CURRENT RESEARCH AND TRENDS IN DENTAL AND MEDICAL TECHNOLOGY

Edited by
Rahmi Amtha, Ferry Sandra, Rosalina Tjandrawinata,
Indrayadi Gunardi and Anggraeny Putri Sekar Palupi

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This book presents cutting-edge research and advancements in the rapidly evolving fields of dental technology and medical sciences. It offers new methodologies, interdisciplinary approaches, and case studies to illustrate real-world applications and scientific achievements in medicine.

It delves into a broad spectrum of topics including digital dentistry, biomaterials, regenerative medicine, diagnostic imaging, public health innovations, and advanced clinical practices. It also offers insights into the integration of technology in improving diagnostic accuracy, treatment outcomes, and healthcare accessibility. Resulting from rigorous research, academic collaboration and a dynamic exchange of ideas, this volume will serve as a reference guide for future innovations in healthcare-related sciences.

This book is intended for researchers, clinicians, educators, students, and policymakers in the fields of dental and medical sciences. It is especially useful for those seeking to stay informed about technological advancements and evidence-based practices in healthcare.

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Current Research and Trends in Dental and Medical Technology

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Preface

It is with great pleasure that we present the proceedings of the **2nd International Conference in Dental Technology and Medical Sciences (ICDenTeMS)**, held on **November 21–22, 2024**, at the **Faculty of Dentistry, Trisakti University**, Jakarta, Indonesia.

With the theme **"Current Research and Trends in Dental and Medical Technology,"** this conference brought together a diverse group of researchers, academics, practitioners, and students to explore the latest innovations, challenges, and breakthroughs in the fields of dental and medical sciences. The hybrid format of this year's conference allowed for broader participation, both onsite and online, reinforcing our commitment to inclusivity and global collaboration.

The contributions compiled in this book reflect the depth and breadth of current research, showcasing original studies, case reports, and literature reviews that span a wide range of topics within dental and medical technology. Each manuscript has undergone a peer-review process, ensuring the quality and relevance of the work presented.

We would like to express our sincere appreciation to all authors, reviewers, speakers, and participants whose enthusiasm and dedication have made this event a success. Special thanks are also extended to the editorial and scientific committees for their tireless efforts in preparing these proceedings.

We hope that the insights shared in this volume will inspire further research and foster ongoing academic collaboration, contributing meaningfully to the advancement of dental and medical technology in Indonesia and beyond.

Jakarta, November 2024
Prof. drg. Rahmi Amtha, MDS, Sp.PM (K), Ph.D.
Chairperson, 2nd ICDenTeMS

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We also sincerely thank all participating educational institutions, both national and international, for their valuable contributions, collaboration, and active involvement in this event. Your participation has greatly enriched the scientific discourse and helped foster a spirit of academic exchange and innovation.

This conference would not have been possible without the collective efforts and dedication of every institution and individual involved. We are truly grateful.

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Current Research and Trends in Dental and Medical Technology – Amtha et al. (Eds)

Attenuation of Streptococcus mutans in orthodontic patients by probiotic

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ABSTRACT: Objective: To determine the effect of consuming *L.reuteri p*robiotic lozenges on *S.mutans* virulence genes (*gbpB*, *gtfB)* in dental biofilms of fixed orthodontic patients. Method: Dental biofilm samples were obtained from 20 subjects before and after consuming $2x10^8$ CFU/ml *L.reuteri* probiotic lozenges (Biogaia, Sweden), once daily for 14 days. RNA was extracted, cDNA was synthesized and subjected to Real-time polymerase chain reaction (RT-PCR) using specific primers for *gbpB* and *gtfB* genes of *S.mutans*. *lepA* gene was used as the housekeeping gene. Statistical analysis was performed using paired t-test with significance level of $p<0.05$. Results: Expression of *gbpB* genes were found to be significantly decreased ($p<0.05$) with fold change before (13.7 ± 17.00) and after (0.46 ± 0.34) consumption, as well as the expression of *gtfB* genes before (4.66 ± 5.59) and after (0.62 ± 0.35) consumption. Conclusion: Probiotic *L. reuteri* consumption may help reduce *S.mutans* virulence genes in dental biofilm of fixed orthodontic patients.

Keywords: Biofilm Formation, Orthodontic, Lactobacillus Reuteri, Probiotic, Streptococcus Mutans

1 INTRODUCTION

White spot lesions (WSLs) are a common and unwanted complication for patients receiving orthodontic treatment.(Toti *et al.* 2022) Multiple studies have shown that WSLs tend to appear within the initial weeks of orthodontic treatment.(Toti *et al.* 2022) Orthodontic appliances create an environment that promotes both qualitative and quantitative alterations in dental biofilms. Specifically fixed orthodontic appliances can obstruct access to the tooth surface, making it more challenging for patients to perform effective mechanical cleaning during orthodontic treatment.(Luengthamchat *et al.* 2022; Thanetchaloempong *et al.* 2022) Significant changes such as high sucrose intake, can shift the balance of bacteria within the dental biofilm, leading to the development of a pathogenic biofilm. Factors like acid production, acid tolerance, and the presence of intracellular and extracellular substances contribute to the cariogenic potential of these dental biofilms.(Luengthamchat *et al.* 2022) Various studies have shown that the population of *Streptococcus mutans* and *Lactobacillus*, increase within the biofilms of patients with orthodontic appliances.(Bozkurt *et al.* 2020; Thanetchaloempong *et al.* 2022) These results were also confirmed by Topaloglu-Ak *et al.* a significant increase in *Streptococcus mutans, Lactobacilli,* and *Candida albicans* has been reported six months after appliance insertion, with a higher prevalence observed in

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patients with fixed appliances in contrast to those with removable appliances.(Santonocito *et al.* 2022)

S. mutans is a gram-positive, cariogenic bacteria that is essential in dental caries.(Lemos *et al.* 2019) *S. mutans* can increase the concentration of saliva containing *Lactobacillus acidophilus* which is a source of acid for enamel demineralization.(Bozkurt *et al.* 2020) These bacteria can produce insoluble extracellular polysaccharide substances, increase adhesion to enamel and lead to biofilm formation on components of orthodontic appliance components. (Bozkurt *et al.* 2020; Thanetchaloempong *et al.* 2022) The expression of *S. mutans* virulence genes can be classified based on genes related to bacterial adhesion (*gbpB* gene), a *Glucan-binding-protein* and *glucosyltransferase*, converts sucrose into glucan which then mediates the adhesion of *S. mutans* to tooth surfaces through the expression of the *gtfB* gene. (Thanetchaloempong *et al.* 2022; Rezaei *et al.* 2023; Luo *et al.* 2024) Due to the formation of pathogenic dental biofilms, patients with fixed orthodontic appliances face an increased risk of developing oral diseases.(Widyarman *et al.* 2022) The most commonly utilized mechanical approach for plaque control is a manual toothbrush, preferably combined with fluoride toothpaste.(Farook *et al.* 2023) Thus, in addition to mechanical oral hygiene practices, supplementary oral hygiene measures are required in orthodontic patients.(Widyarman *et al.* 2022) Probiotics have gained attention for their potential in preventing and treating biofilm-related oral diseases, including caries and periodontal disease.(Widyarman *et al.* 2018)

The probiotic bacterial species, *Lactobacillus reuteri*, has been shown to produce a number of antibacterial compounds. In a previous study, a short-term intervention with probiotic *L. reuteri* helped in maintaining optimal oral health in individuals receiving fixed orthodontic treatment.(Widyarman *et al.* 2022) However, the effect of *L. reuteri* on the expression of *S. mutans* virulence genes in biofilm of patients wearing fixed orthodontic appliances has not been thoroughly studied. Currently, there is no evidence to support the effectiveness of probiotic lozenges on the virulence genes of *S. mutans*. Here, we aimed to evaluate the effect of taking probiotic lozenges containing *L.reuteri* for 14 days on the expression of *S. mutans* virulence genes (*gbpB* and *gtfB*) in the biofilms of fixed orthodontic patients.

2 METHOD

2.1 *Sample collection*

This was an experimental laboratory test study. Dental plaque samples were obtained from previous study consist of 20 patients wearing fixed orthodontic appliances. The study inclusion criteria stipulated that participants need to undergo fixed orthodontic therapy for at least six months, have not consumed probiotics or antibiotics in the three months prior, and over 18 years of age. Patients with hypertension or diabetes, taking systemic medications (including anti-hypertensives, analgesics, hormonal drugs, sedatives, or anti-seizure medications), and individuals with allergies to probiotics were excluded in this study. Patients were instructed to collect dental plaques at RSGMP Universitas Trisakti on the first day before consuming *L. reuteri* probiotic lozenges (concentration: 2 x 10^8 Colony Forming Unit/ml) once daily and after 14 days the patients were recalled for dental plaque sample collection. Ethical clearance for the study was acquired from Ethical Commitee, Faculty of Dentistry, Universitas Trisakti (Approval No.425/S2/KEPK/FKG/10/2020).

2.2 *RNA Extraction, Complimentary DNA (cDNA) Synthesize and cDNA Quantification*

RNA was extracted from dental plaque samples using GENEzol™ reagent (Geneaid, Taiwan) by standard methodology. RNA was subsequently synthesized into cDNA through reverse transcription with ReverTra Ace® qPCR RT Master Mix *with* gDNA Remover

(Toyobo, Japan). cDNA was then quantified by InvitrogenTM Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Massachusetts). The synthesized cDNA was then stored at -20°C.

2.3 *Real-time Polymerase Chain Reaction (RT-PCR)*

A RT-PCR test was carried out using 5 x HOT FIREPol EvaGreen® qPCR Mix Plus (Solis Biodyne, Estonia) in an RT-PCR device (Thermo Fisher Scientific, Massachusetts). The *gbpB* and *gtfB* primer sequences were sourced from Thanetchaloempong *et al.* (Table 1). (Thanetchaloempong *et al.* 2022) The *lepA* gene served as the housekeeping gene.(Do *et al.* 2010) The relative gene expression levels were determined using the $2^{-\Delta\Delta CT}$ equation.

Table 1. Primer sequence.

Gene	Primer Sequence
gpbB	Forward : 5' - CGTGTTTCGGCTATTCGTGAAG -3' Reverse : 5' - TGCTGCTTGATTTTCTTGTTGC- 3'
gtfB	Forward : 5' - AGCAATGCAGCCATCTACAAAT – 3' Reverse : 5' - ACGAACTTTGCCGTTATTGTCA – 3'
lepA (housekeeping gene)	Forward : 5'- CTCTATTATTGCCC- 3' Reverse : 5' – TACATCACCCGTTG – 3'

2.4 *Statistical analysis*

The data obtained were subjected to normality test (Shapiro-Wilk) ($p > 0.05$). For data with a normal distribution, a paired *t*-test was applied. Data considered significant ($p < 0.05$). IBM SPSS statistics software (version 25) were used to perform all statistical analysis. Numerical data are presented in the figures as the mean ± standard error.

3 RESULT

The RT-PCR test of the effect of taking probiotic lozenges containing *L. reuteri* on virulence gene expression (*gbpB* and *gtfB*) showed a reduction of *gbpB* (Figure 1) and *gtfB* (Figure 2) expression after 14 days of consumption. As shown by the results of the Shapiro-Wilk normality tests, all data were normally distributed ($p > 0.05$). Data were then 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 *gbpB* and *gtfB* gene expression before and after the patients consumed *L.reuteri* for 14 days.

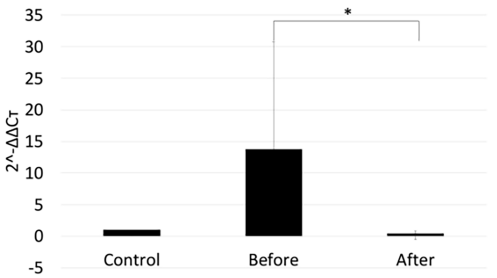

Figure 1. Mean graphs of *gbpB* gene expression before and after consuming *L.reuteri* lozenges based on the $2^{-\Delta\Delta C_T}$ calculation method ($n = 20$). Bars represent the mean ± standart errror of gene expression level relative to that of the expression level before consuming *L.reuteri* lozenges. (*= $p<0.05$)

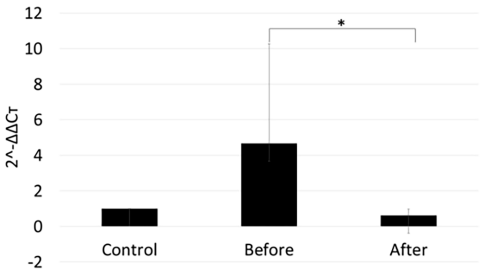

Figure 2. Mean graphs of *gtfB* gene expression before and after consuming *L.reuteri* lozenges based on the $2^{-\Delta\Delta C_T}$ calculation method ($n = 20$). Bars represent the mean ± standart error of gene expression level relative to that of the expression level before consuming *L.reuteri* lozenges. (*= $p<0.05$)

4 DISCUSSION

Increasing public awareness of dental health and the high prevalence of malocclusion have led to an increased demand for orthodontic treatment.(Ratya Utari *et al.* 2019) Fixed orthodontic treatment features components including brackets, wires, springs, that can obstruct access for mechanical cleaning, making it difficult to remove biofilms by brushing teeth and flossing.(Toti *et al.* 2022; Rezaei *et al.* 2023) As a result, WSLs might clinically appear as white patches or lines due to dental biofilms around orthodontic brackets that facilitate rapid enamel demineralization by the action of *S.mutans* or other acidogenic bacterias. (Rajaram *et al.* 2023; Spatafora *et al.* 2024) To maintain optimal oral hygiene, patients with fixed orthodontic appliances need to be instructed to brush their teeth at least twice daily using manual or an electric toothbrush and to replace manual toothbrushes every 3 months.(Aryeetey *et al.* 2024)

Probiotics may aid in the prevention and treatment of oral diseases through various mechanisms by interacting directly with dental plaque.(Pandya 2016) One of them, *Lactobacillus spp*., produces organic acids, hydrogen peroxides, biosurfactants, bacteriocins and adhesion inhibitors. These bacteria have the potential to control caries and prevent invasion by *S.mutans* via various mechanisms including producing antimicrobial compounds, competing with pathogens and modulating the host immune system. (Thanetchaloempong *et al.* 2022) Probiotics can also prevent adhesion by manipulating bacterial virulence gene expression and altering salivary protein composition.(Chew *et al.* 2020)

The present study showed taking L. reuteri probiotic lozenges for 14 days reduced *gbpB* (Figure 1) and *gtfB* (Figure 2) gene expression in plaque biofilms of patients with fixed orthodontic appliances. Primers for *gbpB* and g*tfB* (Table 1) used in this study was obtained from a previous study conducted by Thanetchaloempong *et al.* 2022. Previous study reported that *L.reuteri* strongly inhibited glucosyltransferase in *S.mutans* ATCC 35668 with its biosurfactant content.(Salehi *et al.* 2014) Biosurfactants contribute to biofilm formation, cell motility, and adhesion processes, thereby facilitating microorganisms colony formation. Due to the unique properties of biosurfactants, they can alter the surface tension and physicochemical characteristics of oral health, thereby disrupting microbial adhesion and inhibiting the biofilm formation. Biosurfactants also exhibit antimicrobial properties that directly target the survival and growth of microorganisms and provide an additional barrier to biofilm formation.(Rafael 2023)

In addition to mediating *S. mutans* adhesion, *gtfB* gene expression derived from glucosyltransferase can mediate with other bacteria for persistent colonization on the tooth surface. The *gtf* gene is a well-known virulence factor associated with caries pathogenesis. Thus, g*lucosyltransferases* enabling *S.mutans* to synthesize polysaccharides (intracellular and

extracellular). extracellular polysaccharides or EPS can be divided into water-insoluble glucan which has an essential role in biofilm construction and water-soluble glucan.(Zhang *et al.* 2022) Insoluble glucan in plaque is associated with a higher risk of biofilm formation and caries in the oral cavity.(Salehi *et al.* 2014) Probiotics can inhibit the activity of pathogenic bacteria and their adhesion to surfaces via various mechanisms, such as preventing quorum sensing, disrupting biofilm integrity or quality, and ultimately reducing biofilm formation.(Chew *et al.* 2020)

Decreased expression of the *gpbB* gene affects the primary role of the *gbpB* gene, namely, bacterial adhesion surfaces in the oral cavity. Thus, altering the initial stages of biofilm formation, which involve physiological processes in the transition from planktonic to biofilm.(Duque *et al.* 2011) The outcomes of this study support the presence of decreased transcriptional regulation of the *gbpB* gene. In a previous study, the extracellular amount of *gbpB* gene was found to be correlated with biofilm formation in clinical isolates and to maintain cell walls shape.(Duque *et al.* 2011; Zhang *et al.* 2022)

The results of this study prove that taking lozenges containing *L. reuteri* for 14 days affected formation of *S. mutans* biofilm by regulating the expression of genes that contribute in biofilm formation. Thus, probiotic *L. reuteri* is expected to be an adjuvant therapy in improving oral health. As treatment progresses, patients with fixed orthodontic appliances should be instructed to maintain oral health by brushing teeth at least twice a day. Other additional tools such as dental floss, interdental brushes, and dental water flossers, can be used to achieve optimal oral hygiene. Health practitioners must strive to make patients aware of the need for optimal oral hygiene and motivate them to increase compliance. (Duque *et al.* 2011; Ratya Utari *et al.* 2019)

5 CONCLUSION

These results of this study suggested that taking lozenges containing *L. reuteri* for 14 days affected *S.mutans* biofim in dental plaque of patients with fixed orthodontic appliances by attenuating the expression of *gbpB* and *gtfB* genes. *L. reuteri* may be able to prevent the occurrence of WSLs in patients with fixed orthodontic appliances. However, further studies are warranted to study the specific role of *L. reuteri* in gene expression in dental biofilms and to maximize the intervention protocol. Longitudinal studies of the impact of the consumption *of L.reuteri* on the development of caries are warranted.

AUTHORS' CONTRIBUTION

A.S.W, J.K and D.C planned the study concept. A.S.W collected and prepared the samples. D.C and A.S.W performed measurements and statistical analysis. A.S.W, J.K and D.C wrote the manuscript draft, revised, and approved final version of the full paper.

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Joko Kusnoto FKG

Attenuation of Streptococcus Mutans in Orthodontic Patients by Probiotic

Artikel 1

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CURRENT RESEARCH AND TRENDS IN DENTAL AND MEDICAL TECHNOLOGY

Edited by
Rahmi Amtha, Ferry Sandra, Rosalina Tjandrawinata,
Indrayadi Gunardi and Anggraeny Putri Sekar Palupi

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CURRENT RESEARCH AND TRENDS IN DENTAL AND MEDICAL TECHNOLOGY

This book presents cutting-edge research and advancements in the rapidly evolving fields of dental technology and medical sciences. It offers new methodologies, interdisciplinary approaches, and case studies to illustrate real-world applications and scientific achievements in medicine.

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PROCEEDINGS OF THE SECOND INTERNATIONAL CONFERENCE ON DENTAL TECHNOLOGY AND MEDICAL SCIENCES (ICDENTEMS 2024), JAKARTA, INDONESIA, NOVEMBER 21ST—22ND, 2024

6

Current Research and Trends in Dental and Medical Technology

Edited by

Rahmi Amtha, Ferry Sandra, Rosalina Tjandrawinata, Indrayadi Gunardi and Anggraeny Putri Sekar Palupi
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Preface

It is with great pleasure that we present the proceedings of the **2nd International Conference in Dental Technology and Medical Sciences (ICDenTeMS)**, held on **November 21–22, 2024**, at the **Faculty of Dentistry, Trisakti University**, Jakarta, Indonesia.

With the theme **"Current Research and Trends in Dental and Medical Technology,"** this conference brought together a diverse group of researchers, academics, practitioners, and students to explore the latest innovations, challenges, and breakthroughs in the fields of dental and medical sciences. The hybrid format of this year's conference allowed for broader participation, both onsite and online, reinforcing our commitment to inclusivity and global collaboration.

The contributions compiled in this book reflect the depth and breadth of current research, showcasing original studies, case reports, and literature reviews that span a wide range of topics within dental and medical technology. Each manuscript has undergone a peer-review process, ensuring the quality and relevance of the work presented.

We would like to express our sincere appreciation to all authors, reviewers, speakers, and participants whose enthusiasm and dedication have made this event a success. Special thanks are also extended to the editorial and scientific committees for their tireless efforts in preparing these proceedings.

We hope that the insights shared in this volume will inspire further research and foster ongoing academic collaboration, contributing meaningfully to the advancement of dental and medical technology in Indonesia and beyond.

Jakarta, November 2024
Prof. drg. Rahmi Amtha, MDS, Sp.PM (K), Ph.D.
Chairperson, 2nd ICDenTeMS

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We also sincerely thank all participating educational institutions, both national and international, for their valuable contributions, collaboration, and active involvement in this event. Your participation has greatly enriched the scientific discourse and helped foster a spirit of academic exchange and innovation.

This conference would not have been possible without the collective efforts and dedication of every institution and individual involved. We are truly grateful.

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Session 13: Oral biology and microbiology

Current Research and Trends in Dental and Medical Technology – Amtha et al. (Eds)
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Attenuation of Streptococcus mutans in orthodontic patients by probiotic

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ABSTRACT: Objective: To determine the effect of consuming *L.reuteri* probiotic lozenges on *S.mutans* virulence genes (*gbpB*, *gtfB)* in dental biofilms of fixed orthodontic patients. Method: Dental biofilm samples were obtained from 20 subjects before and after consuming $2x10^8$ CFU/ml *L.reuteri* probiotic lozenges (Biogaia, Sweden), once daily for 14 days. RNA was extracted, cDNA was synthesized and subjected to Real-time polymerase chain reaction (RT-PCR) using specific primers for *gbpB* and *gtfB* genes of *S.mutans*. *lepA* gene was used as the housekeeping gene. Statistical analysis was performed using paired t-test with significance level of $p<0.05$. Results: Expression of *gbpB* genes were found to be significantly decreased ($p<0.05$) with fold change before (13.7 ± 17.00) and after (0.46 ± 0.34) consumption, as well as the expression of *gtfB* genes before (4.66 ± 5.59) and after (0.62 ± 0.35) consumption. Conclusion: Probiotic *L. reuteri* consumption may help reduce *S.mutans* virulence genes in dental biofilm of fixed orthodontic patients.

Keywords: Biofilm Formation, Orthodontic, Lactobacillus Reuteri, Probiotic, Streptococcus Mutans

1 INTRODUCTION

White spot lesions (WSLs) are a common and unwanted complication for patients receiving orthodontic treatment.(Toti *et al.* 2022) Multiple studies have shown that WSLs tend to appear within the initial weeks of orthodontic treatment.(Toti *et al.* 2022) Orthodontic appliances create an environment that promotes both qualitative and quantitative alterations in dental biofilms. Specifically fixed orthodontic appliances can obstruct access to the tooth surface, making it more challenging for patients to perform effective mechanical cleaning during orthodontic treatment.(Luengthamchat *et al.* 2022; Thanetchaloempong *et al.* 2022) Significant changes such as high sucrose intake, can shift the balance of bacteria within the dental biofilm, leading to the development of a pathogenic biofilm. Factors like acid production, acid tolerance, and the presence of intracellular and extracellular substances contribute to the cariogenic potential of these dental biofilms.(Luengthamchat *et al.* 2022) Various studies have shown that the population of *Streptococcus mutans* and *Lactobacillus*, increase within the biofilms of patients with orthodontic appliances.(Bozkurt *et al.* 2020; Thanetchaloempong *et al.* 2022) These results were also confirmed by Topaloglu-Ak *et al.* a significant increase in *Streptococcus mutans, Lactobacilli,* and *Candida albicans* has been reported six months after appliance insertion, with a higher prevalence observed in

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patients with fixed appliances in contrast to those with removable appliances.(Santonocito *et al.* 2022)

S. mutans is a gram-positive, cariogenic bacteria that is essential in dental caries.(Lemos *et al.* 2019) *S. mutans* can increase the concentration of saliva containing *Lactobacillus acidophilus* which is a source of acid for enamel demineralization.(Bozkurt *et al.* 2020) These bacteria can produce insoluble extracellular polysaccharide substances, increase adhesion to enamel and lead to biofilm formation on components of orthodontic appliance components. (Bozkurt *et al.* 2020; Thanetchaloempong *et al.* 2022) The expression of *S. mutans* virulence genes can be classified based on genes related to bacterial adhesion (*gbpB* gene), a *Glucan-binding-protein* and *glucosyltransferase*, converts sucrose into glucan which then mediates the adhesion of *S. mutans* to tooth surfaces through the expression of the *gtfB* gene. (Thanetchaloempong *et al.* 2022; Rezaei *et al.* 2023; Luo *et al.* 2024) Due to the formation of pathogenic dental biofilms, patients with fixed orthodontic appliances face an increased risk of developing oral diseases.(Widyarman *et al.* 2022) The most commonly utilized mechanical approach for plaque control is a manual toothbrush, preferably combined with fluoride toothpaste.(Farook *et al.* 2023) Thus, in addition to mechanical oral hygiene practices, supplementary oral hygiene measures are required in orthodontic patients.(Widyarman *et al.* 2022) Probiotics have gained attention for their potential in preventing and treating biofilm-related oral diseases, including caries and periodontal disease.(Widyarman *et al.* 2018)

The probiotic bacterial species, *Lactobacillus reuteri*, has been shown to produce a number of antibacterial compounds. In a previous study, a short-term intervention with probiotic *L. reuteri* helped in maintaining optimal oral health in individuals receiving fixed orthodontic treatment.(Widyarman *et al.* 2022) However, the effect of *L. reuteri* on the expression of *S. mutans* virulence genes in biofilm of patients wearing fixed orthodontic appliances has not been thoroughly studied. Currently, there is no evidence to support the effectiveness of probiotic lozenges on the virulence genes of *S. mutans*. Here, we aimed to evaluate the effect of taking probiotic lozenges containing *L.reuteri* for 14 days on the expression of *S. mutans* virulence genes (*gbpB* and *gtfB*) in the biofilms of fixed orthodontic patients.

2 METHOD

2.1 *Sample collection*

This was an experimental laboratory test study. Dental plaque samples were obtained from previous study consist of 20 patients wearing fixed orthodontic appliances. The study inclusion criteria stipulated that participants need to undergo fixed orthodontic therapy for at least six months, have not consumed probiotics or antibiotics in the three months prior, and over 18 years of age. Patients with hypertension or diabetes, taking systemic medications (including anti-hypertensives, analgesics, hormonal drugs, sedatives, or anti-seizure medications), and individuals with allergies to probiotics were excluded in this study. Patients were instructed to collect dental plaques at RSGMP Universitas Trisakti on the first day before consuming *L. reuteri* probiotic lozenges (concentration: 2 x 10^8 Colony Forming Unit/ml) once daily and after 14 days the patients were recalled for dental plaque sample collection. Ethical clearance for the study was acquired from Ethical Commitee, Faculty of Dentistry, Universitas Trisakti (Approval No.425/S2/KEPK/FKG/10/2020).

2.2 *RNA Extraction, Complimentary DNA (cDNA) Synthesize and cDNA Quantification*

RNA was extracted from dental plaque samples using GENEzol™ reagent (Geneaid, Taiwan) by standard methodology. RNA was subsequently synthesized into cDNA through reverse transcription with ReverTra Ace® qPCR RT Master Mix *with* gDNA Remover

(Toyobo, Japan). cDNA was then quantified by InvitrogenTM Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Massachusetts). The synthesized cDNA was then stored at -20°C.

2.3 *Real-time Polymerase Chain Reaction (RT-PCR)*

A RT-PCR test was carried out using 5 x HOT FIREPol EvaGreen® qPCR Mix Plus (Solis Biodyne, Estonia) in an RT-PCR device (Thermo Fisher Scientific, Massachusetts). The *gbpB* and *gtfB* primer sequences were sourced from Thanetchaloempong *et al.* (Table 1). (Thanetchaloempong *et al.* 2022) The *lepA* gene served as the housekeeping gene.(Do *et al.* 2010) The relative gene expression levels were determined using the $2^{-\Delta\Delta CT}$ equation.

Table 1. Primer sequence.

Gene	Primer Sequence
gpbB	Forward : 5' - CGTGTTTCGGCTATTCGTGAAG -3' Reverse : 5' - TGCTGCTTGATTTTCTTGTTGC- 3'
gtfB	Forward : 5' - AGCAATGCAGCCATCTACAAAT – 3' Reverse : 5' - ACGAACTTTGCCGTTATTGTCA – 3'
lepA (housekeeping gene)	Forward : 5'- CTCTATTATTGCCC- 3' Reverse : 5' – TACATCACCCGTTG – 3'

2.4 *Statistical analysis*

The data obtained were subjected to normality test (Shapiro-Wilk) ($p > 0.05$). For data with a normal distribution, a paired *t*-test was applied. Data considered significant ($p < 0.05$). IBM SPSS statistics software (version 25) were used to perform all statistical analysis. Numerical data are presented in the figures as the mean ± standard error.

3 RESULT

The RT-PCR test of the effect of taking probiotic lozenges containing *L. reuteri* on virulence gene expression (*gbpB* and *gtfB*) showed a reduction of *gbpB* (Figure 1) and *gtfB* (Figure 2) expression after 14 days of consumption. As shown by the results of the Shapiro-Wilk normality tests, all data were normally distributed ($p > 0.05$). Data were then 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 *gbpB* and *gtfB* gene expression before and after the patients consumed *L.reuteri* for 14 days.

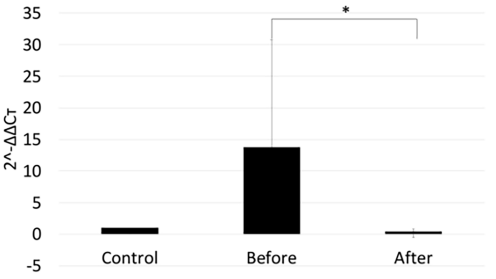

Figure 1. Mean graphs of *gbpB* gene expression before and after consuming *L.reuteri* lozenges based on the $2^{-\Delta\Delta C_T}$ calculation method ($n = 20$). Bars represent the mean ± standart errror of gene expression level relative to that of the expression level before consuming *L.reuteri* lozenges. (*= $p<0.05$)

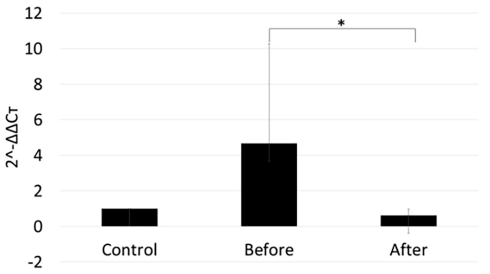

Figure 2. Mean graphs of *gtfB* gene expression before and after consuming *L.reuteri* lozenges based on the $2^{-\Delta\Delta C_T}$ calculation method (n = 20). Bars represent the mean ± standart error of gene expression level relative to that of the expression level before consuming *L.reuteri* lozenges. (*= $p<0.05$)

4 DISCUSSION

Increasing public awareness of dental health and the high prevalence of malocclusion have led to an increased demand for orthodontic treatment.(Ratya Utari *et al.* 2019) Fixed orthodontic treatment features components including brackets, wires, springs, that can obstruct access for mechanical cleaning, making it difficult to remove biofilms by brushing teeth and flossing.(Toti *et al.* 2022; Rezaei *et al.* 2023) As a result, WSLs might clinically appear as white patches or lines due to dental biofilms around orthodontic brackets that facilitate rapid enamel demineralization by the action of *S.mutans* or other acidogenic bacterias. (Rajaram *et al.* 2023; Spatafora *et al.* 2024) To maintain optimal oral hygiene, patients with fixed orthodontic appliances need to be instructed to brush their teeth at least twice daily using manual or an electric toothbrush and to replace manual toothbrushes every 3 months.(Aryeetey *et al.* 2024)

Probiotics may aid in the prevention and treatment of oral diseases through various mechanisms by interacting directly with dental plaque.(Pandya 2016) One of them, *Lactobacillus spp*., produces organic acids, hydrogen peroxides, biosurfactants, bacteriocins and adhesion inhibitors. These bacteria have the potential to control caries and prevent invasion by *S.mutans* via various mechanisms including producing antimicrobial compounds, competing with pathogens and modulating the host immune system. (Thanetchaloempong *et al.* 2022) Probiotics can also prevent adhesion by manipulating bacterial virulence gene expression and altering salivary protein composition.(Chew *et al.* 2020)

The present study showed taking L. reuteri probiotic lozenges for 14 days reduced *gbpB* (Figure 1) and *gtfB* (Figure 2) gene expression in plaque biofilms of patients with fixed orthodontic appliances. Primers for *gbpB* and g*tfB* (Table 1) used in this study was obtained from a previous study conducted by Thanetchaloempong *et al.* 2022. Previous study reported that *L.reuteri* strongly inhibited glucosyltransferase in *S.mutans* ATCC 35668 with its biosurfactant content.(Salehi *et al.* 2014) Biosurfactants contribute to biofilm formation, cell motility, and adhesion processes, thereby facilitating microorganisms colony formation. Due to the unique properties of biosurfactants, they can alter the surface tension and physicochemical characteristics of oral health, thereby disrupting microbial adhesion and inhibiting the biofilm formation. Biosurfactants also exhibit antimicrobial properties that directly target the survival and growth of microorganisms and provide an additional barrier to biofilm formation.(Rafael 2023)

In addition to mediating *S. mutans* adhesion, *gtfB* gene expression derived from glucosyltransferase can mediate with other bacteria for persistent colonization on the tooth surface. The *gtf* gene is a well-known virulence factor associated with caries pathogenesis. Thus, g*lucosyltransferases* enabling *S.mutans* to synthesize polysaccharides (intracellular and

extracellular). extracellular polysaccharides or EPS can be divided into water-insoluble glucan which has an essential role in biofilm construction and water-soluble glucan.(Zhang *et al.* 2022) Insoluble glucan in plaque is associated with a higher risk of biofilm formation and caries in the oral cavity.(Salehi *et al.* 2014) Probiotics can inhibit the activity of pathogenic bacteria and their adhesion to surfaces via various mechanisms, such as preventing quorum sensing, disrupting biofilm integrity or quality, and ultimately reducing biofilm formation.(Chew *et al.* 2020)

Decreased expression of the *gpbB* gene affects the primary role of the *gbpB* gene, namely, bacterial adhesion surfaces in the oral cavity. Thus, altering the initial stages of biofilm formation, which involve physiological processes in the transition from planktonic to biofilm.(Duque *et al.* 2011) The outcomes of this study support the presence of decreased transcriptional regulation of the *gbpB* gene. In a previous study, the extracellular amount of *gbpB* gene was found to be correlated with biofilm formation in clinical isolates and to maintain cell walls shape.(Duque *et al.* 2011; Zhang *et al.* 2022)

The results of this study prove that taking lozenges containing *L. reuteri* for 14 days affected formation of *S. mutans* biofilm by regulating the expression of genes that contribute in biofilm formation. Thus, probiotic *L. reuteri* is expected to be an adjuvant therapy in improving oral health. As treatment progresses, patients with fixed orthodontic appliances should be instructed to maintain oral health by brushing teeth at least twice a day. Other additional tools such as dental floss, interdental brushes, and dental water flossers, can be used to achieve optimal oral hygiene. Health practitioners must strive to make patients aware of the need for optimal oral hygiene and motivate them to increase compliance. (Duque *et al.* 2011; Ratya Utari *et al.* 2019)

5 CONCLUSION

These results of this study suggested that taking lozenges containing *L. reuteri* for 14 days affected *S.mutans* biofim in dental plaque of patients with fixed orthodontic appliances by attenuating the expression of *gbpB* and *gtfB* genes. *L. reuteri* may be able to prevent the occurrence of WSLs in patients with fixed orthodontic appliances. However, further studies are warranted to study the specific role of *L. reuteri* in gene expression in dental biofilms and to maximize the intervention protocol. Longitudinal studies of the impact of the consumption *of L.reuteri* on the development of caries are warranted.

AUTHORS' CONTRIBUTION

A.S.W, J.K and D.C planned the study concept. A.S.W collected and prepared the samples. D.C and A.S.W performed measurements and statistical analysis. A.S.W, J.K and D.C wrote the manuscript draft, revised, and approved final version of the full paper.

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Author index

UNIVERSITAS TRISAKTI

Joko Kusnoto <joko.k@trisakti.ac.id>

ICDenTeMS 2024 : Please Validate Your Email

1 message

ICDenTeMS 2024 <automail@interconf.org> Thu, Aug 1, 2024 at 9:32 PM
Reply-To: icdentems@trisakti.ac.id
To: joko.k@trisakti.ac.id

Dear Dr. drg. Joko Kusnoto,

Thank you for your registration to ICDenTeMS 2024,
please open (copy-paste to browser) the following link to validate your email:

https://interconf.org/2024/icdentems/kfz/pages/activate.php?q=VbYtWTS7R

After email validation, your login code will be sent by our human admin, so please just wait and be patient.
Please also check your SPAM/JUNK email folder.

Thank you.
Best regards,

ICDenTeMS 2024 Organizing Committee
Website : https://scientific-event.fkg.trisakti.ac.id
Email : icdentems@trisakti.ac.id

---
https://konfrenzi.com | Web Systems for Scientific Conferences

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Joko Kusnoto <joko.k@trisakti.ac.id>

ICDenTeMS 2024 : Your Registration has been Approved

1 message

ICDenTeMS 2024 <automail@interconf.org> Fri, Aug 2, 2024 at 8:12 AM
Reply-To: icdentems@trisakti.ac.id
To: joko.k@trisakti.ac.id

Dear Dr. drg. Joko Kusnoto,

Your Registration has been Approved.
User ID: USER-74
Please use this "User ID" in all correspondence (instead of your name).

Login Link : https://interconf.org/2024/icdentems/kfz
Login Email: joko.k@trisakti.ac.id
Login Code : nBfEvusTwp

You need the "Login Code" to login to our site, so please do not delete this email.
Login to submit your abstract and paper.

Please also join the official WhatsApp group of ICDenTeMS 2024 at
https://chat.whatsapp.com/FSYTXvvvUDS3O5DX67Djmz

Thank you.
Best regards,

ICDenTeMS 2024 Organizing Committee
Website : https://scientific-event.fkg.trisakti.ac.id
Email : icdentems@trisakti.ac.id

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Joko Kusnoto <joko.k@trisakti.ac.id>

Fullpaper Revision

1 message

International Conference FKG <icdentems@trisakti.ac.id> Mon, Aug 26, 2024 at 5:28 PM
Bcc: joko.k@trisakti.ac.id

Dear Author
The full paper review process is complete, please check your konfrenzi account to make revisions and upload the latest file.

Steps:
1. Login to your konfrenzi account
2. Select the abstract menu
3. Scroll down to the bottom of the page
4. Check reviewer status
5. Upload the revised paper based on the reviewer's comments.
6. Re-upload the revised paper, even though without revision from reviewers.
7. The deadline for submitting revised full papers is September 5, 2024.

Regards Chairperson,
Prof. Dr. drg. Rahmi Amtha, MDS., Sp.PM., Ph.D.
ICDenTeMS 2024

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Joko Kusnoto <joko.k@trisakti.ac.id>

ICDenTeMS 2024 Important Update

1 message

International Conference FKG <icdentems@trisakti.ac.id> Tue, Oct 1, 2024 at 9:15 PM
Bcc: joko.k@trisakti.ac.id

Dear Presenter,
There are several things that need to be considered regarding ICDenTeMS full paper presentation:

1. The presentation will be held online via Zoom Meeting.
2. Presentations must be recorded through the zoom meeting application and the video link of the presentation must be submitted by November 5, 2024.
3. Presenters are required to be present in the breakout room 15 minutes before the scheduled presentation, during the presentation session, and the Q&A session.
4. The video recording will be shown according to the schedule set by the committee. Each participant's presentation video will be shown for a maximum of 10 minutes, ending with a Q & A session of a maximum of 5 minutes.

Presentation Submission Guidelines

1. Please click the link to the presentation submission https://scientific-event.fkg.trisakti.ac.id/icdentems-2024/presentation-submission/ . Need to login first.
2. Please fill all fields in the presentation submission registration form. Make sure the video presentation link is valid and fully accessible to the committee, then click submit.

Presentation Recording Guidelines

1. Powerpoint template and Virtual Background has been provided by the committee and are required to be used.
2. Recording can be done through the Zoom meeting application.
3. Maximum recording duration is 10 minute.
4. Slides and Presentation are delivered in English.
5. During the presentation, the presenter’s face is clearly visible.
6. Follow all the steps below to record your presentation:
   a. Choose share screen
   b. Click on advanced
   c. Choose Slides as background
   d. Click share
   e. Drag the Presenter to the top right
   f. The Participant box is minimized
   g. Start record

Need help? contact https://wa.me/6282210179741

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Joko Kusnoto <joko.k@trisakti.ac.id>

Schedule for a short lecture 2nd-ICDenTeMS presentation

1 message

International Conference FKG <icdentems@trisakti.ac.id> Fri, Nov 15, 2024 at 10:25 AM
Bcc: joko.k@trisakti.ac.id

Dear Presenter,

There is a schedule for a short lecture 2nd-ICDenTeMS presentation. The schedule will be divided into two days, **Thursday and Friday, November 21 and 22, 2024.** The presentation will be held after the main lecture in two sessions every day, **03.00-04.00 pm and 04.00-05.00 pm**.

Please enter the Zoom meeting at least fifteen minutes before the presentation session time through this link:
https://trisakti-ac-id.zoom.us/j/91060445041?pwd=EtFYHB9GjyYaFzslOm3qiiDbbNMxkc.1
Meeting ID : 910 6044 5041
Passcode : fkgusakti

After entering the Zoom meeting, please select the break-out room according to the schedule, and please change the name with presenter name _ presenter code, for example, Goalbertus _ 1111. Thanks for your attention.

Yours sincerely,
ICDenTeMS 2024 Committee

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ICDenTeMS Short Lecture Schedule.pdf
178K

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Joko Kusnoto <joko.k@trisakti.ac.id>

Proceedings ICDenTeMS 2024

2 messages

icdentems@trisakti.ac.id <icdentems@trisakti.ac.id> Mon, Mar 10, 2025 at 7:36 AM
To: joko.k@trisakti.ac.id

Dear Professors and Esteemed Colleagues,

Joko Kusnoto
Paper Code ABS 64
Faculty of Dentistry, Universitas Trisakti
Title Manuscript : Lactobacillus reuteri Attenuates Streptococcus mutans Virulence Gene Expression in Fixed Orthodontic Patients

Continuing the ICDenTeMS scientific activities, we have reached the stage of publishing papers for Proceedings ICDenTeMS 2024, and collaborating with Taylor & Francis for the publication.

Here we attached the files “The Consent to Publish Form” that need to be filled in and should be signed by the first author of each paper and submitted to us no later than Tuesday, March 11, 2025, by 11.59 PM.

Consent to Publish: https://docs.google.com/document/d/1RknbUMYkhgj35UJm75eCqpBi8X8stQmp/edit?usp=sharing&ouid=101834932524334827043&rtpof=true&sd=true

We greatly appreciate your participation toward the success of ICDenTeMS 2024 proceedings.

With sincere thanks and best regards,
Scientific Committee
2nd ICDenTeMS, Faculty of Dentistry
Universitas Trisakti

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Joko Kusnoto <joko.k@trisakti.ac.id> Mon, Mar 10, 2025 at 12:05 PM
To: icdentems@trisakti.ac.id

Dear ICDenTeMS committee,

Herewith is the signed Consent to Publish Form of our manuscript (paper code ABS 64). Thank you for your kind attention.

Sincerely,
Joko Kusnoto

[Quoted text hidden]

ICDenTeMS 2024_CtP (ABS 64).docx
148K

UNIVERSITAS TRISAKTI

Joko Kusnoto <joko.k@trisakti.ac.id>

Perbaikan Manuskrip Sesuai Template Taylor & Francis

2 messages

icdentems@trisakti.ac.id <icdentems@trisakti.ac.id> Wed, Apr 9, 2025 at 9:52 PM
To: joko.k@trisakti.ac.id

Ysh, Penulis ICDenTeMS Proceeding 2024

Kode : ABS 64
Joko Kusnoto
No Telp : 628159965447
Institusi: Faculty of Dentistry, Universitas Trisakti
Judul Manuskrip: Lactobacillus reuteri Attenuates Streptococcus mutans Virulence Gene Expression in Fixed Orthodontic Patients

Berkaitan dengan proses review oleh Pihak Publisher (Taylor & Francis), dibutuhkan beberapa perbaikan naskah manuskrip.

Berikut kami kirimkan file yang dibutuhkan untuk perbaikan manuskrip, mohon dapat dipelajari dan melakukan perbaikan manuskrip sesuai petunjuk

Agar perbaikan dapat selesai tepat waktu, penulis akan mendapatkan 1 (satu) orang pendamping yang akan membantu proses perbaikan , para penulis dipersilahkan menghubungi melalui email atau whatsapp.

PIC : Eddy
Email : eddydrg@trisakti.ac.id
No telp: 082122621475

Perbaikan manuskrip kami tunggu paling lambat tanggal 23 April 2025 pukul 23.59 wib

Kami akan memberikan penjelasan mengenai tatacara perbaikan manuskrip secara online yang akan dilaksanakan pada :

Hari/Tanggal : Kamis/10 April 2025
Pukul : 21.00 wib

Link zoom :
https://trisakti-ac-id.zoom.us/j/93836890490?pwd=gAwD8S9aIynbXWX0kFNsTWZrWIbeTC.1

Meeting ID: 938 3689 0490
Passcode: 463794

Paper : https://docs.google.com/document/d/1Pf-COdRo5VbDczEQs9MC4K5qp0cQv1Qu/edit?usp=drive_link&ouid=103168639191755896898&rtpof=true&sd=true
Petunjuk Bagi Penulis Makalah : https://docs.google.com/document/d/1O-MAwJiVg-QVXRpe-FaSqVqU0fDL7KLY/edit?usp=drive_link&ouid=103168639191755896898&rtpof=true&sd=true
Original Guideline of Paper's Template: https://docs.google.com/document/d/1Pbis-fVLa8moL4yM5RrBGwZckqLvQZFH/edit?usp=drive_link&ouid=103168639191755896898&rtpof=true&sd=true
Contoh Manuskrip Sesuai Guideline: https://docs.google.com/document/d/1kGwwhCrRt1FHHuzI3UfywPhTsG2LMY_3/edit?usp=drive_link&ouid=103168639191755896898&rtpof=true&sd=true
Permission Verification Form : https://docs.google.com/document/d/1wa-rO5h90YQMI3Lm-27vJNmMEN3Iy5By/edit?usp=drive_link&ouid=103168639191755896898&rtpof=true&sd=true
Artwork: https://drive.google.com/file/d/1_wIk5RFGG8QLX1UFUS47r2VbED0vj1F1/view?usp=sharing
Permission Flowchart: https://drive.google.com/file/d/1giByB_52GK9s36VGV5uCtYGxwbwyrbb4/view?usp=drive_link

Atas perhatian dan kerjasamanya diucapkan terimakasih

Salam,
Panitia ICDenTeMS 2024

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Joko Kusnoto <joko.k@trisakti.ac.id> Wed, Apr 23, 2025 at 8:35 PM
To: icdentems@trisakti.ac.id

Yth Panitia ICDenTeMS 2024,

Bersama ini kami sampaikan revisi manuskrip kami dengan kode ABS 64 dan judul Attenuation of Streptococcus mutans in Orthodontic Patients by Probiotic, serta juga Permission Verification Form dari tim penulis kami. Semoga sudah memenuhi persyaratan untuk diterbitkan pada proceeding ICDenTeMS 2024. Atas perhatiannya kami ucapkan terima kasih.

Hormat kami,
Deandra Carissa Wiriawan, Joko Kusnoto, Armelia Sari Widyarman

[Quoted text hidden]

---

2 attachments

Final-Checked Manuscript ABS-064-Rev_Deandra.docx
139K

Permission Verification Form_Deandra.doc
330K

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Joko Kusnoto <joko.k@trisakti.ac.id>

PAPER CODE 064

2 messages

icdentems@trisakti.ac.id <icdentems@trisakti.ac.id> Wed, Jun 4, 2025 at 8:31 AM
To: joko.k@trisakti.ac.id

Dear D.C. Wiriawan
Postgraduate Student, Faculty of Dentistry, Universitas Trisakti, Indonesia

Berikut kami kirimkan kembali paper untuk dapat direvisi sesuai dengan comment yang kami sisipkan di dalam paper. Jika ada yg belum jelas, bisa langsung menghubungi Dr.Indrayadi (082114727167)

Setelah revisi, paper dapat dikirimkan dengan cara me- REPLY email ini, terimakasih atas bantuan dan kerjasamanya

Best Regards,
Panitia ICDenTeMS 2024

https://docs.google.com/document/d/1WSk3GwaW0aXuq20wjjlqnzE35HZ8cWvh/edit?usp=drive_link&ouid=103168639191755896898&rtpof=true&sd=true

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Joko Kusnoto <joko.k@trisakti.ac.id> Thu, Jun 19, 2025 at 1:18 AM
To: icdentems@trisakti.ac.id

Yth Panitia ICDenTeMS 2024,

Bersama ini kami kirimkan kembali manuskrip kami (ABS 064) yang telah kami revisi (judul file ABS 064R - REVISED). Semoga panitia ICDenTeMS 2024 berkenan dengan revisi yang telah kami lakukan. Atas kerjasamanya kami ucapkan terima kasih.

Hormat kami,
D.C. Wiriawan, J. Kusnoto, dan A.S. Widyarman
[Quoted text hidden]

ABS 064R - REVISED.docx
69K