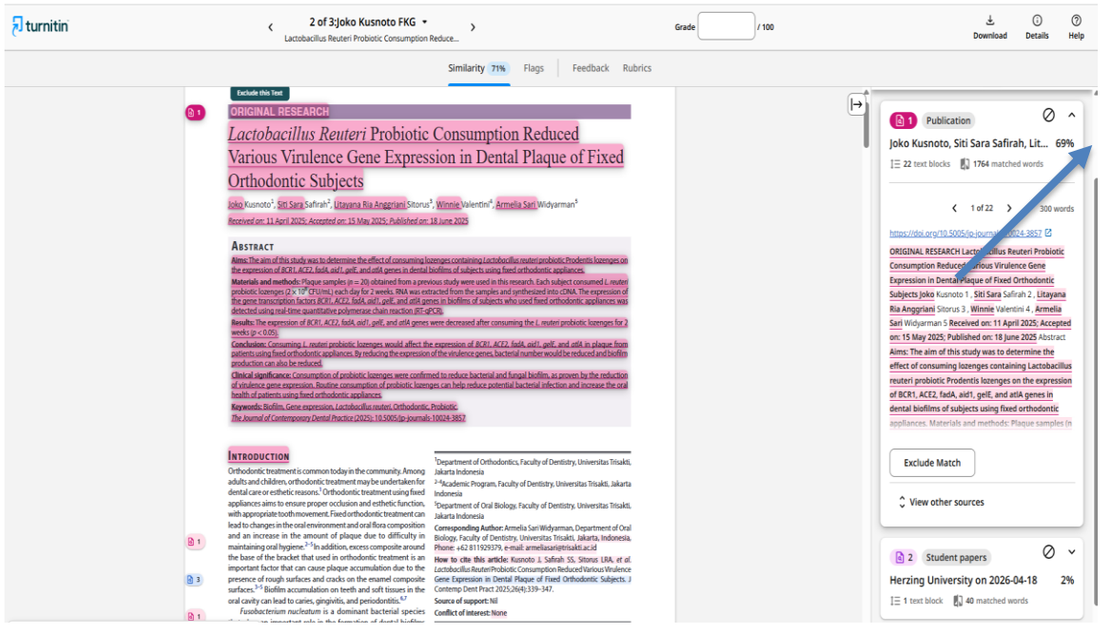
a) *Exclude Bibliography*
b) *Exclude Quotes*
c) *Exclude Small Matches 10 Word*

UNIVERSITAS TRISAKTI
FAKULTAS KEDOKTERAN GIGI
FACULTY OF DENTISTRY – UNIVERSITAS TRISAKTI
KAMPUS B – Jl. Kyai Tapa No. 260 – Grogol – Jakarta Barat 11440 – Indonesia
Telp : +62-21-5672731 (Hunting)
Fax : +62-21-5655787
E-mail : fkg@trisakti.ac.id
Website : https://trisakti.ac.id

❖ Selanjutnya dilakukan "*Exclude Sources*"

Sumber yang di*exclude* beserta alasannya ditampilkan pada tabel berikut;





Exclude 1

Dari Artikel dan Jurnal yang sama

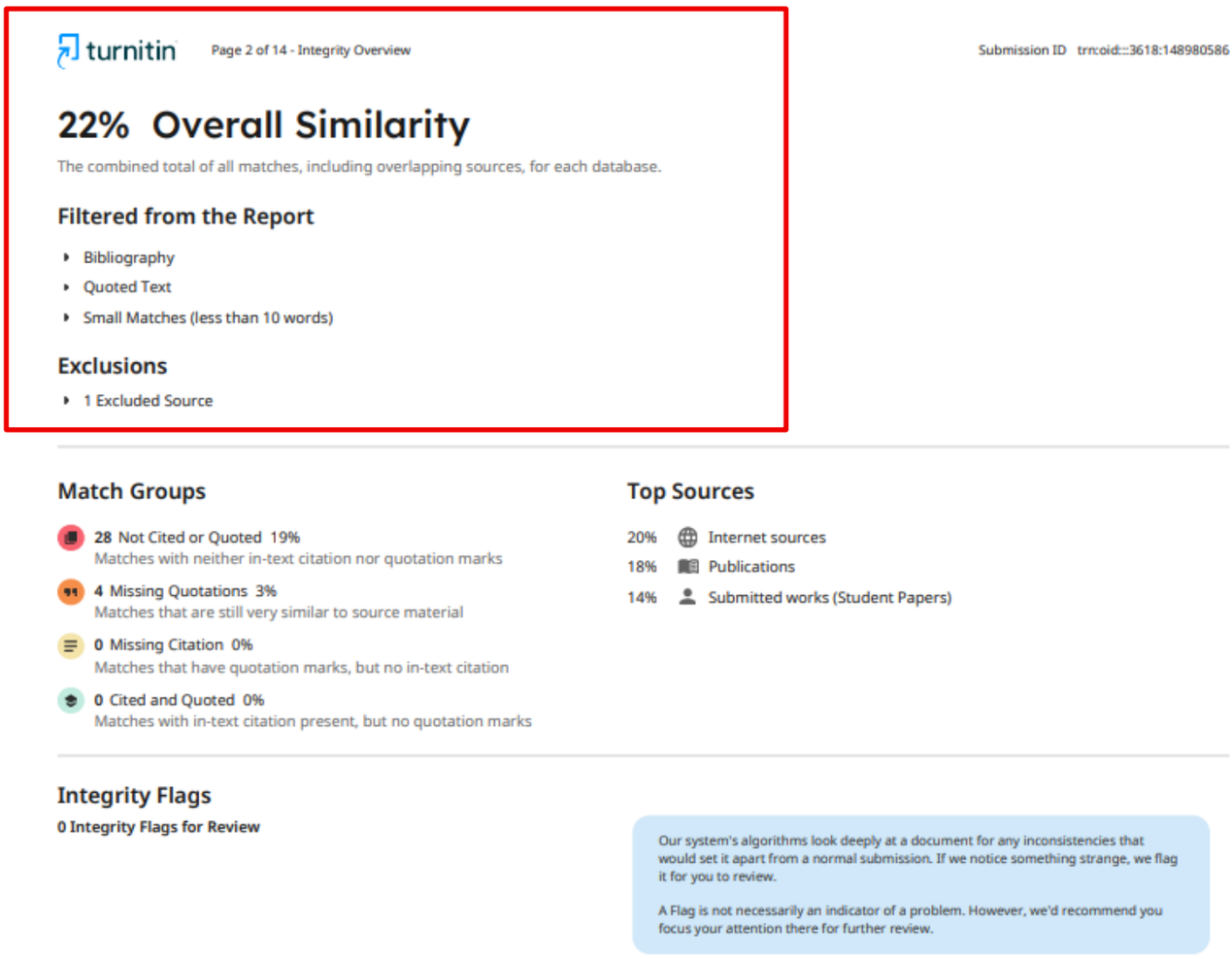
UNIVERSITAS TRISAKTI
FAKULTAS KEDOKTERAN GIGI
FACULTY OF DENTISTRY – UNIVERSITAS TRISAKTI
KAMPUS B – Jl. Kyai Tapa No. 260 – Grogol – Jakarta Barat 11440 – Indonesia
Telp : +62-21-5672731 (Hunting)
Fax : +62-21-5655787
E-mail : fkg@trisakti.ac.id
Website : https://trisakti.ac.id

Keterangan:

Dokumen yang di*exclude* berasal dari sumber jurnal dan artikel yang sama.

UNIVERSITAS TRISAKTI
FAKULTAS KEDOKTERAN GIGI
FACULTY OF DENTISTRY – UNIVERSITAS TRISAKTI
KAMPUS B – Jl. Kyai Tapa No. 260 – Grogol – Jakarta Barat 11440 – Indonesia
Telp : +62-21-5672731 (Hunting)
Fax : +62-21-5655787
E-mail : fkg@trisakti.ac.id
Website : https://trisakti.ac.id

Hasil sesudah diexclude



turnitin Page 2 of 14 - Integrity Overview Submission ID trn:oid:::3618:148980586

22% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Small Matches (less than 10 words)

Exclusions

- 1 Excluded Source

Match Groups

- **28** Not Cited or Quoted 19%
  Matches with neither in-text citation nor quotation marks
- **4** Missing Quotations 3%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

20% Internet sources
18% Publications
14% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

DOKUMEN SEBELUM DIEXCLUDE

Joko Kusnoto FKG

Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodon...

New Assignment

Document Details

Submission ID
trn:oid:::3618:148980586

Submission Date
Aug 21, 2026, 4:25 PM GMT+7

Download Date
Aug 21, 2026, 4:31 PM GMT+7

File Name
Syarat Khusus Joko Kusnoto - 02.pdf

File Size
1.1 MB

9 Pages

4,050 Words

38,393 Characters

71% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Small Matches (less than 10 words)

Match Groups

20 Not Cited or Quoted 60%
Matches with neither in-text citation nor quotation marks

4 Missing Quotations 10%
Matches that are still very similar to source material

0 Missing Citation 0%
Matches that have quotation marks, but no in-text citation

0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

Top Sources

20% Internet sources
71% Publications
14% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

Match Groups

- **20** Not Cited or Quoted 60%
  Matches with neither in-text citation nor quotation marks
- **4** Missing Quotations 10%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

20% Internet sources
71% Publications
14% Submitted works (Student Papers)

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1 Publication

Joko Kusnoto, Siti Sara Safirah, Litayana Ria Anggriani Sitorus, Winnie Valentini, A... **69%**

2 Student papers

Herzing University on 2026-04-18 **2%**

3 Internet

ojs.zu.edu.pk **<1%**

ORIGINAL RESEARCH

Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects

Joko Kusnoto[1], Siti Sara Safirah[2], Litayana Ria Anggriani Sitorus[3], Winnie Valentini[4], Armelia Sari Widyarman[5]

Received on: 11 April 2025; *Accepted on*: 15 May 2025; *Published on*: 18 June 2025

Abstract

Aims: The aim of this study was to determine the effect of consuming lozenges containing *Lactobacillus reuteri* probiotic Prodentis lozenges on the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in dental biofilms of subjects using fixed orthodontic appliances.

Materials and methods: Plaque samples ($n = 20$) obtained from a previous study were used in this research. Each subject consumed *L. reuteri* probiotic lozenges (2×10^8 CFU/mL) each day for 2 weeks. RNA was extracted from the samples and synthesized into cDNA. The expression of the gene transcription factors *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in biofilms of subjects who used fixed orthodontic appliances was detected using real-time quantitative polymerase chain reaction (RT-qPCR).

Results: The expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes were decreased after consuming the *L. reuteri* probiotic lozenges for 2 weeks ($p < 0.05$).

Conclusion: Consuming *L. reuteri* probiotic lozenges would affect the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* in plaque from patients using fixed orthodontic appliances. By reducing the expression of the virulence genes, bacterial number would be reduced and biofilm production can also be reduced.

Clinical significance: Consumption of probiotic lozenges were confirmed to reduce bacterial and fungal biofilm, as proven by the reduction of virulence gene expression. Routine consumption of probiotic lozenges can help reduce potential bacterial infection and increase the oral health of patients using fixed orthodontic appliances.

Keywords: Biofilm, Gene expression, *Lactobacillus reuteri*, Orthodontic, Probiotic.

The Journal of Contemporary Dental Practice (2025): 10.5005/jp-journals-10024-3857

Introduction

Orthodontic treatment is common today in the community. Among adults and children, orthodontic treatment may be undertaken for dental care or esthetic reasons.[1] Orthodontic treatment using fixed appliances aims to ensure proper occlusion and esthetic function, with appropriate tooth movement. Fixed orthodontic treatment can lead to changes in the oral environment and oral flora composition and an increase in the amount of plaque due to difficulty in maintaining oral hygiene.[2–5] In addition, excess composite around the base of the bracket that used in orthodontic treatment is an important factor that can cause plaque accumulation due to the presence of rough surfaces and cracks on the enamel composite surfaces.[3–5] Biofilm accumulation on teeth and soft tissues in the oral cavity can lead to caries, gingivitis, and periodontitis.[6,7]

Fusobacterium nucleatum is a dominant bacterial species that plays an important role in the formation of dental biofilms and periodontal tissue disease. In the formation of biofilms, *F. nucleatum* being a "bridging" or "linking" organism between initial bacterial colonization and final bacterial colonization, which are unable to bind to each other directly. *F. nucleatum* can also co-aggregate with various microbial species in the oral cavity.[8–10] *F. nucleatum* encodes several adhesion genes involved in interspecies interactions, including fusobacterium adhesion A (*fadA*), fusobacterial outer membrane protein A (*fomA*), *radD* (an arginine-inhibitable adhesin), and adherence inducing determinant gene 1 (*aid1*).[8,11–14] Fusobacterium adhesin A (FadA) is known to be involved in *F. nucleatum* invasion and adhesion to host cells and is highly conserved among oral Fusobacterium species.[8,15,16] FadA has been identified has a major virulence factor in *F. nucleatum* in interspecies interactions with *Streptococcus* mediated by *radD*, as it increases the binding specificity of *F. nucleatum* to other microbial species.[14] The arginine-inhibitable adhesion *radD* is required by *F. nucleatum* for co-adherence with various species of Gram-positive bacteria, such as streptococci (early colonizers), and fungal species, such as *Candida*.[11,12,17]

[1]Department of Orthodontics, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia

[2–4]Academic Program, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia

[5]Department of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia

Corresponding Author: Armelia Sari Widyarman, Department of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia, Phone: +62 811929379, e-mail: armeliasari@trisakti.ac.id

How to cite this article: Kusnoto J, Safirah SS, Sitorus LRA, *et al.* *Lactobacillus Reuteri* Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects. J Contemp Dent Pract 2025;26(4):339–347.

Source of support: Nil

Conflict of interest: None

Enterococcus faecalis (*E. faecalis*) is associated with chronic periodontitis and chronic apical periodontitis in failed root canal treatment.[18] *E. faecalis* is a Gram-positive aerobic bacterium. The severity of *E. faecalis* infection depends on the immune response and virulence factors, which can exacerbate infection and play a role in increasing biofilm formation.[19] There are several genes associated with *E. faecalis* biofilm formation, including gelatinase (*gelE*) and autolysin (*atlA*).[20] GelE in *E. faecalis* plaque or saliva isolates showed resistance to antibiotics and high biofilm formation ability.[21] atlA is the main peptidoglycan hydrolase or autolysin of *E. faecalis*.[22] AtlA plays a role in the biofilm maturation stage during which extracellular DNA (eDNA) is released and contributes to biofilm attachment and stability.[23,24]

An increase in the number of colonies of microorganisms also increased, one of which was *Candida albicans*, which causes infections of oral mucosa.[25,26] *C. albicans* has a protein in the form of an adhesive that mediates other microorganisms to adhere to abiotic and host surfaces to form biofilms.[27] Several *C. albicans* gene transcription factors, including biofilm and cell wall regulator 1 (*BCR1*) and angiotensin converting enzyme 2 (*ACE2*), play a role in the formation of biofilms. BCR1 acts as a major regulator of *C. albicans* biofilm formation.[28] The ACE2 transcription factor plays a role in fungal adherence, biofilm formation, and hyphal morphogenesis. In addition, ACE2 plays a role in regulating the expression of genes involved in cell wall separation and metabolism.[29] As shown in previous research, ACE2 is required for filamentation, and it can increase the number of pseudohyphae cells at the time of biofilm formation.[30]

Biofilm formation plays a role in increasing antibiotic resistance in bacterial cells. Therefore, an effective therapy is needed to prevent biofilm formation. The use of probiotics has been suggested as a promising approach to prevent and treat microbial diseases and biofilm activity in the oral cavity.[31,32] Several studies have proven that the use of probiotics has oral cavity health benefits, such as preventing caries and periodontal disease.[32,33] One commercial probiotic proven to be beneficial for oral health is *Lactobacillus reuteri*. The antimicrobial activity of *L. reuteri* inhibits colonization by pathogenic microbes and interacts the inhibition directly with host cells.[34] *L. reuteri* also inhibits the growth of *C. albicans* and *E. faecalis* biofilms.[35,36] However, no studies have investigated the effect of the probiotic *L. reuteri* on the expression of *BCR1*, *ACE2*, *fadA, aid1, gelE*, and *atlA* genes in dental plaque biofilms found in patient's oral environment with fixed orthodontic appliances. By analyzing the virulence genes found in several pathogenic bacteria, we can hypothesize that the downregulation of these genes will lead to less biofilm production and healthier oral health conditions during the duration of fixed orthodontic appliances usage. Thus, to bridge the knowledge gap, the aim of this study was to determine the effect of consuming lozenges containing the probiotic *L. reuteri* on the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in biofilms from subjects using fixed orthodontic appliances.

Materials and Methods

Sample Collection

Subjects undergoing fixed orthodontic therapy were enrolled in this open-label prospective, clinical trial. Ethical approval for the study was granted by the Institutional Review Board of the Faculty of Dentistry, Universitas Indonesia (approval number: 100,701,020). The inclusion criteria specified participants aged 18 years or older who had been undergoing orthodontic treatment with fixed appliances for a minimum duration of 1 year and had not consumed probiotics or antibiotics in the preceding 3 months. The fixed orthodontic appliances used by the subjects are conventional metal braces fixed appliance. Exclusion criteria encompassed individuals with systemic conditions such as hypertension or diabetes, those on systemic medications including antihypertensives, analgesics, hormonal therapies, sedatives, or anti-seizure medications, as well as individuals presenting with severe periodontal disease or known allergies to probiotics.

The study utilized *L. reuteri* Prodentis lozenges, obtained from BioGaia (Stockholm, Sweden). Each lozenge (800 mg) contained a minimum of 2×10^8 live *Limosilactobacillus reuteri* Prodentis (previously classified as *L. reuteri*). This food supplement, specifically formulated for oral health, included a proprietary combination of the *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 strains, which are recognized for their potential to support and maintain oral health.

To standardize oral hygiene practices, all participants were provided with a standardized toothbrush and toothpaste for use throughout the study period. Participants received oral hygiene instructions and were instructed to brush their teeth twice daily. Each participant was administered one probiotic lozenge containing *Limosilactobacillus reuteri* daily for 14 days, to be taken once per day after morning toothbrushing and prior to breakfast. Plaque samples were collected from participants on the day of enrolment, prior to the initiation of probiotic lozenge consumption, and again on the 14th day following the completion of the probiotic regimen.

Before sample collection, mandatory rapid antigen test for SARS-CoV-2 detection had to be taken by the subjects in response to the COVID-19 pandemic (as of 30 September 2020 when collecting the samples) and the test result had to be negative for the subject partaking in the study. In brief, the participants were instructed not to eat or drink anything 2 hours prior to collection of sample. Samples were collected using sterile cotton buds and swabbed from buccal/mesial/distal/lingual/occlusal surfaces of the index teeth of the subjects. The plaque samples were stored in sterile falcon tubes with 5 mL of phosphate-buffered saline (PBS).

Furthermore, clinical data, including the Oral Hygiene Index-Simplified score and the Papilla Bleeding Index, were recorded at each visit. Overall, 20 subjects had enrolled and fulfilled all requirements and inclusion criteria, as 20 subjects were proved to be sufficient to provide valid data as a pilot study.[37,38] Plaque samples from the subjects were continued unto the following downstream analysis.

RNA Extraction, cDNA Synthesis, and Quantification

RNA from the sample was extracted using TRIzol reagent methodology as instructed by the manufacturer (Thermo Fisher, Waltham, MA). The extracted RNA was synthesized into cDNA using ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Toyobo, Japan). The mixture for cDNA synthesis is described in Table 1. The cDNA was then quantified using an Invitrogen™ Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA). The cDNA was stored at –20°C for storage of directly used for downstream analysis.

qPCR Analysis

Amplification and detection by qPCR (Applied Biosystems, Waltham, MA) were performed. The components of the qPCR Master Mix are listed in Table 2. Using a specific kit, namely HOT FIREpol EvaGreen® qPCR Mix (Solis Biodyne, Tartu, Estonia) which was activated by

Table 1: Reagent components for DNase I reaction solution

Component	*Volume*
4 × DN Master Mix	2 mL
RNA template	0.5 pg – 0.5 mg
Nuclease-free water	X mL
Total volume	8 mL

Table 2: Components of Master Mix RT-qPCR

Component	*Volume*
5 × HOT FIREPol EvaGreen® qPCR Mix Plus	4 mL
Primer *Forward*	1 mL
Primer *Reverse*	1 mL
DNA *template*	2 mL
NFW	12 mL
Total	20 mL

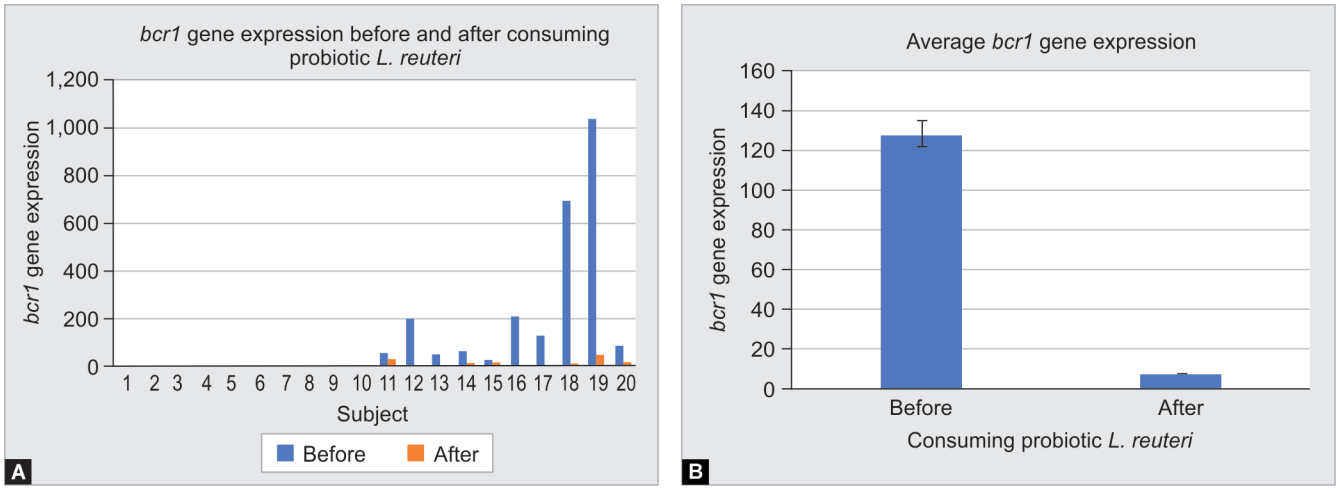
incubation at 95°C for 10 minutes. This was followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing temperature at 60–65°C (Table 3), and elongation at 72°C for 20 seconds. For templates longer than 150 bp, the annealing and elongation times were extended to 30 seconds. Actin gene encoding as the housekeeping gene was used for normalization purposes. qPCR was performed on cDNA. qPCR was performed using the primers listed in Table 3.

Data Analysis and Outcome Analysis

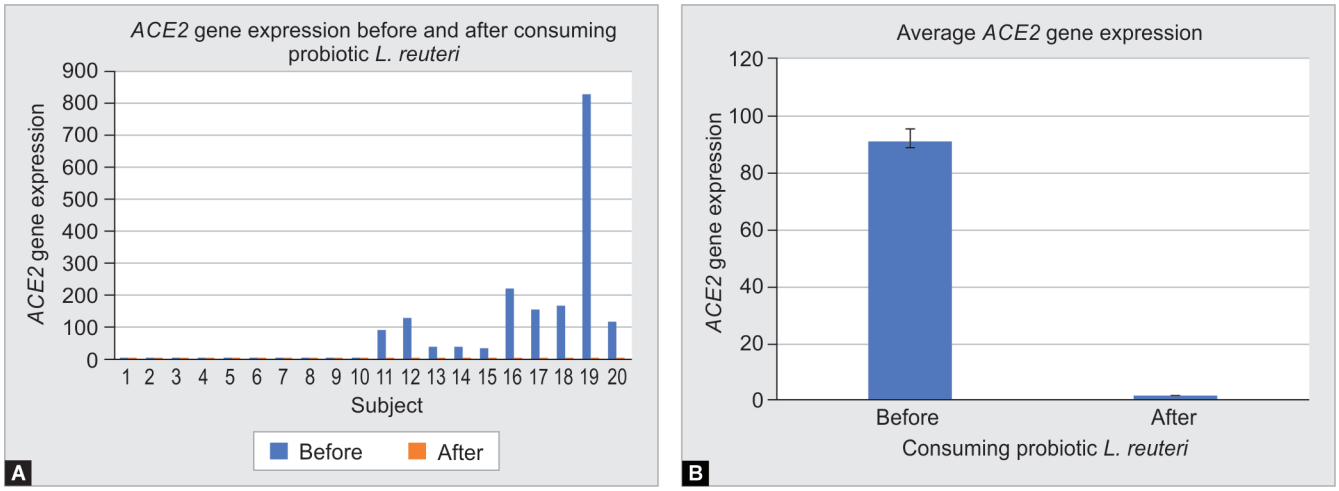
The data were analyzed using the Shapiro–Wilk normality test ($p > 0.05$). For data with a normal distribution, a paired *t*-test was applied ($p < 0.05$). The software used for the analysis is Statistical Package for the Social Sciences (SPSS) version 27 (IBM, Armonk, NY). The outcome analysis are the quantitative data presented from the RT-qPCR instrument. This data can then be correlated with previous study that also conducted such research to ensure the validity of this data.

Table 3: *Primer sequence* for RT-qPCR

Genes	*Primer sequence*	*Temperature and cycle settings*
BCR1[39]	*forward*: 5′-CTTCAGCAGCTTCATTAACACCTA-3′ *reverse*: 5′-TCTTGGATCAGGTGTACTTTTCAA-3′	Initial denaturation of 95°C for 5 minutes; 40 cycles of denaturation at 95°C for 1 minute and annealing at 58°C for 1 minute.
ACE2[40]	*forward* 5′-AGAATTGACCGTTGTCCGTGTAAG-3′ *reverse*: 5′-AATGGGTGAATAAATCCCTCCCTAA-3′	Initial denaturation 95°C for 2 minutes; 40 cycles of denaturation at 95°C for 30 seconds and annealing at 60°C for 1 minute.
Housekeeping gene C. albicans: *ACT1*[41]	*forward*: 5′-TTTCATCTTCTGTATCAGAGGAACTTATTT-3′ *reverse*: 5′-ATGGGATGAATCATCAAACAAGAG-3′	Initial denaturation 95°C for 10 minutes; 40 cycles of denaturing 95°C for 15 seconds and annealing 60°C for 1 minute.
fadA[15]	*forward*: 5′-CAC AAG CTG ACG CTG CTA GA- 3′ *reverse*: 5′-TTA CCA GCT CTT AAA GCT TG-3′	Initial incubation for 4 minutes at 94°C followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55.8°C for 30 seconds, and elongation at 72°C for 40 seconds and the final elongation for 6 minutes.[14]
aid1[14]	*forward:* 5′-TACAGGAG GTGCCGTAGCAG-3′ *reverse:* 5′-TTTTTGTTAATTCT CCAGCTCCA-3′	Initial incubation for 10 minutes at 95°C followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and elongation at 60°C for 1 minute.[13]
Housekeeping gene *F. nucleatum*: *rpoB*[42]	*forward:* 5′-GGYTWYGAAGTNCGHGACGTDCA-3′ *reverse:* 5′-TGACGYTGCATGTTBGMR CCCATMA-3′	Initial incubation for 10 minutes at 95°C followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and elongation at 60°C for 1 minute.
gelE[43]	*forward:* 5′-CGGAACATACTGCCGGTTTAGA-3′ *reverse:* 5′-TGGATTAGATGCACCCGAAAT -3′	Initial denaturation at 95°C for 3 minutes, 40 cycles of denaturation at 95°C for 5 seconds, and annealing at 60°C for 30 seconds.
atlA[44]	*forward:* 5′-AATAATCAATCAGGAACGAATACG-3′ *reverse:* 5′-GCCACACTAACACCGAAT-3′	Initial denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 60 seconds.
Housekeeping gene E. faecalis: *rpoA*[44]	*forward:* 5′-GTGAAACCTGGTCGTGGCTA-3′ *reverse:* 5′-CGACGAACGGGTGTGTAGAT-3′	Initial denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 60 seconds.



Figs 1A and B: (A) A graph showing *bcr1* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *bcr1* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 2A and B: (A) A graph showing *ACE2* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *ACE2* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

Results

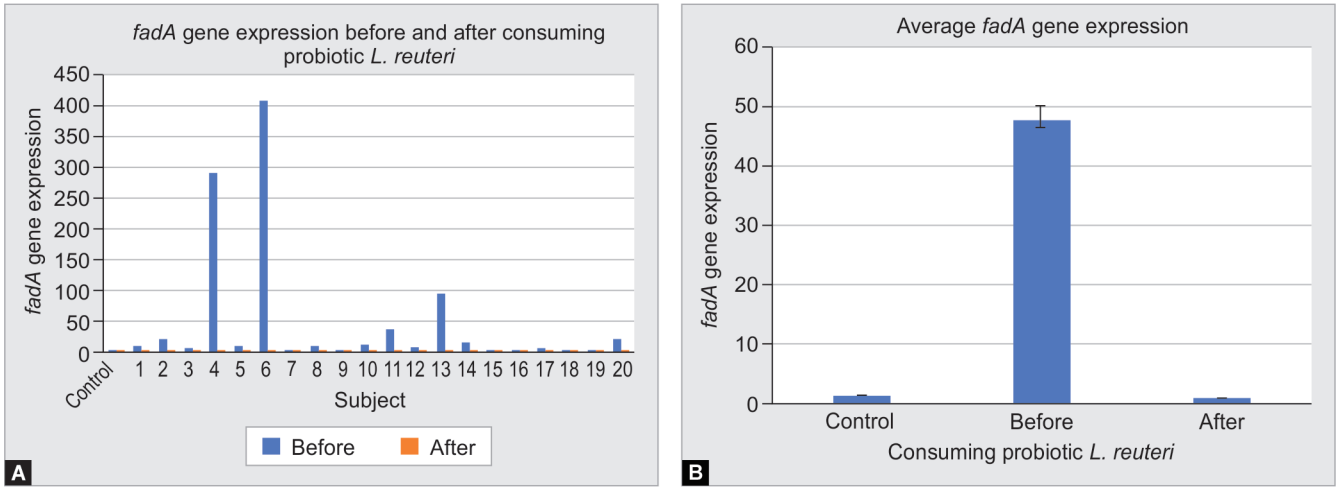
Based on the results of the qPCR test, the expression of *BCR1* (Fig. 1), *ACE2* (Fig. 2), *fadA* (Fig. 3), *aid1* (Fig. 4), *gelE* (Fig. 5), and *atlA* (Fig. 6), on average, were decreased when comparing the day-0 prior to probiotic consumption and day-14 after the subjects consumed the probiotic *L. reuteri*.

As shown by the results of the Shapiro–Wilk normality test, all the data were normally distributed ($p > 0.05$). The data were analyzed using a paired *t*-test, with a significance level of $p < 0.05$. The results of the paired *t*-test revealed a significant difference in the comparison of the *BCR1* and *ACE2* gene expression data. There was a significant difference ($p < 0.05$) in the expression of *BCR1, ACE2, fadA, aid1, gelE*, and *atlA* genes after consuming the probiotic *L. reuteri*.

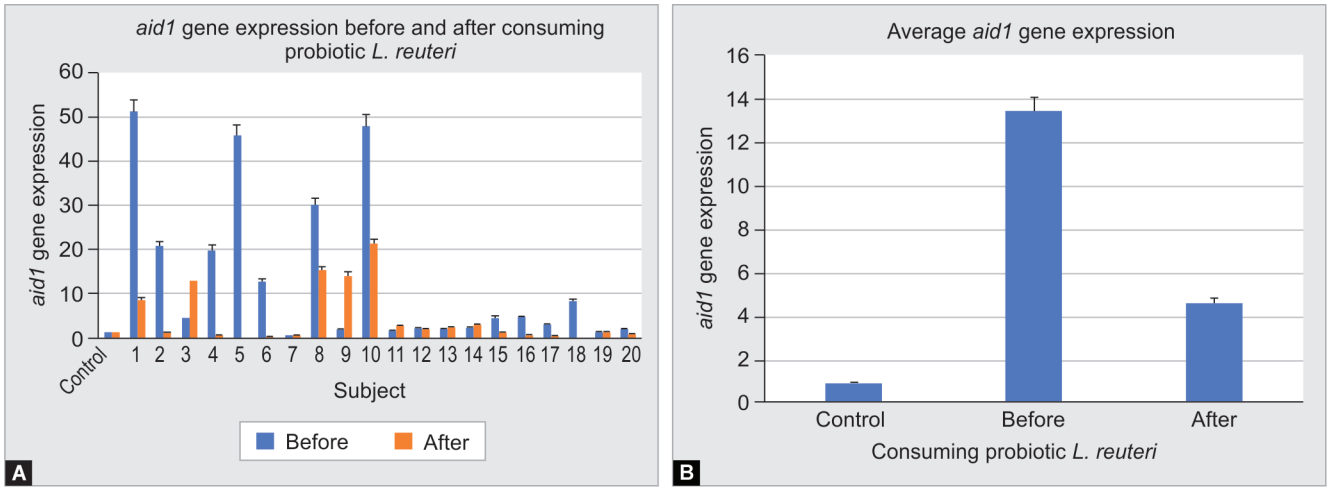
Discussion

The use of fixed orthodontic appliances often causes poor oral hygiene, thus facilitating microorganism accumulation and various pathological conditions in the oral cavity, such as fungal infections.[45] A previous study revealed an increase in *Candida* in saliva after using fixed orthodontic appliances. The authors attributed this to the design of fixed orthodontic appliances, which creates a space for the retention of food waste.[46] Thus, patients must be instructed about good oral hygiene practices after orthodontic treatment.[38] Fixed orthodontic appliances also induce changes in buffer capacity, salivary flow rates, and acidity (pH), leading to plaque accumulation and an increase in caries and periodontal disease.[47–49] Based on this information, we attempted this study to prove the benefits of consuming probiotics lozenges as a supplement for patients using fixed orthodontic appliances.

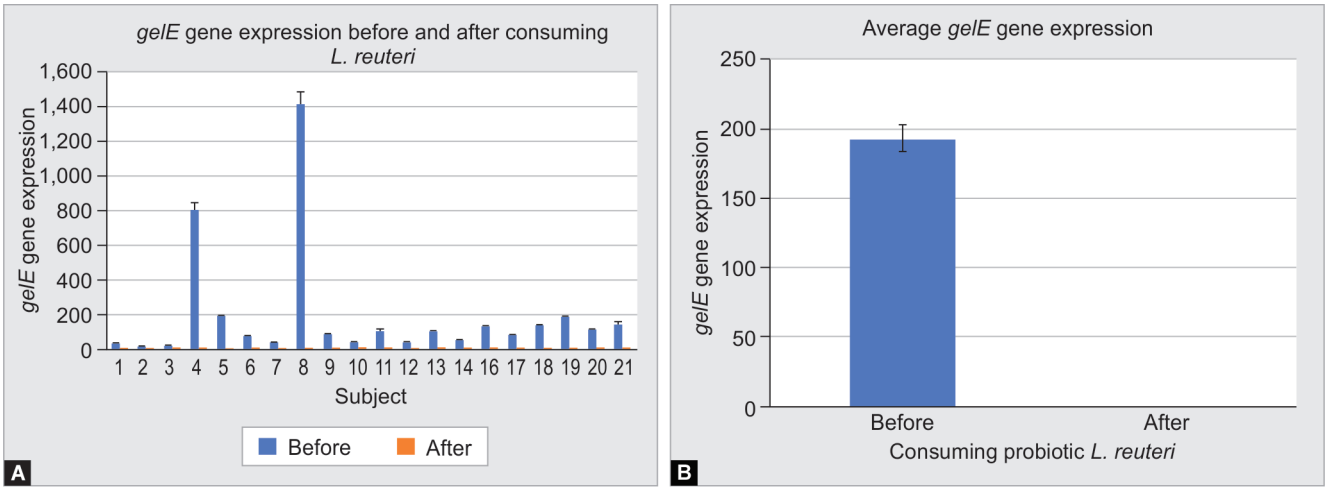
Biofilm formation is an important virulence factor of *F. nucleatum* due to its higher resistance to host defense or antibacterial agents compared to planktonic cells.[50] Several studies detected increased numbers of *Porphyromonas gingivalis, F. nucleatum, P. intermedia,* and *Tannerella forsythia* after the use of fixed orthodontic appliances.[15,51,52] They also reported that *F. nucleatum* increased



Figs 3A and B: (A) A graph showing *fadA* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *fadA* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

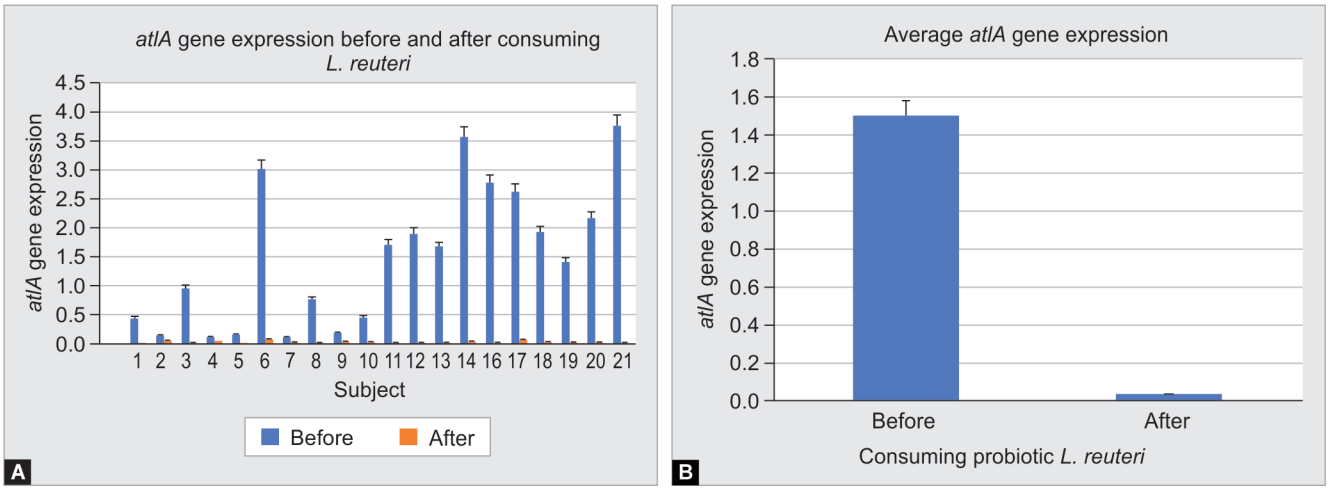


Figs 4A and B: (A) A graph showing *aid1* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *aid1* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 5A and B: (A) A graph showing *gelE* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *gelE* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

turnitin Page 9 of 12 - Integrity Submission Submission ID trn:oid:::3618:148980586



Figs 6A and B: (A) A graph showing *atlA* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *atlA* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

the risk of periodontitis in orthodontic patients due to a conducive environment for anaerobic bacteria.[15,51,52] Biofilm formation is also a contributing factor to *E. faecalis* colonization and infection. Biofilms develop through various processes by which bacteria adhere to surfaces, decompose complex matrices, and develop into bacterial colonies, which adhere to surfaces.[53]

In this study, we used the qPCR method and $2^{-\Delta\Delta CT}$ formula to calculate target gene expression. As shown by the results, the probiotic *L. reuteri* affected the expression of *BCR1, ACE2, fadA, aid1, gelE*, and *atlA* genes and the formation of *C. albicans, F. nucleatum*, and *E. faecalis* biofilms. Based on the Shapiro–Wilk normality test, all the data were normally distributed, with $p > 0.05$. The paired *t*-test results revealed significant differences in the expression *of BCR1, ACE2, fadA, aid1, gelE*, and *atlA* genes (all $p < 0.05$).

Based on the results of this study, the probiotic *L. reuteri* significantly downregulated the transcription of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes. In gene expression, the process of translating genetic information in the form of sequence of bases of DNA or RNA into proteins.[54] Through gene expression measurement, it is possible to assess qualitatively and quantitatively the effect of a treatment, such as the administration of a drug compound.[55] Gene expression in microorganisms is involved in regulating cell-cell communication, carbohydrate metabolism, adherence, and adaptation to the surrounding environment. A decrease in the expression of specific genes can reduce microorganism colonization and microorganism numbers.[56]

BCR1, a major gene transcription factor, produces an adhesin protein, which facilitates *C. albicans* attachment to mucosal surfaces, which is a critical stage of infection.[39] Deletion of *BCR1* eliminates *C. albicans* gene function, resulting in a decrease in biofilm formation.[57] This was supported by the results of the present study, which revealed a statistically significant decrease in *BCR1* gene expression after consuming the probiotic.

The expression of *ACE2* also decreased based on the results of the statistical tests. With deletion of *ACE2*, *C. albicans* is unable to form hyphal cells, and thus biofilm formation is inhibited.[30] An earlier in vitro study showed that probiotics have antifungal effects against *C. albicans* in the oral cavity. Regular use of probiotics helped to inhibit *Candida* biofilms and reduced *Candida* colonization in the oral cavity, thereby reducing the possibility of candidiasis infection.[58]

FadA protein is the main *F. nucleatum* virulence factor and mediates microbial attachment and colonization.[8] Based on the results of this study, the probiotic *L. reuteri* appears to influence the pathogenicity of *F. nucleatum* adhesion molecules and colonization and affect biofilm formation through decreased expression of the *fadA* gene.[35] Various *F. nucleatum* adhesins mediate adhesion and aggregation and function as coaggregation intermediaries in the formation and maturation of dental biofilms.[59] The interaction of Fusobacterium with other species is largely mediated by the adhesin genes *radD* and *aid1*.[60] The *aid1* gene plays a role in interspecies interactions, colonization, and aggregation of *F. nucleatum*. In a previous study, inactivation of the *aid1* gene decreased the ability of *F. nucleatum* to aggregate, especially with *Streptococcus* spp. or *E. faecalis*.[14] As shown in earlier studies, probiotics can affect the expression of genes involved in cell adhesion, quorum sensing (QS), virulence factors, and biofilm formation.[61,62]

The *E. faecalis gelE* gene has the ability to hydrolyse gelatine, collagen, fibrin, and other peptides.[63] Gene *gelE* is a virulence factor in infection formation through bacterial attachment and biofilm formation.[22] In *E. faecalis*, biofilm formation is regulated by QS, where Fsr regulates the expression of the *gelE* gene.[64] Fsr regulates the formation of *E. faecalis* biofilms through its product *gelE* and serine proteases.[43] QS is a molecular mechanism by which bacterial cells communicate with each other via signaling molecules in biofilms. If the protease encoded by a signaling factor is decreased, communication between bacteria and biofilm formation will be disrupted.[62] GelE can also activate *atlA*, which is responsible for eDNA release at the biofilm maturation stage.[65]

AtlA is involved in the hydrolysis of peptidoglycan, which plays an important role in separating cells division after replication.[22] AtlA plays a role in the biofilm maturation stage of *E. faecalis*, during which eDNA is released and contributes to biofilm attachment and stability, biofilm defects in primary attachment, and decreased biofilm production.[20,23] This study focused on the probiotic *L. reuteri*, which has ability to secrete antimicrobial substances and compete with oral pathogens for adhesion to mucosa. In addition, *L. reuteri* can adapt and change the pH of the surrounding environment, thereby inhibiting the growth of oral pathogens.[66] The antimicrobial substances secreted by *L. reuteri* are reuterin and

turnitin Page 9 of 12 - Integrity Submission Submission ID trn:oid:::3618:148980586

reutericycline.[67] Reuterin and reutericycline are broad-spectrum antimicrobial agents that are effective against Gram-positive and -negative bacteria, fungi, and protozoa by inhibiting microbial DNA synthesis.[35,47]

Many dental and oral health care products used daily now include probiotics. The use of probiotics is increasing due to their advantages over chemical agents, namely reducing the risk of antibiotic resistance.[68] Probiotics work by modulating the immune system, producing antimicrobial substances, and inhibiting certain pathogenic organisms by interfering with adhesion, colonization, and biofilm formation. They inhibit the growth of pathogens via the production of various substances, such as lactic acid and acetic acid, which penetrate the bacterial cell membrane and lower the cytoplasmic pH of pathogenic bacteria. Hydrogen peroxide and bacteriocin can destroy the cell membrane of pathogenic bacteria and inhibit the synthesis of pathogenic DNA.[32,33,35,69] Based on the results of this study, 2 weeks of daily consumption of the probiotic *L. reuteri* affected the process of biofilm formation by downregulating the expression of *BCR1*, *ACE2, fadA, aid1, gelE,* and *atlA* genes, which function as adherent regulators and regulators of hyphae formation in biofilm formation. Many previous studies demonstrated that the addition of probiotics to dental and oral health care products. Probiotic can reduce pathogenic microorganisms in plaque samples from patients using fixed orthodontic appliances.[47,68,70–72] Therefore, it can be stated that the probiotic *L. reuteri* has good ability as an additional treatment for dental and oral health in patients using fixed orthodontic appliances.

The limitation of this study is the small sample size and the fact that the sampling had to be done during COVID-19 pandemic. Although the sample size is small, the result of this study can still provide concise and significant result. In the future, there should be larger sample size for conducting this research so the results can more accurately represent the actual population. Daily consumption of probiotic lozenges duration can also be increased for more precise and accurate results. On the other hand, consumption of probiotic lozenges research can also be conducted for other aspect of oral health diseases to promote the functionality and health-inducing aspect of probiotic lozenges, such as antiinflammation.

Conclusion

Within the limitation of this study, it can be concluded that the probiotic *L. reuteri* influences the expression of *BCR1*, *ACE2*, *fadA, aid1, gelE,* and *atlA* genes in biofilm formation. By reducing the expression of those genes, the probiotic *L. reuteri* can reduce biofilm formation, such as dental plaque, in patients using fixed orthodontic appliances.

Clinical Significance

Consumption of probiotic lozenges were confirmed to reduce bacterial and fungal biofilm, as proven by the reduction of virulence gene expression, hence, helping increasing oral health of consumer. The results of this study help clinicians provide probiotic lozenges for patients to promote and maintain their oral health.

References

1. Sihotang BM, Panjaitan M, et al. Perbedaan oral hygiene pemakaian pesawat ortodonti cekat dan pesawat ortodonti lepasan pada pasien klinik spesialis RS Herna. J Prima Medika Sains 2015;2(1):1–5. DOI: 10.34012/jpms.v2i1.840.
2. Gkantidis N, Christou P, Topouzelis N. The orthodontic-periodontic interrelationship in integrated treatment challenges: A systematic review. J Oral Rehabil 2010;37(5):377–390. DOI: 10.1111/j.1365-2842.2010.02068.x.
3. Oktaviani V, Santoso O. Perbedaan Indeks Higiene Oral dan pH Plak Kelompok Pemakai dan Bukan Pemakai Pesawat Ortodonti Cekat 2016;5(1):8.
4. Bowen WH, Burne RA, Wu H, et al. Oral biofilms: pathogens, matrix and polymicrobial interactions in microenvironments. Trends Microbiol 2018;26(3):229–242. DOI: 10.1016/j.tim.2017.09.008.
5. Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm. Virulence 2011;2(5):435–444. DOI: 10.4161/viru.2.5.16140.
6. Verrusio C, Iorio-Siciliano V, Blasi A, et al. The effect of orthodontic treatment on periodontal tissue inflammation: A systematic review. Quintessence Int 2018;49(1):69–77. DOI: 10.3290/j.qi.a39225.
7. Perkowski K, Baltaza W, Conn DB, et al. Examination of oral biofilm microbiota in patients using fixed orthodontic appliances in order to prevent risk factors for health complications. Ann Agric Environ Med 2019;26(2):231–235. DOI: 10.26444/aaem/105797.
8. Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol 2015;23:141–147. DOI: 10.1016/j.mib.2014.11.013.
9. Rickard AH, Gilbert P, High NJ, et al. Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends Microbiol 2003;11(2):94–100. DOI: 10.1016/s0966-842x(02)00034-3.
10. Thurnheer T, Karygianni L, Flury M, et al. Fusobacterium species and subspecies differentially affect the composition and architecture of supra- and subgingival biofilms models. Front Microbiol 2019;10:1716. DOI: 10.3389/fmicb.2019.01716.
11. Chew J, Zilm PS, Fuss JM, et al. A proteomic investigation of Fusobacterium nucleatum alkaline-induced biofilms. BMC Microbiol 2012;12(1):189. DOI: 10.1186/1471-2180-12-189.
12. Kaplan CW, Lux R, Haake SK, et al. The Fusobacterium nucleatum outer membrane protein RadD is an arginine-inhibitable adhesin required for inter-species adherence and the structured architecture of multi-species biofilm. Mol Microbiol 2009;71(1):35–47. DOI: 10.1111/j.1365-2958.2008.06503.x.
13. Cochrane K, Robinson AV, Holt RA, et al. A survey of Fusobacterium nucleatum genes modulated by host cell infection. Microb Genom 2020;6(2):e000300. DOI: 10.1099/mgen.0.000300.
14. Kaplan A, Kaplan CW, He X, et al. Characterization of aid1, a novel gene involved in Fusobacterium nucleatum interspecies interactions. Microb Ecol 2014;68(2):379–387. DOI: 10.1007/s00248-014-0400-y.
15. Liu P, Liu Y, Wang J, et al. Detection of Fusobacterium nucleatum and fadA adhesin gene in patients with orthodontic gingivitis and non-orthodontic periodontal inflammation. PLOS One 2014;9(1):e85280. DOI: 10.1371/journal.pone.0085280.
16. Brennan CA, Garrett WS. Fusobacterium nucleatum – symbiont, opportunist and oncobacterium. Nat Rev Microbiol 2019;17(3):156–166. DOI: 10.1038/s41579-018-0129-6.
17. Wu T, Cen L, Kaplan C, et al. Cellular components mediating coadherence of Candida albicans and Fusobacterium nucleatum. J Dent Res 2015;94(10):1432–1438. DOI: 10.1177/0022034515593706.
18. Wang Q-Q, Zhang C-F, Chu C-H, et al. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci 2012;4(1):19–23. DOI: 10.1038/ijos.2012.17.
19. Najafi K, Gholizadeh P, Tanomand A, et al. Oral cavity infection by Enterococcus faecalis: Virulence factors and pathogenesis. Rev Med Microbiol 2019;30(2). DOI: 10.1097/MRM.0000000000000168.
20. Ch'ng J-H, Chong KKL, Lam LN, et al. Biofilm-associated infection by enterococci. Nat Rev Microbiol 2019;17(2):82–94. DOI: 10.1038/s41579-018-0107-z.
21. Anderson AC, Jonas D, Huber I, et al. Enterococcus faecalis from food, clinical specimens, and oral sites: prevalence of virulence factors in association with biofilm formation. Front Microbiol 2016;6:1534. DOI: 10.3389/fmicb.2015.01534.
22. Stinemetz EK, Gao P, Pinkston KL, et al. Processing of the major autolysin of E. faecalis, AtlA, by the zinc-metalloprotease, GelE,

impacts AtlA septal localization and cell separation. PLoS One 2017;12(10):e0186706.
23. Guiton PS, Hung CS, Kline KA, et al. Contribution of autolysin and sortase A during Enterococcus faecalis DNA-dependent biofilm development. Infect Immun 2009;77(9):3626–3638. DOI: 10.1128/IAI.00219-09.
24. Paganelli FL, Willems RJL, Jansen P, et al. Enterococcus faecium biofilm formation: identification of major autolysin AtlAEfm, associated Acm surface localization, and AtlAEfm-independent extracellular DNA release. mBio 2013;4(2):e00154. DOI: 10.1128/mBio.00154-13.
25. Putri MH, Herijulianti E. Ilmu Pencegahan Penyakit Jaringan Keras dan Jaringan Pendukung Gigi | OPAC Integrasi | Online Public Access Catalog | Universitas Gadjah Mada. RGC; 2010.
26. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev 2007;20(1):133–163. DOI: 10.1128/CMR.00029-06.
27. Garcia MC, Lee JT, Ramsook CB, et al. A role for amyloid in cell aggregation and biofilm formation. PLoS One 2011;6(3):e17632. DOI: 10.1371/journal.pone.0017632.
28. Nobile CJ, Mitchell AP. Regulation of cell-surface genes and biofilm formation by the C. albicans transcription factor Bcr1p. Curr Biol 2005;15(12):1150–1155. DOI: 10.1016/j.cub.2005.05.047.
29. Mulhern SM, Logue ME, Butler G. Candida albicans transcription factor Ace2 regulates metabolism and is required for filamentation in hypoxic conditions. Eukaryot Cell 2006;5(12):2001–2013. DOI: 10.1128/EC.00155-06.
30. Saputo S, Kumar A, Krysan DJ. Efg1 directly regulates ACE2 expression to mediate cross talk between the cAMP/PKA and RAM pathways during Candida albicans morphogenesis. Eukaryot Cell 2014;13(9):1169–1180. DOI: 10.1128/EC.00148-14.
31. Widyarman AS, Bachtiar EW, Bachtiar BM, et al. Inhibitory effect of probiotic lactobacilli against Streptococcus mutans and Porphyromonas gingivalis biofilms. Sci Dental J 2019;3(2):50. DOI: 10.4103/SDJ.SDJ_8_19.
32. Widyarman AS, Ramajayanti T, Theodorea CF. Indonesian strain of probiotic reduces the initial biofilm colonization. The Open Dent J 2020;14(1). DOI: 10.2174/1874210602014010544.
33. Widyarman AS, Theodorea CF. Novel indigenous probiotic Lactobacillus reuteri strain produces anti-biofilm reuterin against pathogenic periodontal bacteria. Eur J Dent 2021;16(1):96–101. DOI: 10.1055/s-0041-1731591.
34. Mu Q, Tavella VJ, Luo XM. Role of Lactobacillus reuteri in human health and diseases. Front Microbiol 2018;9:757. DOI: 10.3389/fmicb.2018.00757.
35. Sari Widyarman A, Kiky Elysia Lazaroni N. Persistent endodontics pathogens biofilm inhibited by Lactobacillus reuteri Indonesian strain. J Dent Indones 2019;26(3). Available from: https://scholarhub.ui.ac.id/jdi/vol26/iss3/7/.
36. Collado MC, Meriluoto J, Salminen S. Adhesion and aggregation properties of probiotic and pathogen strains. Eur Food Res Technol 2008;226(5):1065–1073. DOI: 10.1007/s00217-007-0632-x.
37. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. Pharm Stat 2005;4:287–291. DOI: 10.1002/pst.185.
38. Johanson GA, Brooks GP. Initial scale development: Sample size for pilot studies. Educ Psychol Meas 2010;70:394–400. DOI: 10.1177/0013164409355692.
39. Nikoomanesh F, Roudbarmohammadi S, Roudbary M, et al. Investigation of bcr1 gene expression in Candida albicans isolates by RT-PCR technique and its impact on biofilm formation. iem 2016;2(1):22–24. DOI: 10.7508/iem.2016.01.007.
40. Ranjith K, Kalyana Chakravarthy S, Adicherla H, et al. Temporal expression of genes in biofilm-forming ocular *Candida albicans* isolated from patients with keratitis and orbital cellulitis. Invest Ophthalmol Vis Sci 2018;59(1):528–538. DOI: 10.1167/iovs.17-22933.
41. Nailis H, Coenye T, Van Nieuwerburgh F, et al. Development and evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. BMC Mol Biol 2006;7:25. DOI: 10.1186/1471-2199-7-25.
42. Vos M, Quince C, Pijl AS, et al. A Comparison of rpoB and 16S rRNA as markers in pyrosequencing studies of bacterial diversity. PLoS One 2012;7(2):e30600. DOI: 10.1371/journal.pone.0030600.
43. Wang L, Dong M, Zheng J, et al. Relationship of biofilm formation and gelE gene expression in Enterococcus faecalis recovered from root canals in patients requiring endodontic retreatment. J Endod 2011;37(5):631–636. DOI: 10.1016/j.joen.2011.02.006.
44. Zheng J-X, Wu Y, Lin Z-W, et al. Characteristics of and virulence factors associated with biofilm formation in clinical Enterococcus faecalis isolates in China. Front Microbiol 2017;8:2338. DOI: 10.3389/fmicb.2017.02338.
45. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. J Oral Microbiol 2011;3. DOI: 10.3402/jom.v3i0.5771.
46. Khanpayeh E, Jafari AA, Tabatabaei Z. Comparison of salivary Candida profile in patients with fixed and removable orthodontic appliances therapy. Iran J Microbiol 2014;6(4):263–268. PMID: 25802710.
47. Widyarman AS, Hartono V, Marjani LI, et al. Lactobacillus reuteri containing probiotic Lozenges consumption reduces Streptococcus mutans, Streptococcus sobrinus, Porphyromonas gingivalis, and Aggregatibacter actinomycetemcomitans in orthodontic patients. J Int Dent Med Res 2018;11(2):628–633.
48. Restrepo M, Bussaneli DG, Jeremias F, et al. Control of white spot lesions with use of fluoride varnish or chlorhexidine gel during orthodontic treatment A randomized clinical trial. J Clin Pediatr Dent 2016;40(4):274–280. DOI: 10.17796/1053-4628-40.4.274.
49. Lu H, Tang H, Zhou T, et al. Assessment of the periodontal health status in patients undergoing orthodontic treatment with fixed appliances and Invisalign system. Medicine (Baltimore) 2018;97(13):e0248. DOI: 10.1097/MD.0000000000010248.
50. Fitriasari N, Wahyuni IS. POTENSI PROBIOTIK DALAM TATALAKSANA ORAL CANDIDIASIS: Ulasan Sistematik. Odonto : Dent J 2021;8(1):34–44. DOI: 10.30659/odj.8.1.34-44.
51. Guo L, Feng Y, Guo H-G, et al. Consequences of orthodontic treatment in malocclusion patients: clinical and microbial effects in adults and children. BMC Oral Health 2016;16(1):112. DOI: 10.1186/s12903-016-0308-7.
52. Alqerban A. Efficacy of antimicrobial photodynamic and photobiomodulation therapy against Treponema denticola, fusobacterium nucleatum and human beta defensin-2 levels in patients with gingivitis undergoing fixed orthodontic treatment: a clinic-laboratory study. Photodiagnosis Photodyn Ther 2020;29:101659.
53. Willett JLE, Ji MM, Dunny GM. Exploiting biofilm phenotypes for functional characterization of hypothetical genes in Enterococcus faecalis. NPJ Biofilms Microbiomes 2019;5(1):23. DOI: 10.1038/s41522-019-0099-0.
54. Sinaga E. Regulasi Ekspresi Gen. Regulasi Ekspresi Gen 2011;1–24.
55. Van Peer G, Mestdagh P, Vandesompele J. Accurate RT-qPCR gene expression analysis on cell culture lysates. Sci Rep 2012;2:222. DOI: 10.1038/srep00222.
56. Shemesh M, Tam A, Steinberg D. Expression of biofilm-associated genes of Streptococcus mutans in response to glucose and sucrose. J Med Microbiol 2007;56(11):1528–1535. DOI: 10.1099/jmm.0.47146-0.
57. Fanning S, Xu W, Solis N, et al. Divergent targets of Candida albicans biofilm regulator Bcr1 in vitro and in vivo. Eukaryot Cell 2012;11(7):896–904. DOI: 10.1128/EC.00103-12.
58. Matsubara VH, Bandara HMHN, Mayer MPA, et al. Probiotics as antifungals in mucosal candidiasis. Clin Infect Dis 2016;62(9):1143–1153. DOI: 10.1093/cid/ciw038.
59. Li Q, Tan L, Wang H, et al. Fusobacterium nucleatum interaction with Pseudomonas aeruginosa induces biofilm-associated antibiotic tolerance via Fusobacterium adhesin A. ACS Infect Dis 2020;6(7):1686–1696. DOI: 10.1021/acsinfecdis.9b00402.
60. Kaplan A. Identification and characterization of a novel lipoprotein in Fusobacterium nucleatum. UCLA; 2015 Available from: https://escholarship.org/uc/item/3r90n33c.
61. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25(4):402–408.

62. Barzegari A, Kheyrolahzadeh K, Hosseiniyan Khatibi SM, et al. The battle of probiotics and their derivatives against biofilms. Infect Drug Resist 2020;13:659–672. DOI: 10.2147/IDR.S232982.
63. Guneser MB, Eldeniz AU. The effect of gelatinase production of Enterococcus faecalis on adhesion to dentin after irrigation with various endodontic irrigants. Acta Biomater Odontol Scand 2016;2(1):144–149. DOI: 10.1080/23337931.2016.1256212.
64. Hashem YA, Amin HM, Essam TM, et al. Biofilm formation in enterococci: Genotype-phenotype correlations and inhibition by vancomycin. Sci Rep 2017;7(1):5733. DOI: 10.1038/s41598-017-05901-0.
65. Ali L, Goraya MU, Arafat Y, et al. Molecular mechanism of quorum-sensing in Enterococcus faecalis: its role in virulence and therapeutic approaches. Int J Mol Sci 2017;18(5):960. DOI: 10.3390/ijms1805 0960.
66. Bonifait L, Chandad F, Grenier D. Probiotics for oral health: myth or reality? J Can Dent Assoc 2009;75(8):585–590. PMID: 19840501
67. Urbańska M, Szajewska H. The efficacy of Lactobacillus reuteri DSM 17938 in infants and children: A review of the current evidence. Eur J Pediatr 2014;173(10):1327–1337. DOI: 10.1007/s00431-014-2328-0.
68. Benic GZ, Farella M, Morgan XC, et al. Oral probiotics reduce halitosis in patients wearing orthodontic braces: A randomized, triple-blind, placebo-controlled trial. J Breath Res 2019;13(3):036010. DOI: 10.1088/1752-7163/ab1c81.
69. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, et al. Probiotic mechanisms of action. Ann Nutr Metab 2012;61(2):160–174. DOI: 10.1159/000342079.
70. Widyarman AS, Yunita ST, Prasetyadi T. Consumption of yogurt containing probiotic *Bifidobacterium lactis* reduces *Streptococcus mutans* in orthodontic patients. Sci Dent J2018;2(1):19–25. DOI: 10.26912/sdj.v2i1.1913.
71. Alp S, Baka ZM. Effects of probiotics on salivary *Streptecoccus mutans* and Lactobacillus levels in orthodontic patients. Am J Orthod Dentofacial Orthop 2018;154(4):517–523. DOI: 10.1016/j.ajodo.2018.01.010.
72. Goyal N, Shamanna PU, Varughese ST, et al. Effects of amine fluoride and probiotic mouthwash on levels of *Porphyromonas gingivalis* in orthodontic patients: a randomized controlled trial. J Indian Soc Periodontol 2019;23(4):339–344. DOI: 10.4103/jisp.jisp_551_18.

DOKUMEN SESUDAH DIEXCLUDE

Joko Kusnoto FKG

Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodon...

New Assignment

Document Details

Submission ID
trn:oid:::3618:148980586

Submission Date
Aug 21, 2026, 4:25 PM GMT+7

Download Date
Aug 21, 2026, 4:43 PM GMT+7

File Name
Syarat Khusus Joko Kusnoto - 02.pdf

File Size
1.1 MB

9 Pages

4,050 Words

38,393 Characters

22% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Small Matches (less than 10 words)

Exclusions

- 1 Excluded Source

Match Groups

28 Not Cited or Quoted 19%
Matches with neither in-text citation nor quotation marks

4 Missing Quotations 3%
Matches that are still very similar to source material

0 Missing Citation 0%
Matches that have quotation marks, but no in-text citation

0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

Top Sources

20% Internet sources
18% Publications
14% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

Match Groups

- **28** Not Cited or Quoted 19%
  Matches with neither in-text citation nor quotation marks
- **4** Missing Quotations 3%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

20% Internet sources
18% Publications
14% Submitted works (Student Papers)

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	Publication	
Rahmi Amtha, Ferry Sandra, Rosalina Tjandrawinata, Indrayadi Gunardi, Anggrae...		2%
2	Student papers	
Herzing University on 2026-04-18		2%
3	Internet	
www.thejcdp.com		2%
4	Internet	
pdffox.com		1%
5	Internet	
www.aging-us.com		1%
6	Internet	
www.osti.gov		1%
7	Internet	
ojs.zu.edu.pk		1%
8	Internet	
www.nature.com		1%
9	Internet	
docs.wixstatic.com		<1%
10	Internet	
warm.dovepress.com		<1%

11	Publication	
Shupei Qiao, Yufang Zhao, Hui Tian, Ishara Manike, Liang Ma, Hongji Yan, Weimin...		<1%
12	Student papers	
Imperial College of Science, Technology and Medicine on 2013-03-12		<1%
13	Internet	
gutpathogens.biomedcentral.com		<1%
14	Student papers	
Universiti Sains Malaysia on 2014-12-19		<1%
15	Internet	
openrepository.aut.ac.nz		<1%
16	Internet	
www.mdpi.com		<1%
17	Internet	
ies.ijo.cn		<1%
18	Internet	
www.plosone.org		<1%
19	Internet	
escholarship.org		<1%
20	Internet	
pmc.ncbi.nlm.nih.gov		<1%
21	Student papers	
Universidad de Lleida on 2026-07-08		<1%
22	Internet	
e-crt.org		<1%
23	Internet	
docplayer.net		<1%
24	Internet	
www.karyailmiah.trisakti.ac.id		<1%

25 Internet

nl.growell.co.uk <1%

26 Internet

openaccess.hacettepe.edu.tr:8080 <1%

ORIGINAL RESEARCH

Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects

Joko Kusnoto[1], Siti Sara Safirah[2], Litayana Ria Anggriani Sitorus[3], Winnie Valentini[4], Armelia Sari Widyarman[5]

Received on: 11 April 2025; *Accepted on*: 15 May 2025; *Published on*: 18 June 2025

Abstract

Aims: The aim of this study was to determine the effect of consuming lozenges containing *Lactobacillus reuteri* probiotic Prodentis lozenges on the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in dental biofilms of subjects using fixed orthodontic appliances.

Materials and methods: Plaque samples ($n = 20$) obtained from a previous study were used in this research. Each subject consumed *L. reuteri* probiotic lozenges (2×10^8 CFU/mL) each day for 2 weeks. RNA was extracted from the samples and synthesized into cDNA. The expression of the gene transcription factors *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in biofilms of subjects who used fixed orthodontic appliances was detected using real-time quantitative polymerase chain reaction (RT-qPCR).

Results: The expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes were decreased after consuming the *L. reuteri* probiotic lozenges for 2 weeks ($p < 0.05$).

Conclusion: Consuming *L. reuteri* probiotic lozenges would affect the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* in plaque from patients using fixed orthodontic appliances. By reducing the expression of the virulence genes, bacterial number would be reduced and biofilm production can also be reduced.

Clinical significance: Consumption of probiotic lozenges were confirmed to reduce bacterial and fungal biofilm, as proven by the reduction of virulence gene expression. Routine consumption of probiotic lozenges can help reduce potential bacterial infection and increase the oral health of patients using fixed orthodontic appliances.

Keywords: Biofilm, Gene expression, *Lactobacillus reuteri*, Orthodontic, Probiotic.

The Journal of Contemporary Dental Practice (2025): 10.5005/jp-journals-10024-3857

Introduction

Orthodontic treatment is common today in the community. Among adults and children, orthodontic treatment may be undertaken for dental care or esthetic reasons.[1] Orthodontic treatment using fixed appliances aims to ensure proper occlusion and esthetic function, with appropriate tooth movement. Fixed orthodontic treatment can lead to changes in the oral environment and oral flora composition and an increase in the amount of plaque due to difficulty in maintaining oral hygiene.[2–5] In addition, excess composite around the base of the bracket that used in orthodontic treatment is an important factor that can cause plaque accumulation due to the presence of rough surfaces and cracks on the enamel composite surfaces.[3–5] Biofilm accumulation on teeth and soft tissues in the oral cavity can lead to caries, gingivitis, and periodontitis.[6,7]

Fusobacterium nucleatum is a dominant bacterial species that plays an important role in the formation of dental biofilms and periodontal tissue disease. In the formation of biofilms, *F. nucleatum* being a "bridging" or "linking" organism between initial bacterial colonization and final bacterial colonization, which are unable to bind to each other directly. *F. nucleatum* can also co-aggregate with various microbial species in the oral cavity.[8–10] *F. nucleatum* encodes several adhesion genes involved in interspecies interactions, including fusobacterium adhesion A (*fadA*), fusobacterial outer membrane protein A (*fomA*), *radD* (an arginine-inhibitable adhesin), and adherence inducing determinant gene 1 (*aid1*).[8,11–14] Fusobacterium adhesin A (FadA) is known to be involved in *F. nucleatum* invasion and adhesion to host cells and is highly conserved among oral Fusobacterium species.[8,15,16] FadA has been identified has a major virulence factor in *F. nucleatum* in interspecies interactions with *Streptococcus* mediated by *radD*, as it increases the binding specificity of *F. nucleatum* to other microbial species.[14] The arginine-inhibitable adhesion *radD* is required by *F. nucleatum* for co-adherence with various species of Gram-positive bacteria, such as streptococci (early colonizers), and fungal species, such as *Candida*.[11,12,17]

[1]Department of Orthodontics, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia

[2–4]Academic Program, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia

[5]Department of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia

Corresponding Author: Armelia Sari Widyarman, Department of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia, Phone: +62 811929379, e-mail: armeliasari@trisakti.ac.id

How to cite this article: Kusnoto J, Safirah SS, Sitorus LRA, *et al*. *Lactobacillus Reuteri* Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects. J Contemp Dent Pract 2025;26(4):339–347.

Source of support: Nil

Conflict of interest: None

Enterococcus faecalis (*E. faecalis*) is associated with chronic periodontitis and chronic apical periodontitis in failed root canal treatment.[18] *E. faecalis* is a Gram-positive aerobic bacterium. The severity of *E. faecalis* infection depends on the immune response and virulence factors, which can exacerbate infection and play a role in increasing biofilm formation.[19] There are several genes associated with *E. faecalis* biofilm formation, including gelatinase (*gelE*) and autolysin (*atlA*).[20] GelE in *E. faecalis* plaque or saliva isolates showed resistance to antibiotics and high biofilm formation ability.[21] atlA is the main peptidoglycan hydrolase or autolysin of *E. faecalis*.[22] AtlA plays a role in the biofilm maturation stage during which extracellular DNA (eDNA) is released and contributes to biofilm attachment and stability.[23,24]

An increase in the number of colonies of microorganisms also increased, one of which was *Candida albicans*, which causes infections of oral mucosa.[25,26] *C. albicans* has a protein in the form of an adhesive that mediates other microorganisms to adhere to abiotic and host surfaces to form biofilms.[27] Several *C. albicans* gene transcription factors, including biofilm and cell wall regulator 1 (*BCR1*) and angiotensin converting enzyme 2 (*ACE2*), play a role in the formation of biofilms. BCR1 acts as a major regulator of *C. albicans* biofilm formation.[28] The ACE2 transcription factor plays a role in fungal adherence, biofilm formation, and hyphal morphogenesis. In addition, ACE2 plays a role in regulating the expression of genes involved in cell wall separation and metabolism.[29] As shown in previous research, ACE2 is required for filamentation, and it can increase the number of pseudohyphae cells at the time of biofilm formation.[30]

Biofilm formation plays a role in increasing antibiotic resistance in bacterial cells. Therefore, an effective therapy is needed to prevent biofilm formation. The use of probiotics has been suggested as a promising approach to prevent and treat microbial diseases and biofilm activity in the oral cavity.[31,32] Several studies have proven that the use of probiotics has oral cavity health benefits, such as preventing caries and periodontal disease.[32,33] One commercial probiotic proven to be beneficial for oral health is *Lactobacillus reuteri*. The antimicrobial activity of *L. reuteri* inhibits colonization by pathogenic microbes and interacts the inhibition directly with host cells.[34] *L. reuteri* also inhibits the growth of *C. albicans* and *E. faecalis* biofilms.[35,36] However, no studies have investigated the effect of the probiotic *L. reuteri* on the expression of *BCR1*, *ACE2*, *fadA, aid1, gelE*, and *atlA* genes in dental plaque biofilms found in patient's oral environment with fixed orthodontic appliances. By analyzing the virulence genes found in several pathogenic bacteria, we can hypothesize that the downregulation of these genes will lead to less biofilm production and healthier oral health conditions during the duration of fixed orthodontic appliances usage. Thus, to bridge the knowledge gap, the aim of this study was to determine the effect of consuming lozenges containing the probiotic *L. reuteri* on the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in biofilms from subjects using fixed orthodontic appliances.

Materials and Methods

Sample Collection

Subjects undergoing fixed orthodontic therapy were enrolled in this open-label prospective, clinical trial. Ethical approval for the study was granted by the Institutional Review Board of the Faculty of Dentistry, Universitas Indonesia (approval number: 100,701,020). The inclusion criteria specified participants aged 18 years or older who had been undergoing orthodontic treatment with fixed appliances for a minimum duration of 1 year and had not consumed probiotics or antibiotics in the preceding 3 months. The fixed orthodontic appliances used by the subjects are conventional metal braces fixed appliance. Exclusion criteria encompassed individuals with systemic conditions such as hypertension or diabetes, those on systemic medications including antihypertensives, analgesics, hormonal therapies, sedatives, or anti-seizure medications, as well as individuals presenting with severe periodontal disease or known allergies to probiotics.

The study utilized *L. reuteri* Prodentis lozenges, obtained from BioGaia (Stockholm, Sweden). Each lozenge (800 mg) contained a minimum of 2×10^8 live *Limosilactobacillus reuteri* Prodentis (previously classified as *L. reuteri*). This food supplement, specifically formulated for oral health, included a proprietary combination of the *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 strains, which are recognized for their potential to support and maintain oral health.

To standardize oral hygiene practices, all participants were provided with a standardized toothbrush and toothpaste for use throughout the study period. Participants received oral hygiene instructions and were instructed to brush their teeth twice daily. Each participant was administered one probiotic lozenge containing *Limosilactobacillus reuteri* daily for 14 days, to be taken once per day after morning toothbrushing and prior to breakfast. Plaque samples were collected from participants on the day of enrolment, prior to the initiation of probiotic lozenge consumption, and again on the 14th day following the completion of the probiotic regimen.

Before sample collection, mandatory rapid antigen test for SARS-CoV-2 detection had to be taken by the subjects in response to the COVID-19 pandemic (as of 30 September 2020 when collecting the samples) and the test result had to be negative for the subject partaking in the study. In brief, the participants were instructed not to eat or drink anything 2 hours prior to collection of sample. Samples were collected using sterile cotton buds and swabbed from buccal/mesial/distal/lingual/occlusal surfaces of the index teeth of the subjects. The plaque samples were stored in sterile falcon tubes with 5 mL of phosphate-buffered saline (PBS).

Furthermore, clinical data, including the Oral Hygiene Index-Simplified score and the Papilla Bleeding Index, were recorded at each visit. Overall, 20 subjects had enrolled and fulfilled all requirements and inclusion criteria, as 20 subjects were proved to be sufficient to provide valid data as a pilot study.[37,38] Plaque samples from the subjects were continued unto the following downstream analysis.

RNA Extraction, cDNA Synthesis, and Quantification

RNA from the sample was extracted using TRIzol reagent methodology as instructed by the manufacturer (Thermo Fisher, Waltham, MA). The extracted RNA was synthesized into cDNA using ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Toyobo, Japan). The mixture for cDNA synthesis is described in Table 1. The cDNA was then quantified using an Invitrogen™ Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA). The cDNA was stored at –20°C for storage of directly used for downstream analysis.

qPCR Analysis

Amplification and detection by qPCR (Applied Biosystems, Waltham, MA) were performed. The components of the qPCR Master Mix are listed in Table 2. Using a specific kit, namely HOT FIREpol EvaGreen® qPCR Mix (Solis Biodyne, Tartu, Estonia) which was activated by

Table 1: Reagent components for DNase I reaction solution

Component	*Volume*
4 × DN Master Mix	2 mL
RNA template	0.5 pg – 0.5 mg
Nuclease-free water	X mL
Total volume	8 mL

Table 2: Components of Master Mix RT-qPCR

Component	*Volume*
5 × HOT FIREPol EvaGreen® qPCR Mix Plus	4 mL
Primer *Forward*	1 mL
Primer *Reverse*	1 mL
DNA *template*	2 mL
NFW	12 mL
Total	20 mL

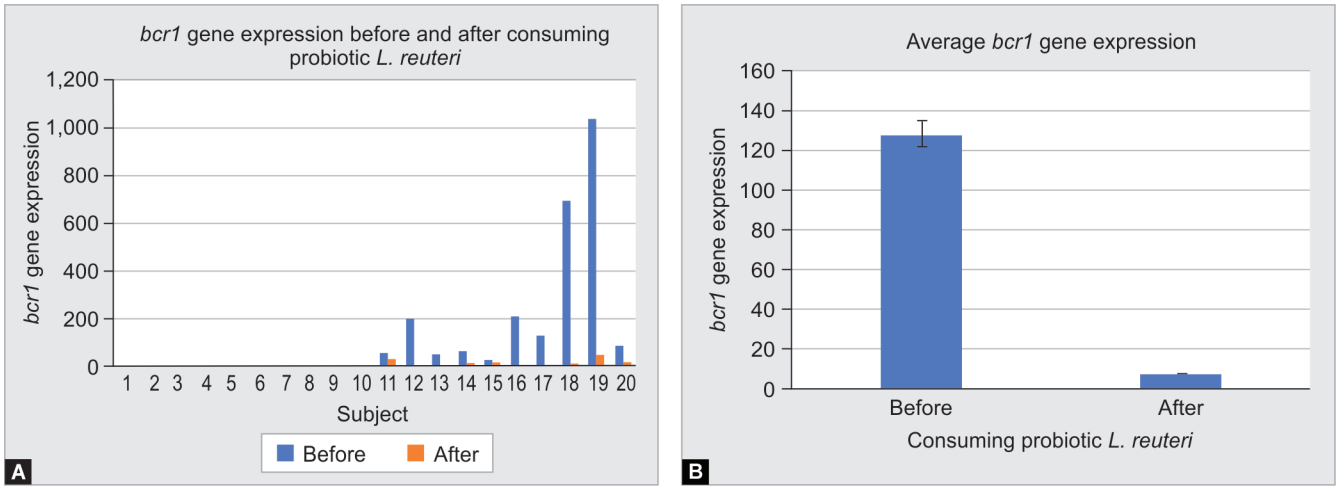
incubation at 95°C for 10 minutes. This was followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing temperature at 60–65°C (Table 3), and elongation at 72°C for 20 seconds. For templates longer than 150 bp, the annealing and elongation times were extended to 30 seconds. Actin gene encoding as the housekeeping gene was used for normalization purposes. qPCR was performed on cDNA. qPCR was performed using the primers listed in Table 3.

Data Analysis and Outcome Analysis

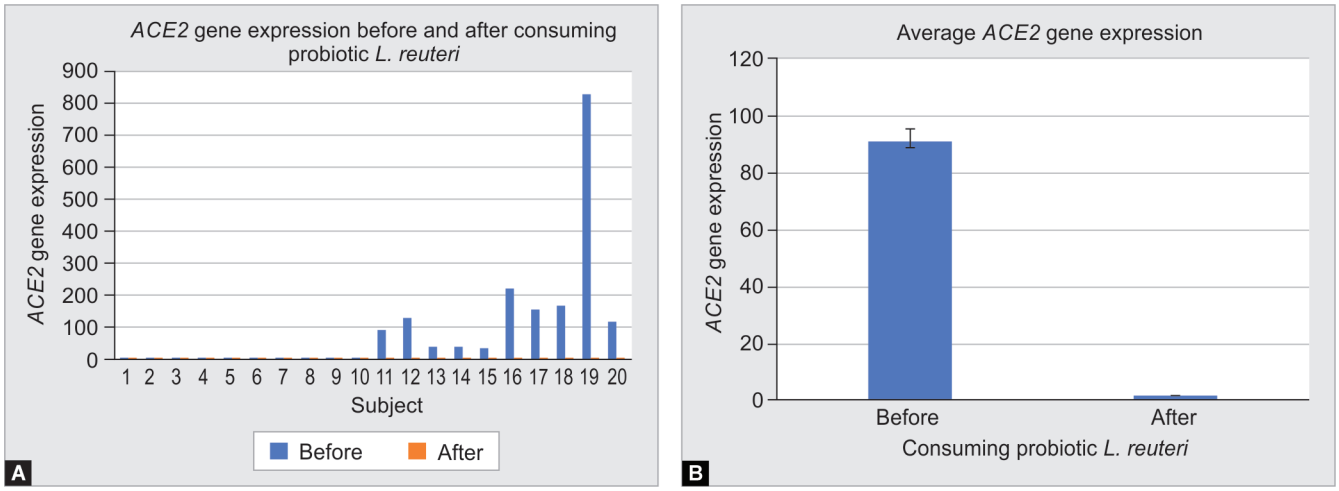
The data were analyzed using the Shapiro–Wilk normality test ($p > 0.05$). For data with a normal distribution, a paired *t*-test was applied ($p < 0.05$). The software used for the analysis is Statistical Package for the Social Sciences (SPSS) version 27 (IBM, Armonk, NY). The outcome analysis are the quantitative data presented from the RT-qPCR instrument. This data can then be correlated with previous study that also conducted such research to ensure the validity of this data.

Table 3: *Primer sequence* for RT-qPCR

Genes	*Primer sequence*	*Temperature and cycle settings*
BCR1[39]	*forward*: 5′-CTTCAGCAGCTTCATTAACACCTA-3′ *reverse*: 5′-TCTTGGATCAGGTGTACTTTTCAA-3′	Initial denaturation of 95°C for 5 minutes; 40 cycles of denaturation at 95°C for 1 minute and annealing at 58°C for 1 minute.
ACE2[40]	*forward* 5′-AGAATTGACCGTTGTCCGTGTAAG-3′ *reverse*: 5′-AATGGGTGAATAAATCCCTCCCTAA-3′	Initial denaturation 95°C for 2 minutes; 40 cycles of denaturation at 95°C for 30 seconds and annealing at 60°C for 1 minute.
Housekeeping gene *C. albicans*: *ACT1*[41]	*forward*: 5′-TTTCATCTTCTGTATCAGAGGAACTTATTT-3′ *reverse*: 5′-ATGGGATGAATCATCAAACAAGAG-3′	Initial denaturation 95°C for 10 minutes; 40 cycles of denaturing 95°C for 15 seconds and annealing 60°C for 1 minute.
fadA[15]	*forward*: 5′-CAC AAG CTG ACG CTG CTA GA- 3′ *reverse*: 5′-TTA CCA GCT CTT AAA GCT TG-3′	Initial incubation for 4 minutes at 94°C followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55.8°C for 30 seconds, and elongation at 72°C for 40 seconds and the final elongation for 6 minutes.[14]
aid1[14]	*forward:* 5′-TACAGGAG GTGCCGTAGCAG-3′ *reverse:* 5′-TTTTTGTTAATTCT CCAGCTCCA-3′	Initial incubation for 10 minutes at 95°C followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and elongation at 60°C for 1 minute.[13]
Housekeeping gene *F. nucleatum*: *rpoB*[42]	*forward:* 5′-GGYTWYGAAGTNCGHGACGTDCA-3′ *reverse:* 5′-TGACGYTGCATGTTBGMR CCCATMA-3′	Initial incubation for 10 minutes at 95°C followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and elongation at 60°C for 1 minute.
gelE[43]	*forward:* 5′-CGGAACATACTGCCGGTTTAGA-3′ *reverse:* 5′-TGGATTAGATGCACCCGAAAT -3′	Initial denaturation at 95°C for 3 minutes, 40 cycles of denaturation at 95°C for 5 seconds, and annealing at 60°C for 30 seconds.
atlA[44]	*forward:* 5′-AATAATCAATCAGGAACGAATACG-3′ *reverse:* 5′-GCCACACTAACACCGAAT-3′	Initial denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 60 seconds.
Housekeeping gene *E. faecalis*: *rpoA*[44]	*forward:* 5′-GTGAAACCTGGTCGTGGCTA-3′ *reverse:* 5′-CGACGAACGGGTGTGTAGAT-3′	Initial denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 60 seconds.



Figs 1A and B: (A) A graph showing *bcr1* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *bcr1* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 2A and B: (A) A graph showing *ACE2* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *ACE2* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

Results

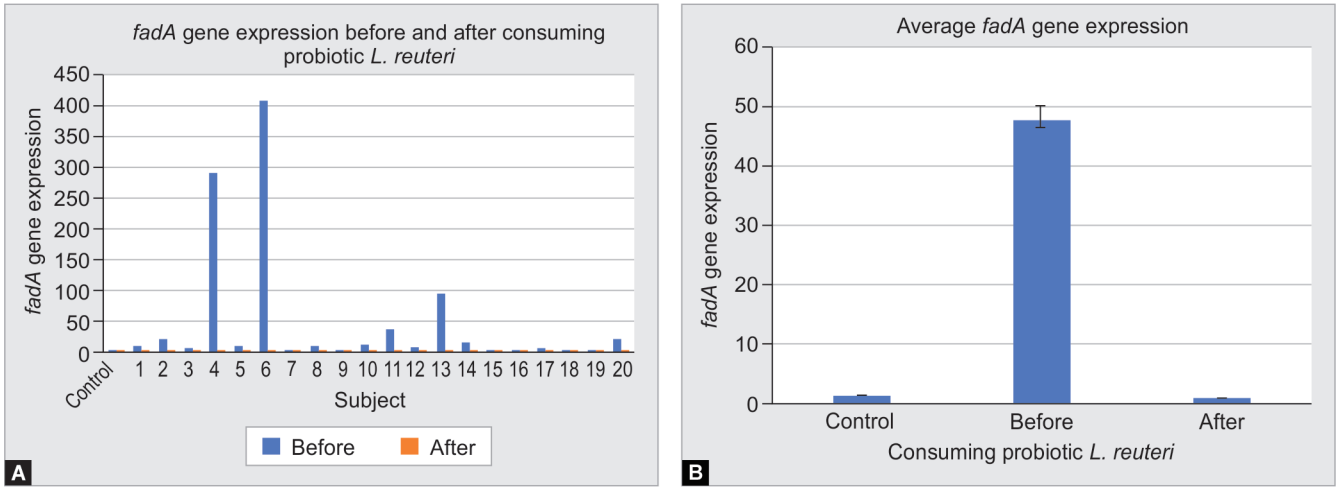
Based on the results of the qPCR test, the expression of *BCR1* (Fig. 1), *ACE2* (Fig. 2), *fadA* (Fig. 3), *aid1* (Fig. 4), *gelE* (Fig. 5), and *atlA* (Fig. 6), on average, were decreased when comparing the day-0 prior to probiotic consumption and day-14 after the subjects consumed the probiotic *L. reuteri*.

As shown by the results of the Shapiro–Wilk normality test, all the data were normally distributed ($p > 0.05$). The data were analyzed using a paired *t*-test, with a significance level of $p < 0.05$. The results of the paired *t*-test revealed a significant difference in the comparison of the *BCR1* and *ACE2* gene expression data. There was a significant difference ($p < 0.05$) in the expression of *BCR1, ACE2, fadA, aid1, gelE*, and *atlA* genes after consuming the probiotic *L. reuteri*.

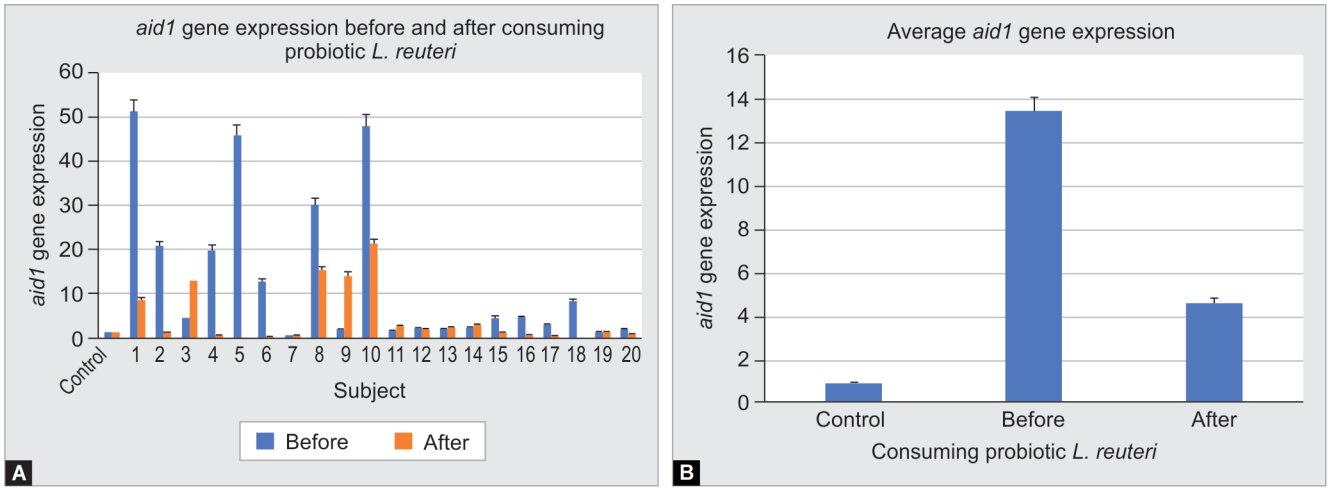
Discussion

The use of fixed orthodontic appliances often causes poor oral hygiene, thus facilitating microorganism accumulation and various pathological conditions in the oral cavity, such as fungal infections.[45] A previous study revealed an increase in *Candida* in saliva after using fixed orthodontic appliances. The authors attributed this to the design of fixed orthodontic appliances, which creates a space for the retention of food waste.[46] Thus, patients must be instructed about good oral hygiene practices after orthodontic treatment.[38] Fixed orthodontic appliances also induce changes in buffer capacity, salivary flow rates, and acidity (pH), leading to plaque accumulation and an increase in caries and periodontal disease.[47–49] Based on this information, we attempted this study to prove the benefits of consuming probiotics lozenges as a supplement for patients using fixed orthodontic appliances.

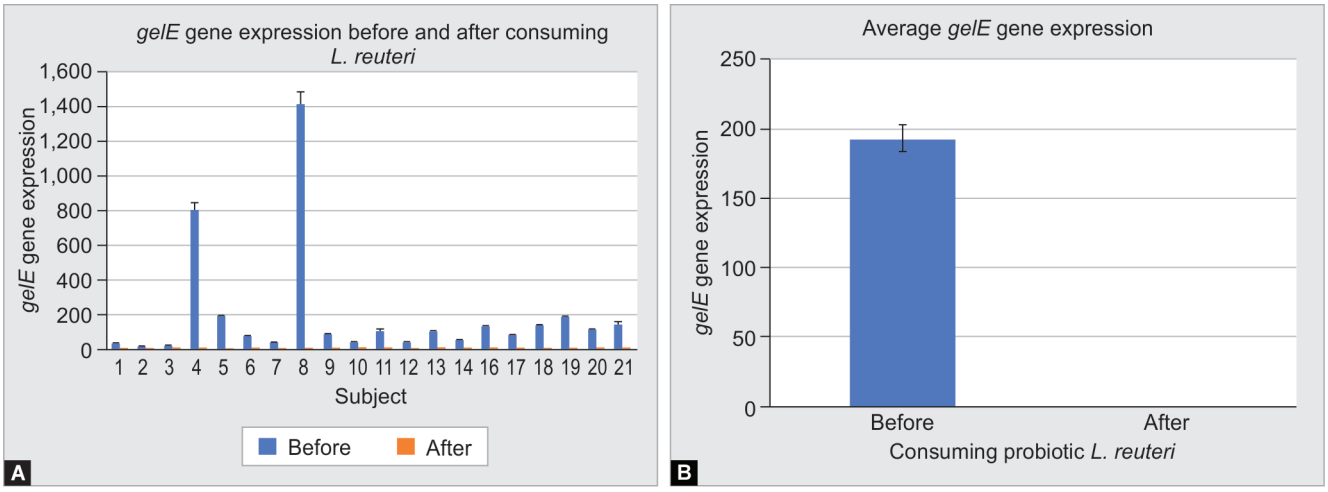
Biofilm formation is an important virulence factor of *F. nucleatum* due to its higher resistance to host defense or antibacterial agents compared to planktonic cells.[50] Several studies detected increased numbers of *Porphyromonas gingivalis, F. nucleatum, P. intermedia,* and *Tannerella forsythia* after the use of fixed orthodontic appliances.[15,51,52] They also reported that *F. nucleatum* increased



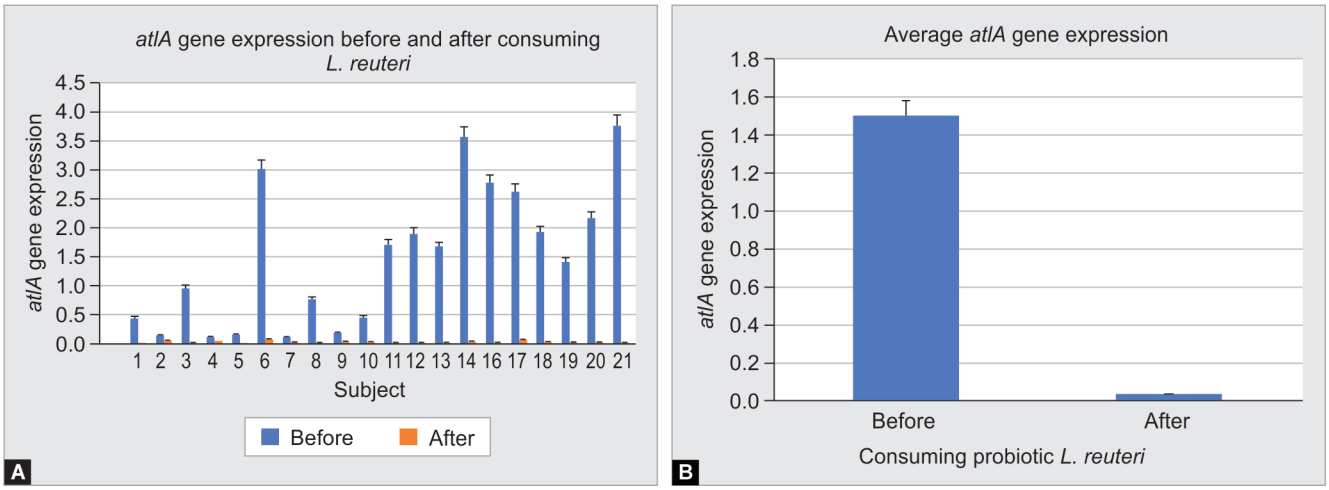
Figs 3A and B: (A) A graph showing *fadA* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *fadA* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 4A and B: (A) A graph showing *aid1* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *aid1* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 5A and B: (A) A graph showing *gelE* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *gelE* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 6A and B: (A) A graph showing *atlA* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *atlA* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

the risk of periodontitis in orthodontic patients due to a conducive environment for anaerobic bacteria.[15,51,52] Biofilm formation is also a contributing factor to *E. faecalis* colonization and infection. Biofilms develop through various processes by which bacteria adhere to surfaces, decompose complex matrices, and develop into bacterial colonies, which adhere to surfaces.[53]

In this study, we used the qPCR method and $2^{-\Delta\Delta CT}$ formula to calculate target gene expression. As shown by the results, the probiotic *L. reuteri* affected the expression of *BCR1, ACE2, fadA*, *aid1*, *gelE*, and *atlA* genes and the formation of *C. albicans, F. nucleatum*, and *E. faecalis* biofilms. Based on the Shapiro–Wilk normality test, all the data were normally distributed, with $p > 0.05$. The paired *t*-test results revealed significant differences in the expression *of BCR1, ACE2, fadA*, *aid1*, *gelE*, and *atlA* genes (all $p < 0.05$).

Based on the results of this study, the probiotic *L. reuteri* significantly downregulated the transcription of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes. In gene expression, the process of translating genetic information in the form of sequence of bases of DNA or RNA into proteins.[54] Through gene expression measurement, it is possible to assess qualitatively and quantitatively the effect of a treatment, such as the administration of a drug compound.[55] Gene expression in microorganisms is involved in regulating cell-cell communication, carbohydrate metabolism, adherence, and adaptation to the surrounding environment. A decrease in the expression of specific genes can reduce microorganism colonization and microorganism numbers.[56]

BCR1, a major gene transcription factor, produces an adhesin protein, which facilitates *C. albicans* attachment to mucosal surfaces, which is a critical stage of infection.[39] Deletion of *BCR1* eliminates *C. albicans* gene function, resulting in a decrease in biofilm formation.[57] This was supported by the results of the present study, which revealed a statistically significant decrease in *BCR1* gene expression after consuming the probiotic.

The expression of *ACE2* also decreased based on the results of the statistical tests. With deletion of *ACE2*, *C. albicans* is unable to form hyphal cells, and thus biofilm formation is inhibited.[30] An earlier in vitro study showed that probiotics have antifungal effects against *C. albicans* in the oral cavity. Regular use of probiotics helped to inhibit *Candida* biofilms and reduced *Candida* colonization in the oral cavity, thereby reducing the possibility of candidiasis infection.[58]

FadA protein is the main *F. nucleatum* virulence factor and mediates microbial attachment and colonization.[8] Based on the results of this study, the probiotic *L. reuteri* appears to influence the pathogenicity of *F. nucleatum* adhesion molecules and colonization and affect biofilm formation through decreased expression of the *fadA* gene.[35] Various *F. nucleatum* adhesins mediate adhesion and aggregation and function as coaggregation intermediaries in the formation and maturation of dental biofilms.[59] The interaction of Fusobacterium with other species is largely mediated by the adhesin genes *radD* and *aid1*.[60] The *aid1* gene plays a role in interspecies interactions, colonization, and aggregation of *F. nucleatum*. In a previous study, inactivation of the *aid1* gene decreased the ability of *F. nucleatum* to aggregate, especially with *Streptococcus* spp. or *E. faecalis*.[14] As shown in earlier studies, probiotics can affect the expression of genes involved in cell adhesion, quorum sensing (QS), virulence factors, and biofilm formation.[61,62]

The *E. faecalis gelE* gene has the ability to hydrolyse gelatine, collagen, fibrin, and other peptides.[63] Gene *gelE* is a virulence factor in infection formation through bacterial attachment and biofilm formation.[22] In *E. faecalis*, biofilm formation is regulated by QS, where Fsr regulates the expression of the *gelE* gene.[64] Fsr regulates the formation of *E. faecalis* biofilms through its product *gelE* and serine proteases.[43] QS is a molecular mechanism by which bacterial cells communicate with each other via signaling molecules in biofilms. If the protease encoded by a signaling factor is decreased, communication between bacteria and biofilm formation will be disrupted.[62] GelE can also activate *atlA*, which is responsible for eDNA release at the biofilm maturation stage.[65]

AtlA is involved in the hydrolysis of peptidoglycan, which plays an important role in separating cells division after replication.[22] AtlA plays a role in the biofilm maturation stage of *E. faecalis*, during which eDNA is released and contributes to biofilm attachment and stability, biofilm defects in primary attachment, and decreased biofilm production.[20,23] This study focused on the probiotic *L. reuteri*, which has ability to secrete antimicrobial substances and compete with oral pathogens for adhesion to mucosa. In addition, *L. reuteri* can adapt and change the pH of the surrounding environment, thereby inhibiting the growth of oral pathogens.[66] The antimicrobial substances secreted by *L. reuteri* are reuterin and

reutericycline.[67] Reuterin and reutericycline are broad-spectrum antimicrobial agents that are effective against Gram-positive and -negative bacteria, fungi, and protozoa by inhibiting microbial DNA synthesis.[35,47]

Many dental and oral health care products used daily now include probiotics. The use of probiotics is increasing due to their advantages over chemical agents, namely reducing the risk of antibiotic resistance.[68] Probiotics work by modulating the immune system, producing antimicrobial substances, and inhibiting certain pathogenic organisms by interfering with adhesion, colonization, and biofilm formation. They inhibit the growth of pathogens via the production of various substances, such as lactic acid and acetic acid, which penetrate the bacterial cell membrane and lower the cytoplasmic pH of pathogenic bacteria. Hydrogen peroxide and bacteriocin can destroy the cell membrane of pathogenic bacteria and inhibit the synthesis of pathogenic DNA.[32,33,35,69] Based on the results of this study, 2 weeks of daily consumption of the probiotic *L. reuteri* affected the process of biofilm formation by downregulating the expression of *BCR1*, *ACE2, fadA, aid1, gelE,* and *atlA* genes, which function as adherent regulators and regulators of hyphae formation in biofilm formation. Many previous studies demonstrated that the addition of probiotics to dental and oral health care products. Probiotic can reduce pathogenic microorganisms in plaque samples from patients using fixed orthodontic appliances.[47,68,70–72] Therefore, it can be stated that the probiotic *L. reuteri* has good ability as an additional treatment for dental and oral health in patients using fixed orthodontic appliances.

The limitation of this study is the small sample size and the fact that the sampling had to be done during COVID-19 pandemic. Although the sample size is small, the result of this study can still provide concise and significant result. In the future, there should be larger sample size for conducting this research so the results can more accurately represent the actual population. Daily consumption of probiotic lozenges duration can also be increased for more precise and accurate results. On the other hand, consumption of probiotic lozenges research can also be conducted for other aspect of oral health diseases to promote the functionality and health-inducing aspect of probiotic lozenges, such as antiinflammation.

24

Conclusion

Within the limitation of this study, it can be concluded that the probiotic *L. reuteri* influences the expression of *BCR1*, *ACE2*, *fadA, aid1, gelE,* and *atlA* genes in biofilm formation. By reducing the expression of those genes, the probiotic *L. reuteri* can reduce biofilm formation, such as dental plaque, in patients using fixed orthodontic appliances.

Clinical Significance

Consumption of probiotic lozenges were confirmed to reduce bacterial and fungal biofilm, as proven by the reduction of virulence gene expression, hence, helping increasing oral health of consumer. The results of this study help clinicians provide probiotic lozenges for patients to promote and maintain their oral health.

References

1. Sihotang BM, Panjaitan M, et al. Perbedaan oral hygiene pemakaian pesawat ortodonti cekat dan pesawat ortodonti lepasan pada pasien klinik spesialis RS Herna. J Prima Medika Sains 2015;2(1):1–5. DOI: 10.34012/jpms.v2i1.840.
2. Gkantidis N, Christou P, Topouzelis N. The orthodontic-periodontic interrelationship in integrated treatment challenges: A systematic review. J Oral Rehabil 2010;37(5):377–390. DOI: 10.1111/j.1365-2842.2010.02068.x.
3. Oktaviani V, Santoso O. Perbedaan Indeks Higiene Oral dan pH Plak Kelompok Pemakai dan Bukan Pemakai Pesawat Ortodonti Cekat 2016;5(1):8.
4. Bowen WH, Burne RA, Wu H, et al. Oral biofilms: pathogens, matrix and polymicrobial interactions in microenvironments. Trends Microbiol 2018;26(3):229–242. DOI: 10.1016/j.tim.2017.09.008.
5. Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm. Virulence 2011;2(5):435–444. DOI: 10.4161/viru.2.5.16140.
6. Verrusio C, Iorio-Siciliano V, Blasi A, et al. The effect of orthodontic treatment on periodontal tissue inflammation: A systematic review. Quintessence Int 2018;49(1):69–77. DOI: 10.3290/j.qi.a39225.
7. Perkowski K, Baltaza W, Conn DB, et al. Examination of oral biofilm microbiota in patients using fixed orthodontic appliances in order to prevent risk factors for health complications. Ann Agric Environ Med 2019;26(2):231–235. DOI: 10.26444/aaem/105797.
8. Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol 2015;23:141–147. DOI: 10.1016/j.mib.2014.11.013.
9. Rickard AH, Gilbert P, High NJ, et al. Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends Microbiol 2003;11(2):94–100. DOI: 10.1016/s0966-842x(02)00034-3.
10. Thurnheer T, Karygianni L, Flury M, et al. Fusobacterium species and subspecies differentially affect the composition and architecture of supra- and subgingival biofilms models. Front Microbiol 2019;10:1716. DOI: 10.3389/fmicb.2019.01716.
11. Chew J, Zilm PS, Fuss JM, et al. A proteomic investigation of Fusobacterium nucleatum alkaline-induced biofilms. BMC Microbiol 2012;12(1):189. DOI: 10.1186/1471-2180-12-189.
12. Kaplan CW, Lux R, Haake SK, et al. The Fusobacterium nucleatum outer membrane protein RadD is an arginine-inhibitable adhesin required for inter-species adherence and the structured architecture of multi-species biofilm. Mol Microbiol 2009;71(1):35–47. DOI: 10.1111/j.1365-2958.2008.06503.x.
13. Cochrane K, Robinson AV, Holt RA, et al. A survey of Fusobacterium nucleatum genes modulated by host cell infection. Microb Genom 2020;6(2):e000300. DOI: 10.1099/mgen.0.000300.
14. Kaplan A, Kaplan CW, He X, et al. Characterization of aid1, a novel gene involved in Fusobacterium nucleatum interspecies interactions. Microb Ecol 2014;68(2):379–387. DOI: 10.1007/s00248-014-0400-y.
15. Liu P, Liu Y, Wang J, et al. Detection of Fusobacterium nucleatum and fadA adhesin gene in patients with orthodontic gingivitis and non-orthodontic periodontal inflammation. PLOS One 2014;9(1):e85280. DOI: 10.1371/journal.pone.0085280.
16. Brennan CA, Garrett WS. Fusobacterium nucleatum – symbiont, opportunist and oncobacterium. Nat Rev Microbiol 2019;17(3):156–166. DOI: 10.1038/s41579-018-0129-6.
17. Wu T, Cen L, Kaplan C, et al. Cellular components mediating coadherence of Candida albicans and Fusobacterium nucleatum. J Dent Res 2015;94(10):1432–1438. DOI: 10.1177/0022034515593706.
18. Wang Q-Q, Zhang C-F, Chu C-H, et al. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci 2012;4(1):19–23. DOI: 10.1038/ijos.2012.17.
19. Najafi K, Gholizadeh P, Tanomand A, et al. Oral cavity infection by Enterococcus faecalis: Virulence factors and pathogenesis. Rev Med Microbiol 2019;30(2). DOI: 10.1097/MRM.0000000000000168.
20. Ch'ng J-H, Chong KKL, Lam LN, et al. Biofilm-associated infection by enterococci. Nat Rev Microbiol 2019;17(2):82–94. DOI: 10.1038/s41579-018-0107-z.
21. Anderson AC, Jonas D, Huber I, et al. Enterococcus faecalis from food, clinical specimens, and oral sites: prevalence of virulence factors in association with biofilm formation. Front Microbiol 2016;6:1534. DOI: 10.3389/fmicb.2015.01534.
22. Stinemetz EK, Gao P, Pinkston KL, et al. Processing of the major autolysin of E. faecalis, AtlA, by the zinc-metalloprotease, GelE,

impacts AtlA septal localization and cell separation. PLoS One 2017;12(10):e0186706.
23. Guiton PS, Hung CS, Kline KA, et al. Contribution of autolysin and sortase A during Enterococcus faecalis DNA-dependent biofilm development. Infect Immun 2009;77(9):3626–3638. DOI: 10.1128/IAI.00219-09.
24. Paganelli FL, Willems RJL, Jansen P, et al. Enterococcus faecium biofilm formation: identification of major autolysin AtlAEfm, associated Acm surface localization, and AtlAEfm-independent extracellular DNA release. mBio 2013;4(2):e00154. DOI: 10.1128/mBio.00154-13.
25. Putri MH, Herijulianti E. Ilmu Pencegahan Penyakit Jaringan Keras dan Jaringan Pendukung Gigi | OPAC Integrasi | Online Public Access Catalog | Universitas Gadjah Mada. RGC; 2010.
26. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev 2007;20(1):133–163. DOI: 10.1128/CMR.00029-06.
27. Garcia MC, Lee JT, Ramsook CB, et al. A role for amyloid in cell aggregation and biofilm formation. PLoS One 2011;6(3):e17632. DOI: 10.1371/journal.pone.0017632.
28. Nobile CJ, Mitchell AP. Regulation of cell-surface genes and biofilm formation by the C. albicans transcription factor Bcr1p. Curr Biol 2005;15(12):1150–1155. DOI: 10.1016/j.cub.2005.05.047.
29. Mulhern SM, Logue ME, Butler G. Candida albicans transcription factor Ace2 regulates metabolism and is required for filamentation in hypoxic conditions. Eukaryot Cell 2006;5(12):2001–2013. DOI: 10.1128/EC.00155-06.
30. Saputo S, Kumar A, Krysan DJ. Efg1 directly regulates ACE2 expression to mediate cross talk between the cAMP/PKA and RAM pathways during Candida albicans morphogenesis. Eukaryot Cell 2014;13(9):1169–1180. DOI: 10.1128/EC.00148-14.
31. Widyarman AS, Bachtiar EW, Bachtiar BM, et al. Inhibitory effect of probiotic lactobacilli against Streptococcus mutans and Porphyromonas gingivalis biofilms. Sci Dental J 2019;3(2):50. DOI: 10.4103/SDJ.SDJ_8_19.
32. Widyarman AS, Ramajayanti T, Theodorea CF. Indonesian strain of probiotic reduces the initial biofilm colonization. The Open Dent J 2020;14(1). DOI: 10.2174/1874210602014010544.
33. Widyarman AS, Theodorea CF. Novel indigenous probiotic Lactobacillus reuteri strain produces anti-biofilm reuterin against pathogenic periodontal bacteria. Eur J Dent 2021;16(1):96–101. DOI: 10.1055/s-0041-1731591.
34. Mu Q, Tavella VJ, Luo XM. Role of Lactobacillus reuteri in human health and diseases. Front Microbiol 2018;9:757. DOI: 10.3389/fmicb.2018.00757.
35. Sari Widyarman A, Kiky Elysia Lazaroni N. Persistent endodontics pathogens biofilm inhibited by Lactobacillus reuteri Indonesian strain. J Dent Indones 2019;26(3). Available from: https://scholarhub.ui.ac.id/jdi/vol26/iss3/7/.
36. Collado MC, Meriluoto J, Salminen S. Adhesion and aggregation properties of probiotic and pathogen strains. Eur Food Res Technol 2008;226(5):1065–1073. DOI: 10.1007/s00217-007-0632-x.
37. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. Pharm Stat 2005;4:287–291. DOI: 10.1002/pst.185.
38. Johanson GA, Brooks GP. Initial scale development: Sample size for pilot studies. Educ Psychol Meas 2010;70:394–400. DOI: 10.1177/0013164409355692.
39. Nikoomanesh F, Roudbarmohammadi S, Roudbary M, et al. Investigation of bcr1 gene expression in Candida albicans isolates by RT-PCR technique and its impact on biofilm formation. iem 2016;2(1):22–24. DOI: 10.7508/iem.2016.01.007.
40. Ranjith K, Kalyana Chakravarthy S, Adicherla H, et al. Temporal expression of genes in biofilm-forming ocular *Candida albicans* isolated from patients with keratitis and orbital cellulitis. Invest Ophthalmol Vis Sci 2018;59(1):528–538. DOI: 10.1167/iovs.17-22933.
41. Nailis H, Coenye T, Van Nieuwerburgh F, et al. Development and evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. BMC Mol Biol 2006;7:25. DOI: 10.1186/1471-2199-7-25.
42. Vos M, Quince C, Pijl AS, et al. A Comparison of rpoB and 16S rRNA as markers in pyrosequencing studies of bacterial diversity. PLoS One 2012;7(2):e30600. DOI: 10.1371/journal.pone.0030600.
43. Wang L, Dong M, Zheng J, et al. Relationship of biofilm formation and gelE gene expression in Enterococcus faecalis recovered from root canals in patients requiring endodontic retreatment. J Endod 2011;37(5):631–636. DOI: 10.1016/j.joen.2011.02.006.
44. Zheng J-X, Wu Y, Lin Z-W, et al. Characteristics of and virulence factors associated with biofilm formation in clinical Enterococcus faecalis isolates in China. Front Microbiol 2017;8:2338. DOI: 10.3389/fmicb.2017.02338.
45. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. J Oral Microbiol 2011;3. DOI: 10.3402/jom.v3i0.5771.
46. Khanpayeh E, Jafari AA, Tabatabaei Z. Comparison of salivary Candida profile in patients with fixed and removable orthodontic appliances therapy. Iran J Microbiol 2014;6(4):263–268. PMID: 25802710.
47. Widyarman AS, Hartono V, Marjani LI, et al. Lactobacillus reuteri containing probiotic Lozenges consumption reduces Streptococcus mutans, Streptococcus sobrinus, Porphyromonas gingivalis, and Aggregatibacter actinomycetemcomitans in orthodontic patients. J Int Dent Med Res 2018;11(2):628–633.
48. Restrepo M, Bussaneli DG, Jeremias F, et al. Control of white spot lesions with use of fluoride varnish or chlorhexidine gel during orthodontic treatment A randomized clinical trial. J Clin Pediatr Dent 2016;40(4):274–280. DOI: 10.17796/1053-4628-40.4.274.
49. Lu H, Tang H, Zhou T, et al. Assessment of the periodontal health status in patients undergoing orthodontic treatment with fixed appliances and Invisalign system. Medicine (Baltimore) 2018;97(13):e0248. DOI: 10.1097/MD.0000000000010248.
50. Fitriasari N, Wahyuni IS. POTENSI PROBIOTIK DALAM TATALAKSANA ORAL CANDIDIASIS: Ulasan Sistematik. Odonto : Dent J 2021;8(1):34–44. DOI: 10.30659/odj.8.1.34-44.
51. Guo L, Feng Y, Guo H-G, et al. Consequences of orthodontic treatment in malocclusion patients: clinical and microbial effects in adults and children. BMC Oral Health 2016;16(1):112. DOI: 10.1186/s12903-016-0308-7.
52. Alqerban A. Efficacy of antimicrobial photodynamic and photobiomodulation therapy against Treponema denticola, fusobacterium nucleatum and human beta defensin-2 levels in patients with gingivitis undergoing fixed orthodontic treatment: a clinic-laboratory study. Photodiagnosis Photodyn Ther 2020;29:101659.
53. Willett JLE, Ji MM, Dunny GM. Exploiting biofilm phenotypes for functional characterization of hypothetical genes in Enterococcus faecalis. NPJ Biofilms Microbiomes 2019;5(1):23. DOI: 10.1038/s41522-019-0099-0.
54. Sinaga E. Regulasi Ekspresi Gen. Regulasi Ekspresi Gen 2011;1–24.
55. Van Peer G, Mestdagh P, Vandesompele J. Accurate RT-qPCR gene expression analysis on cell culture lysates. Sci Rep 2012;2:222. DOI: 10.1038/srep00222.
56. Shemesh M, Tam A, Steinberg D. Expression of biofilm-associated genes of Streptococcus mutans in response to glucose and sucrose. J Med Microbiol 2007;56(11):1528–1535. DOI: 10.1099/jmm.0.47146-0.
57. Fanning S, Xu W, Solis N, et al. Divergent targets of Candida albicans biofilm regulator Bcr1 in vitro and in vivo. Eukaryot Cell 2012;11(7):896–904. DOI: 10.1128/EC.00103-12.
58. Matsubara VH, Bandara HMHN, Mayer MPA, et al. Probiotics as antifungals in mucosal candidiasis. Clin Infect Dis 2016;62(9):1143–1153. DOI: 10.1093/cid/ciw038.
59. Li Q, Tan L, Wang H, et al. Fusobacterium nucleatum interaction with Pseudomonas aeruginosa induces biofilm-associated antibiotic tolerance via Fusobacterium adhesin A. ACS Infect Dis 2020;6(7):1686–1696. DOI: 10.1021/acsinfecdis.9b00402.
60. Kaplan A. Identification and characterization of a novel lipoprotein in Fusobacterium nucleatum. UCLA; 2015 Available from: https://escholarship.org/uc/item/3r90n33c.
61. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25(4):402–408.

62. Barzegari A, Kheyrolahzadeh K, Hosseiniyan Khatibi SM, et al. The battle of probiotics and their derivatives against biofilms. Infect Drug Resist 2020;13:659–672. DOI: 10.2147/IDR.S232982.
63. Guneser MB, Eldeniz AU. The effect of gelatinase production of Enterococcus faecalis on adhesion to dentin after irrigation with various endodontic irrigants. Acta Biomater Odontol Scand 2016;2(1):144–149. DOI: 10.1080/23337931.2016.1256212.
64. Hashem YA, Amin HM, Essam TM, et al. Biofilm formation in enterococci: Genotype-phenotype correlations and inhibition by vancomycin. Sci Rep 2017;7(1):5733. DOI: 10.1038/s41598-017-05901-0.
65. Ali L, Goraya MU, Arafat Y, et al. Molecular mechanism of quorum-sensing in Enterococcus faecalis: its role in virulence and therapeutic approaches. Int J Mol Sci 2017;18(5):960. DOI: 10.3390/ijms1805 0960.
66. Bonifait L, Chandad F, Grenier D. Probiotics for oral health: myth or reality? J Can Dent Assoc 2009;75(8):585–590. PMID: 19840501
67. Urbańska M, Szajewska H. The efficacy of Lactobacillus reuteri DSM 17938 in infants and children: A review of the current evidence. Eur J Pediatr 2014;173(10):1327–1337. DOI: 10.1007/s00431-014-2328-0.
68. Benic GZ, Farella M, Morgan XC, et al. Oral probiotics reduce halitosis in patients wearing orthodontic braces: A randomized, triple-blind, placebo-controlled trial. J Breath Res 2019;13(3):036010. DOI: 10.1088/1752-7163/ab1c81.
69. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, et al. Probiotic mechanisms of action. Ann Nutr Metab 2012;61(2):160–174. DOI: 10.1159/000342079.
70. Widyarman AS, Yunita ST, Prasetyadi T. Consumption of yogurt containing probiotic *Bifidobacterium lactis* reduces *Streptococcus mutans* in orthodontic patients. Sci Dent J2018;2(1):19–25. DOI: 10.26912/sdj.v2i1.1913.
71. Alp S, Baka ZM. Effects of probiotics on salivary *Streptecoccus mutans* and Lactobacillus levels in orthodontic patients. Am J Orthod Dentofacial Orthop 2018;154(4):517–523. DOI: 10.1016/j.ajodo.2018.01.010.
72. Goyal N, Shamanna PU, Varughese ST, et al. Effects of amine fluoride and probiotic mouthwash on levels of *Porphyromonas gingivalis* in orthodontic patients: a randomized controlled trial. J Indian Soc Periodontol 2019;23(4):339–344. DOI: 10.4103/jisp.jisp_551_18.