Probiotics Vs. Bacteria: How Lactobacillus Casei Battles Streptococcus Mutans Biofilm (In Vitro)

*Rahmawati Sri Praptiningsih**, Rina Kartika Sari***, Rama Putranto****, Renanda Rifki Ikhsandarujati**

** Faculty of Dentistry, Sultan Agung Islamic University*
*** Department of Oral Biology, Faculty of Dentistry, Sultan Agung Islamic University*
**** Department of Oral medicine, Faculty of Dentistry, Sultan Agung Islamic University*
***** Department of Orthodontics, Faculty of Dentistry, Sultan Agung Islamic University*

***Correspondence*: rahmawati@unissula.ac.id**

Received 27 December 2024; Accepted 15 January 2025; Published online 20 February 2025

Keywords:

Biofilm Thickness; Probiotics Lactobacillus casei; Streptococcus mutans.

ABSTRACT

Background:*Caries formation is influenced by several factors such as teeth, microorganisms, biofilm, and also time. The initial bacteria that play an important role in biofilm formation is Streptococcus mutans. Probiotics made from these bacteria are considered capable of overcoming dental and oral health problems because they are able to suppress the biofilm which will coat and protect the mucosa from invasion by cariogenic bacteria such as caries. To determine the effect of the probioticLactobacillus casei in reducing Streptococcus mutans biofilm growth (in vitro).*
Method:*The research design carried out was experimental research with a post test only control group design. The research samples were divided into 3 groups, namely the chlorhexidine, distilled water and probiotic groups. Biofilm thickness testing was carried out by OD (Optical density) using an ELISA-reader. Parametric statistical test analysis using the Kruskal Wallis Test.*
Results:*The results of the Kruskal Wallis statistical test showed P= 0.000 there is a significant difference in the 3 Streptococcus mutans thickness test groups.*
Conclusion:*The probioticLactobacillus casei has the effect of reducing the thickness of the Streptococcus mutans biofilm*

DOI: http://dx.doi.org/10.30659/medali.7.1.12-21
2337-6937/ 2460-4151 ©2025 National Research and Innovation Agency

How to Cite: *Praptiningsih et al*. Probiotics Vs. Bacteria: How Lactobacillus Casei Battles Streptococcus Mutans Biofilm (In Vitro). MEDALI Jurnal: Media Dental Intelektual, v.7, n.1, p.12-21, February 2025.

INTRODUCTION

Dental caries is a dental disorder that often occurs in society. Based on the 2023 Riskesdas, 56.9% of the population aged over 3 years experienced dental and oral health problems, and only11.2% of them went to the doctor for treatment. This needs serious attention because there has been no significant change in the last 5 years when compared to the results of the 2018 Riskesdas. The 2023 Riskesdas also stated that Indonesian people experience dental and oral health problems with a caries prevalence of 43.6%(RISKESDA, 2023). Dental caries occurs due to a process of demineralization of the tooth structure caused by microorganisms in a colony called a biofilm. Microorganisms produce acid which forms cavities in the tooth structure, namely on the surface of enamel, dentin, or cementum.(Sibarani,2014). They can live and reproduce in an acidic environment and adhere to the tooth surface. Streptococcus mutans is one of the microorganisms responsible for the formation of dental caries.(Komansilan et al., 2015).

The cause of tooth decay begins with the presence of sucrose and bacteria that stick to the surface of the teeth and then form a thin layer in the form of a biofilm.(Listrianah et al., 2019). Biofilm is a thin layer that occurs due to colonization of bacteria and is coated by an extracellular polysaccharide matrix that supports bacterial survival. Streptococcus mutans is the main colonizer of caries formation because it is cariogenic. This bacteria is acidogenic, resistant to low pH, and also stores polysaccharide reserves such as glycogen.(Fina Maghfirah, Dewi Saputri, 2017).

The right way to reduce biofilm accumulation on the tooth surface is by mechanical means or toothbrush and chemically using mouthwash.(Karyadi & Roza, 2021). If only using a toothbrush, the fissure, buccal pit and interproximal gingival margin area cannot be reached with a toothbrush alone, then it can be helped by using mouthwash to clean the parts that cannot be reached with a toothbrush (Karyadi, 2020). Research by(Kumar et al., 2013)said that Chlorhexidine (CHX) is the 'gold standard' mouthwash and is most widely used to help clean areas that cannot be reached with a toothbrush. Long-term use of chlorhexidine can cause stains on teeth, taste disorders, ulceration in the oral cavity, and also swelling of the parotid glands both unilaterally and bilaterally(Kumar et al., 2013). The use of chlorhexidine also has side effects such as discoloration of the teeth, changes in tooth color, sensations, and unpleasant tastes.(Kumar et al., 2013).

From this, an alternative is needed that can be used to reduce microorganisms that cause caries and hard tissue diseases in teeth, namely by using probiotics. Probiotics are a collection of microorganisms that when given in sufficient quantities will provide benefits to the host and are able to fight infections.(Fina Maghfirah, Dewi Saputri, 2017). Probiotics made from bacteria are considered capable of overcoming dental and oral health problems such as caries, gingivitis, periodontitis, and halitosis.(Sugiman, 2014). One example of probiotic bacteria is Lactobacillus cassei which is found in fermented milk drinks, namely yakult and yogurt.

Study in (Lindawati&Simanjuntak,2020) showed that chlorhexidine and probiotic mouthwash lactobacillus casei have antimicrobial effects on plaque accumulation of Streptococcus mutans. This study explains that chlorhexidine, sodium fluoride, and probiotic mouthwash reduce the levels of Streptococcus mutans. Probiotic mouthwash is effective and equivalent to chlorhexidine and sodium fluoride mouthwash, but chlorhexidine mouthwash has some side effects on

dental and oral health.(Lindawati & Simanjuntak, 2020). Other research by(Fina Maghfirah, Dewi Saputri, 2017)also explains the formation of Streptococcus mutans and Candida albicans biofilm mass given probiotic drinks containing Lactobacillus casei proven to show the thickness of Streptococcus mutans biofilm decreasing, on the other hand this study is still uncertain which bacteria can reduce the number of Streptococcus mutans colonies among the bacteria in yakult. So it is necessary to conduct research on the effect of one of the bacteria in yakult. Therefore, in this study the author chose to try to use Lactobacillus casei bacteria in reducing the growth of Streptococcus mutans biofilm (in vitro).

Considering this background, the author wanted to conduct a study to determine the effect of probiotic Lactobacillus casei in reducing the growth of Streptococcus mutans biofilm.

In Islamic teachings, Allah SWT teaches us to maintain cleanliness, as according to the following hadith was narrated from Sa'ad bin Abi Waqas, from his father, that Rasulullah SAW said: "Indeed, Allah SWT is Most Holy and loves purity, He is Most Clean and loves cleanliness, He is Most Noble and loves glory, and He is Most Beautiful and loves beauty. Therefore, clean your places". Based on this hadith, we can conclude that Allah SWT encourages us to maintain our health and cleanliness, both spiritually and physically, because Allah SWT loves people who maintain their health and cleanliness, and cleanliness is part of faith.

RESEARCH METHODS

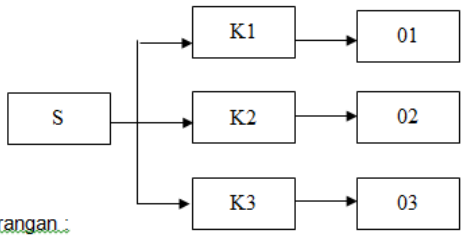
This study used a laboratory experimental design with a posttest-only control group design, involving three treatment groups: probiotic Lactobacillus casei (K1), 0.2% chlorhexidine as a positive control (K2), and aquades as a negative control (K3).

The independent variable in this study was the administration of Lactobacillus casei probiotics, while the dependent variable was the thickness of Streptococcus mutans biofilm. Operational definitions include measuring biofilm thickness using the microtiter plate biofilm assay method with reading the results using an ELISA Reader at a wavelength of 620 nm.

The research process includes preparation of tools and materials, biofilm making, and formation of probiotic and test bacterial suspensions. Sterilization of tools was carried out using an autoclave at a temperature of 121°C with a pressure of 2 atm for 15 minutes. Streptococcus mutans biofilm was cultured in TYS20B media under anaerobic conditions for 48 hours at a temperature of 37°C. Lactobacillus casei probiotics were cultured using MRS Broth media supplemented with pure milk and incubated for 48 hours at the same temperature. Chlorhexidine 0.2% and distilled water were prepared as controls.

This study involved taking saliva from respondents using the spitting out method after stimulation with xylitol chewing gum. Respondents aged 18-25 years were selected based on inclusion and exclusion criteria, and were asked to sign an

Secara skematis rancangan penelitian dapat ditabelkan sebagai berikut :



Keterangan :

- S = subjek
- K1 = kelompok 1 (probiotik *Lactobacillus casei*)
- K2 = kelompok 2 (chlorhexidine)
- K3 = kelompok 3 (akuades)
- 01 = hasil kelompok 01
- 02 = hasil kelompok 02
- 03 = hasil kelompok 03

informed consent. The study procedure involved mixing Streptococcus mutans solution and saliva in a well plate, administering test solutions (probiotics, chlorhexidine, or aquadest), incubation, staining using gentian violet crystals, and reading OD results using an ELISA Reader.

Data processing was carried out through normality tests (Shapiro-Wilk) and homogeneity (Levene's Test). If the data is normally distributed and homogeneous, parametric statistical analysis was performed using the One Way ANOVA test. Otherwise, the nonparametric Kruskal-Wallis test was used. Data analysis was performed using SPSS software.

This study was conducted in September and October 2024 at the Microbiology and Biology Laboratory of the Faculty of Medicine, Sultan Agung Islamic University, Semarang. Ethical clearance for this study was obtained from the Research Ethics Committee of the Faculty of Dentistry, Sultan Agung Islamic University. The study was conducted in accordance with the procedures and ethical principles of scientific research.

RESEARCH RESULT

This study was conducted to determine the effect of giving probiotics Lactobacillus casei, 0.2% chlorhexidine, and distilled water on the thickness of Streptococcus mutans biofilm. Biofilm making is done by culturing Streptococcus mutans bacteria assisted by pure patient saliva, the function of saliva here is used to help stimulate the formation of biofilm so that the biofilm can form and stick to the well plate wall. After that, three control group influences will be given, namely Lactobacillus casei, 0.2% chlorhexidine, and distilled water, then reading and measuring the thickness of the biofilm will be carried out using an ELISA-reader (Indriyanti et al, 2019)**.**

The results of the Streptococcus mutans biofilm thickness test show the average value of the number of Streptococcus mutans with 3 treatment groups given Lactobacillus casei probiotics, aquades, and the treatment group given 0.2% chlorhexidine in table 1.

Table1 Mean and standard deviation of thickness*Streptococcus mutans*

Group	N	Mean	Standard Deviation
Probiotics	9	0.07515	± 0.001771
Chlorhexidine	9	0.07726	± 0.002881
Aquadest	9	0.09332	± 0.007246

The lowest average thickness of Streptococcus mutans biofilm was in the Lactobacillus casei control group, while the highest thickness of Streptococcus mutans biofilm was in the aquadest control group.

The data was tested using the Shapiro-Wilk test to determine its normality and also tested using the Lavene Statistic test to determine its homogeneity. The results of the normality test and homogeneity test can be seen in table 2.

Table2 Normality Test and Homogeneity Test

Group	Normality		Homogeneity	
Probiotics	0.178	Data Normal	0.028	Non-Homogeneous Data
Chlorhexidine	0.726			
Aquadest	0.077			

Based on table 2 shows the results of the normality test all data are normally distributed ($p \geq 0.05$). The data was then tested for homogeneity using the Levene Statistic test. The homogeneity test in table 2, obtained a value of 0.028 ($p < 0.05$) which indicates that the thickness data of Streptococcus mutans is not homogeneous.

Based on these data, it is known that the data is normally distributed and not homogeneous, so the next stage is the Kruskal Wallis parametric test to determine the difference in the thickness of the Streptococcus mutans biofilm in each group, then continued with the Mann Whitney post-hoc test.

Table 3 Test*Post-hoc Mann Whitney and Kruskal Wallis Tests*

	Mann Whitney Post-hoc Test		Kruskal Wallis Test
Chlorhexidine-casein	0.342	There is no significant difference	
Chlorhexidine-aquades	0,000	There is a significant difference	0,000
casei-Aquades	0,000	There is a significant difference	

(a) **(b)** **(c)**

The results of the significance number in the Kruskal Wallis Test shown in the table are 0.000 ($p < 0.05$) so it can be concluded that there is a significant difference between the chlorhexidine, aquades, and probiotic groups on the absorbance value of Streptococcus mutans biofilm. To find out which group has the most significant difference, a Mann Whitney post-hoc test was conducted. In the Mann Whitney post-hoc test above, it can be concluded that there is a significant difference between the chlorhexidine-aquades and casei-aquades groups, while chlorhexidine-casei has no significant difference on the absorbance value of Streptococcus mutans biofilm with a p value> 0.05.

Based on the results of the statistical analysis above, it can be said that there is a significant difference between the administration of Lactobacillus casei, Chlorhexidine, and aquades on the thickness of Streptococcus mutans biofilm.

DISCUSSION

The results of the study showed that there was a significant difference between the administration of Lactobacillus casei bacteria, 0.2% chlorhexidine, and distilled water on the average optical density of the thickness of Streptococcus mutans biofilm. Table 4.1 shows the lowest average

value of Streptococcus mutans biofilm thickness in the Lactobacillus casei control group, while the highest Streptococcus mutans biofilm thickness was in the distilled water control group. Based on research fromFahruddin et al. (2019), explains that the lower the optical density value, the lower the thickness of the Streptococcus mutans biofilm formed.

Further post hoc Mann Whitney test showed that there was no significant difference in the thickness of mutans biofilm between Lactobacillus casei bacteria and 0.2% chlorhexidine. This is because both control groups can influence each other in inhibiting the thickness of s. mutans biofilm. In the analysis of the results of this study, it was found that casei was proven to be more able to reduce the thickness of s. mutans biofilm compared to chlorhexidine, but on the other hand, it should be remembered that this study only used s. mutans biofilm and L. casei probiotics here are only effective as an alternative to reduce microorganisms that cause caries, not as a substitute for chlorhexidine. Because between chlorhexidine and L. casei probiotics there are differences in mechanisms and how to use them is also different. Chlorhexidine works by disrupting the bacterial cell membrane and inactivating important proteins in cells, while probiotics function more to balance the microbiota and prevent the growth of pathogens than by directly destroying existing biofilms.(Widani et al., 2015).

In the Mann Whitney post hoc analysis test, it also showed that there was a significant difference between L. casei and chlorhexidine against aquades. Based on research from Anatje et al, (2022) it was stated that aquades as a negative control did not form a clear zone in the well. This clear zone or bacterial inhibition zone is a clear area that forms around the well of bacterial growth media that is not overgrown with bacteria. This is because aquades does not have antibacterial properties. The reason for using aquades is as a comparison to see whether or not there is an effect of the solvent on bacterial growth. Unlike L. casei and chlorhexidine which can inhibit biofilm growth, L. cassei with its protective mechanism of action while chlorhexidine with its antibacterial properties(Aritonang, N et al., 2019). Simple use of distilled water can still have an effect on maintaining dental and oral health because one of the main benefits of distilled water or plain water is its ability to help maintain pH balance in the mouth (Anatje et al, 2022). These three control groups, namely L. casei, Chlorhexidine, and distilled water, have their own characteristics, meaning that in the three control groups of this study they have different functions, methods of use, times of use, and mechanisms of action.

Biofilm growth can be inhibited by using a probiotic solution of Lactobacillus casei which produces organic acids which are bacteriostatic against Streptococcus mutans biofilms.(Jothika et al. 2015). This study shows that probiotic Lactobacillus casei is effective in reducing the thickness of Streptococcus mutans biofilm. This occurs because the content of probiotic lactobacillus casei produces compounds that can inhibit the growth of Streptococcus mutans. Probiotics produce antagonistic substances such asbiosurfactants, bacteriocins, exopolysaccharides (EPS), organic acids (lactic acid, acetic acid, propionate and succinate), hydrogen peroxide which can inhibit the activity of pathogenic bacteria and their adhesion to the surface and the formation of unfavorable environmental conditions for pathogenic bacteria such as decreased pH and nutrient competition*(Barzegari et al.,*2020). The mechanism of probiotics by producing biosurfactants will disrupt biofilms because the adsorption of biosurfactants to the substrate can

disrupt the adhesion and desorption processes of microbes. Biosurfactants have bactericidal behavior by disrupting the cytoplasmic membrane of pathogens or disrupting biofilm proteins, causing cell lysis, and metabolite leakage. Biosurfactants can disrupt the cell division process and affect the expression of biofilm-related genes, thereby disrupting the release of signaling molecules in the quorum sensing (QS) system and subsequent biofilm formation.(Carvalho et al., 2021).

Chlorhexidine 0.2% solution was used as a positive control because it has bacteriostatic and bactericidal effects against various types of microbes. Chlorhexidine works effectively in inhibiting and killing Gram-positive and Gram-negative bacteria because its molecules are positively charged (cations), while bacteria have a negative charge (anion)(Pratiwi et al., 2022). This molecular interaction allows chlorhexidine to penetrate bacterial cells and form a strong bond with their cell membranes. As a result, the permeability of the bacterial cytoplasmic membrane is disrupted, allowing intracellular compounds to exit the cell. This damage renders the bacterial cell dysfunctional, allowing chlorhexidine to penetrate the entire plaque surface more effectively.(Panesa et al., 2018).

*Chlorhexidine*and probiotic Lactobacillus casei can be used to control bacterial biofilms, but with different mechanisms. Chlorhexidine works by disrupting the bacterial cell membrane and inactivating important proteins in the cell, and has the ability to penetrate the biofilm and kill bacteria, making it more effective in reducing biofilms.(Sinaredi et al., 2014)Meanwhile, probiotics work by competing with pathogenic bacteria for resources and adhesion sites, and can produce antimicrobial substances.(Aritonang, N et al., 2019).

The limitation of this study is that it did not use other types of bacteria, so it is less similar and less representative of the environment in the oral cavity.

CONCLUSION

Based on the results of this study it can be concluded that's the probiotic Lactobacillus casei has been shown to have an effect on the thickness of Streptococcus mutans biofilm and the lowest thickness of Streptococcus mutans biofilm was in the influence of the Lactobacillus casei control group, while the highest thickness of Streptococcus mutans biofilm was in the influence of the aquadest control group. There was a significant difference between the chlorhexidine-aquades and casei-aquades groups, whereas chlorhexidine-casei did not show a significant difference in the thickness of the Streptococcus mutans biofilm.

We would like to express our gratitude to Allah SWT for His grace, so that this research can be completed properly. We would like to express our gratitude to Dr. drg. Yayun Siti Rohmah, Sp.BM, Dean of the Faculty of Dentistry, Sultan Agung Islamic University, Semarang, as well as to the first supervisor, Dr. drg. Hj. Rahmawati Sri Praptiningsih, M.MedEd, and the second supervisor, drg. Rina Kartika Sari, Sp.PM, who have provided guidance, support, and valuable input in the preparation of this paper. We would also like to thank the Microbiology Laboratory of the Faculty of Dentistry, UNISSULA, which has facilitated this research. We would also like to express our gratitude to our family, friends, and colleagues at the Faculty of Dentistry who have provided encouragement, prayers, and motivation. Hopefully, this work will be useful for the development of science, especially in the field of dentistry.

REFERENCE

1. RISKESDA. SKI 2023. 2023;1–68.
2. Sibarani M. Dental Caries: Etiology, Clinical Characteristics, and Management. Maj Kedokt UKI. 2014;30(1):14–22.
3. Komansilan JG, Mintjelungan CN, Waworuntu O. INHIBITORY POWER OF MANGOSTEEN (Garcinia Mangostana L.) PEEL EXTRACT AGAINST Streptococcus mutans. e-GIGI. 2015;3(2).
4. Listrianah L, Zainur RA, Hisata LS. Description of First Permanent Molar Caries in Elementary School Students 13 Palembang in 2018. JPP (Health Journal of Palembang Health Polytechnic). 2019;13(2):136–49.
5. Fina Maghfirah, Dewi Saputri B. Biofilm Formation Activity of Streptococcus Mutans and Candida Albicans After Exposure to Cigarette Smoke Condensate and Probiotic Drink. J Caninus Denstistry Vol 2, Number 1 (February 2017) 12 - 19 Act. 2017;01(February):1–23.
6. Karyadi E, Roza MA. The Effect of Chewing Manalagi Apples on Reducing Plaque Index in Children Aged 9-12 Years. JIKG (Journal of Dentistry). 2021;3(2).
7. Kumar M, Nagpal R, Verma V, Kumar A, Kaur N, Hemalatha R, et al. Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr Rev. 2013;71(1):23–34.
8. Sugiman drg. VK. Benefits of Yogurt Acidity in Preventing Dental Caries. Maranatha Christian University. 2014. p. 107–8.
9. Lindawati Y, Simanjuntak DV. Differences in pH values and number of Streptococcus species colonies before and after consuming probiotic drinks. Padjadjaran J Dent Res Students October. 2020;4(2):116–20.
10. Lee SH, Kim YJ. A comparative study of the effect of probiotics on cariogenic biofilm models for preventing dental caries. Arch Microbiol. 2014;196(8):601–9.
11. Popova M, Molimard P, Courau S, Crociani J, Dufour C, Le Vacon F, et al. Beneficial effects of probiotics in upper respiratory tract infections and their mechanical actions to antagonize pathogens. J Appl Microbiol. 2012;113(6):1305–18.
12. Shah SS, Nambiar S, Kamath D, Suman E, Unnikrishnan B, Desai A, et al. Comparative Evaluation of Plaque Inhibitory and Antimicrobial Efficacy of Probiotic and Chlorhexidine Oral Rinses in Orthodontic Patients: A Randomized Clinical Trial. Int J Dent. Hindawi; 2019;2019:1964158.
13. Pujoraharjo P, Medicine D, Children G, Dental K, Herdiyati Y. Effectiveness of herbal plant antibacterial against streptococcus mutans in children's caries. Indones J Paediatr Dent. 2018;1(1):51–6.
14. Gartika M, Satari MH. Several Natural Ingredients as Alternative Caries Prevention Materials. 2014;(1):63.
15. Doychinova M, Kussovski V, Tonchev T, Dimitrov S. Photodynamic inactivation of human dental biofilm Isolated Streptococcus Mutans With 2 Photosensitizers - an In Vitro Study. Scr Sci Medica. 2015;47(1):32.
16. Maulana I, Hasanah AU, Tyas R, Rizkita AD. Effectiveness Test of Mouthwash Preparations from Ethanol Extract of Prasman Leaves (Eupatorium Triplinerve Vahl) Against Streptococcus Mutans. J Kesehat Mahardika. 2022;9(1):28–34.
17. Warganegara E, Restina D. Jatropha curcas L. Sap as the Growth of Streptococcus mutans Inhibitor in Dental Caries. :1–6.
18. Rahayu YC. The Role of Remineralization Agents in Early Caries Lesions. Stomatognatic (JKG Unej). 2013;10(1):25–30.
19. Bebe ZA, Susanto HS, Martini. Risk Factors for Dental Caries in Adults Aged 20-39 Years in Dadapsari Village, North Semarang District, Semarang City. J Public Health. 2018;6(1):2356–3346.
20. Napitupulu RLY, Adhani R, Erlita I. The Relationship between Tooth Brushing Behavior, Water Acidity, Dental Health Services and Caries in Man 2 Batola. Dentin J Dentistry. 2019;III(1):17–22.
21. Wahyuni S, Bikarindrasari R, Fauziah MN. The Effect Of Isotonic Solution Immersion On Tooth Enamel Hardness After Topical Application Of CPP-ACPF And fTCP. J Dental Health. 2022;9(1):42–6.
22. Listrianah. Dental caries index reviewed from general diseases and saliva secretion in children at State Elementary School 30 Palembang 2017. JPP (Palembang Health Journal). 2017;12(2):136–48.
23. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The Biology

of Streptococcus mutans. Microbiol Spectr. 2019;7(1):1–26.
24. Prasasti CA, G BT, Hasibuan SY, Hutagalung MH., Molek M. Comparison of Bacang Mango Leaf Extract with Papaya Leaf Extract in Inhibiting the Growth of Streptococcus Mutans. J Ilm Kesehat Sandi Husada. 2021;10(1):235–40.
25. Djais AA, Putri N, Jemmy, Putri AR, Darwita RR, Bachtiar BM. Effect of propolis on Streptococcus mutans biofilm formation. Pesqui Bras Odontopediatria Clin Integr. 2019;19(1).
26. Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. The virulence of Streptococcus mutans and the ability to form biofilms. Eur J Clin Microbiol Infect Dis. 2014;33(4):499–515.
27. Sunaryanto R, Martius E, Marwoto B. TEST OF THE ABILITY OF Lactobacillus casei AS A PROBIOTIC AGENT. J Bioteknol Biosains Indones. 2014;1(1):9.
28. Zawistowska-Rojek A, Tyski S. Are probiotics really safe for humans? Polish J Microbiol. 2018;67(3):251–8.
29. Markowiak P, Ślizewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 2017;9(9).
30. Aritonang, NS, Roza E, Rossi E. Probiotics and Prebiotics From Soybeans for Functional Foods. World Gastroenterology Global Guidelines. 2019. 1-18 p.
31. Pramanik S, Venkatraman S, Karthik P, Vaidyanathan VK. A systematic review on selection characterization and implementation of probiotics in human health. Food Sci Biotechnol. Springer Nature Singapore; 2023;32(4):423–40.
32. Montero E, Iniesta M, Roldán S, Herrera D. Probiotics and oral health. Probiotics Child. 2016;2(1):135–60.
33. Suseno P, Surjoseputro S, Indarto Suseno TP, Anita and K. Probiotic Drink Niia Siwalan (Thomas lndarto Lactobacillus casei On Several Pathogenic Bacteria). J Technol Food and Nutrition. 2020;1(1):1–13.
34. Ilyas M, Phielip C. Yogurt consumption reduces the number of cariogenic bacterial colonies in saliva in adolescents. Makassar Dent J. 2018;1(1):1.
35. Hamsar A, Ramadhan ES. The Use of Chlorhexidine Mouthwash in Improving the Dental Hygiene Index of Employees of the Indonesian Ministry of Health Polytechnic of Medan. J Dental Health. 2019;6(2):99–103.
36. Faradiba A, Gunadi A, Praharani D. Antibacterial Power of Tamarind Leaf Infusion (Tamarindus indica Linn) against Streptococcus mutans. e-Journal of Health Library. 2016;4(1):55–60.
37. Luh Widani N, Nasution Y. Comparison of Oral Care Using Povidone Iodine 1% with Chlorhexidine 0.2% on the Number of Bacteria in the Mouth in Patients with Decreased Consciousness. J Nursing Padjadjaran. 2015;v3(n3):185–92.
38. Kuntari LM, Hadriyanto W, Mulyawati E. Differences in antibacterial power of 2% chlorhexidine and various concentrations of sodium hypochlorite combined with 8.5% omeprazole against Enterococcus faecalis. J Dentistry. 2014;5(2):139–49.
39. Sinaredi BR, Pradopo S, Wibowo TB. Antibacterial Power of Chlorhexidine Mouthwash, Povidone Iodine, Fluoride Supplemented Zinc Against Streptococcus mutans and Porphyromonas gingivalis. Maj. Dentistry. 2014;47(4):211.
40. Widani NL, Nasution Y, Tinggi S, Kesehatan I, Jakarta SC, Masyarakat FK. Comparison of Oral Care Using Povidone Iodine 1% with Chlorhexidine 0.2% to the Number of Bacteria in the Mouth in Patients with Decreased Consciousness The Comparison of Oral Care Using Povidone-iodine 1% and Chlorhexidine 0.2% to the Amount of Bacteria. J Nursing UNPAD. 2015;3:185–92.
41. Sinaredi BR, Pradopo S, Wibowo TB. Antibacterial effect of mouth washes containing chlorhexidine, povidone iodine, fluoride plus zinc on Streptococcus mutans and Porphyromonas gingivalis. Dent J. 2014;47(4):211–4.
42. Indriyanti R, Hasan F, Gartika M. DIFFERENCE IN THE DECREASE IN BIOFILM MASS OF Streptococcus mutans BETWEEN THE GIVING OF N-HEXANE AND ETHYL ACETATE FRACTIONS OF ETHANOL EXTRACT OF SINGLE CLOVE GARLIC (Allium sativum L.). ODONTO Dent J. 2019;6:21.
43. Astuti SI, Arso SP, Wigati PA. Biofilm Formation Activity of Streptococcus Mutans and Candida Albicans After Exposure to Cigarette Smoke Condensate and Probiotic Drink. Analysis of Minimum Service Standards in Outpatient Installation at Semarang City Hospital. 2015;3(February):103–11.
44. Fahruddin, Haedar N, Santosa S, Wahyuni S. Test of the growth ability of

bacterial isolates from water and sediment of the Tallo River against lead (Pb) metal. J Natural and Environmental Sciences. 2019;10(2):58–64.
45. Barzegari A, Kheyrolahzadeh K, Mahdi S, Khatibi H, Sharifi S, Memar MY, et al. The battle of probiotics and their derivatives against biofilms. Infect Drug Resist. 2020;13:659–72.
46. Carvalho FM, Teixeira-Santos R, Mergulhão FJM, Gomes LC. The use of probiotics to fight biofilms in medical devices: A systematic review and meta-analysis. Microorganisms. 2021;9(1):1–26.
47. Pratiwi R, Nursyaputri F, Indraswary R, Ratnawati ID. the Effectiveness of Phaleria Macrocarpaâ€TMS Leaf Nanoemulsion Gel on Staphylococcus Aureus Biofilm Thickness (in Vitro). ODONTO Dent J. 2022;9(1):69.
48. Panesa MR, Saputera D, Budiarti L yulia. Effectiveness of Inhibitory Power of Ethanol Extract of Cherry Leaves Compared to Chlorhexidine Gluconate 0.2% Against Staphylococcus aureus. Dentin J Dent. 2018;II(1):79–84.