

The effect of probiotic *Lactobacillus casei* supplementation on the secretory immunoglobulin A level in the saliva of wistar rats



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ABSTRACT

Introduction: Secretory immunoglobulin A (SIgA) is an antibody that plays an important role in mucosal immunity by blocking epithelial receptors inhibiting the attachment of pathogenic bacteria, especially *Streptococcus mutans* to epithelial cells. *Lactobacillus* is one of the bacteria contained in probiotics, which can increase the proliferation of SIgA-producing cells. This study aims to determine the effect of probiotic *Lactobacillus casei* supplementation on increasing SIgA levels in saliva of wistar rats.

Methods: This study used a laboratory prospective experimental research design, with a simple randomized design in which thirty-six male wistar rats were adapted for 7 days. After adaptation, the experimental animals that fulfilled the inclusion criteria were divided

into 2 groups randomly, i.e., the treatment group who received probiotic supplement *Lactobacillus casei* 1.17ml / 200gBW twice daily, and the control group receiving only standard water and food, the intervention was performed for 14 days. Examination of SIgA levels in saliva was conducted with ELISA.

Results: Based on Mann-Whitney analysis, the results showed significant differences in SIgA levels between treatment and control groups with median SIgA values of 26.668 ng/ml and 4.463 ng/ml, with p values being 0.033 (p < 0.05).

Conclusion: Probiotic supplementation of *Lactobacillus casei* could increase SIgA levels in saliva of wistar rats.

Keywords: Probiotic *Lactobacillus casei*, secretory immunoglobulin A (SIgA), *Streptococcus mutans*

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INTRODUCTION

The ability of a bacterium to form complex multi-dimensional structures or biofilms plays an important role in some diseases. The most common disease found in the oral cavity is dental caries. *Streptococcus mutans* are one of many etiological factors of dental caries disease. These bacteria can acquire new properties for the determinants of pathogenic expression that determine their virulence under certain conditions. Through the adhesion mechanism to the solid surface, *Streptococcus mutans* can colonize within the oral cavity and form a bacterial biofilm. Also, these bacteria are also able to survive in acidic environmental conditions and interact with other specific microorganisms in the oral cavity.¹

The cariogenic properties of *Streptococcus mutans* are derived from the enzyme glucosyltransferase. This enzyme has virulence factor in pathogenesis caries, because it can convert sucrose to glucan. Glucan is a component of the dental plaque matrix structure and serves as an early bacterial attachment medium on the tooth surface, facilitating bacterial accumulation and as a source of extracellular polysaccharide reserves.^{1,2}

Another protein produced by *Streptococcus mutans* is the glucan binding protein. This protein

serves to bind the glucan in bacteria, thus contributing to the adhesion of microorganisms and the formation of biofilms. Also, *Streptococcus mutans* are also known to have surface proteins called antigen I/II (Ag I/II), that have adhesive properties and play a role in the attachment of *Streptococcus mutans* with acquired pellicles on tooth surfaces.³

A mucosal barrier is a form of innate immune system in the oral cavity. Antibodies or immunoglobulins are adaptive immune systems that provide the body's defense against various pathogenic microorganisms. Secretion immunoglobulin A (SIgA) is a major class of antibodies contained in secretions of body fluids such as saliva, tears and mucus from the digestive tract. Secretion immunoglobulin A is the dominant adaptive immune defense in the oral cavity. These immunoglobulins can inhibit the attachment of *Streptococcus* to oral mucosal epithelial cells, agglutinate bacteria, neutralize pathogens in the oral cavity and serve as a mucosal barrier and macrophage activation.⁴⁻⁶

The use of antibiotics in treating inflammation caused by infection of the teeth, causes many adverse effects to the human body. The adverse effects of current use of antibiotics include the emergence of allergic reactions, toxicity, bacterial

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resistance, and superinfection caused by resistant organisms. Other side effects are stomach disorders, hematology, neurology and dermatology can also occur.^{7,8}

It has been proven in recent years that the loss of effectiveness of antibiotics is associated with the evolution of pathogenic bacteria that become resistant. This is the cause of treatment failure. Therefore it is necessary to continue to look for alternative drug replacement antibiotics, namely probiotics that can provide health benefits for the body with minimal side effects. In addition, the probiotic

production process is more economical than chemicals in other drugs so that it can be developed in various forms of therapy.⁹

Probiotics have long been known for their benefits in the field of health, but the use of probiotics in the field of dentistry is still very little. Therefore, today there is a tendency to try clinical trials of probiotics to reduce the side effects of antibiotics. Probiotics are supplements that contain living microorganisms that can actively improve health by improving the balance of oral microflora. The benefits of probiotics for the health of the body can be through three mechanisms of function which are protective function, immune system function and probiotic metabolite function. Protective function is the ability to inhibit pathogenic bacteria in the oral cavity, with the presence of colonization of probiotic bacteria in the oral cavity, there will be competition of nutrition and competition where attachment between probiotic bacteria and pathogen bacteria.⁹

Probiotic bacteria can also produce antibacterial components (bacteriocins) that can suppress the growth of pathogenic bacteria, so it can be used to control the number of Streptococcus mutans in plaque and oral cavity, as the main bacteria that cause caries. The benefits of probiotics in improving the body's immune system is through the ability of probiotics to induce SIgA formation, macrophage activation, modulation of pro-inflammatory cytokines, producing vitamins and lactic acid that function as antioxidants.^{9,10}

Probiotic bacteria are found in many foods and dairy products, whether contained or added to the product. Microorganisms that are often used in probiotics are lactic acid bacteria such as Lactobacillus and Bifidobacteria. Lactic acid bacteria are a group of bacteria that produce bacteriocin, an antimicrobial peptide compound which has bactericidal or bacteriostatic properties against other species.^{9,12}

Several studies in the field of dentistry previously stated that probiotic bacteria Lactobacillus rhamnosus and Lactobacillus reuteri in interaction with Streptococcus mutans could reduce the number of pathogenic bacteria. Other studies have suggested that Lactobacillus acidophilus present in yogurt can colonize and adhere to the enamel surface of the tooth. This shows the role of probiotics in dental caries prophylaxis. Administering Lactobacillus paracasei for two weeks through oral shows a very effective probiotic action. The treatment of Lactobacillus fermentum and Lactobacillus plantarum for two weeks in rats was able to increase SIgA level in the small intestine mucosa.^{11,14,15}

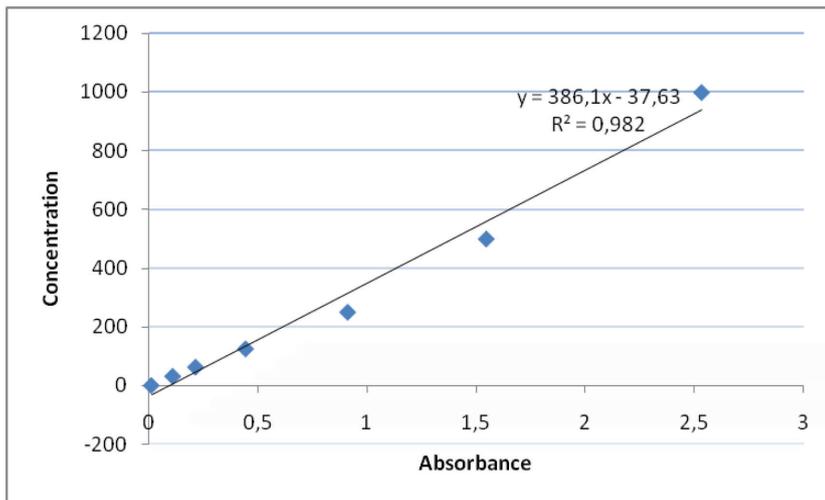


Figure 1 The standard curve of SIgA level

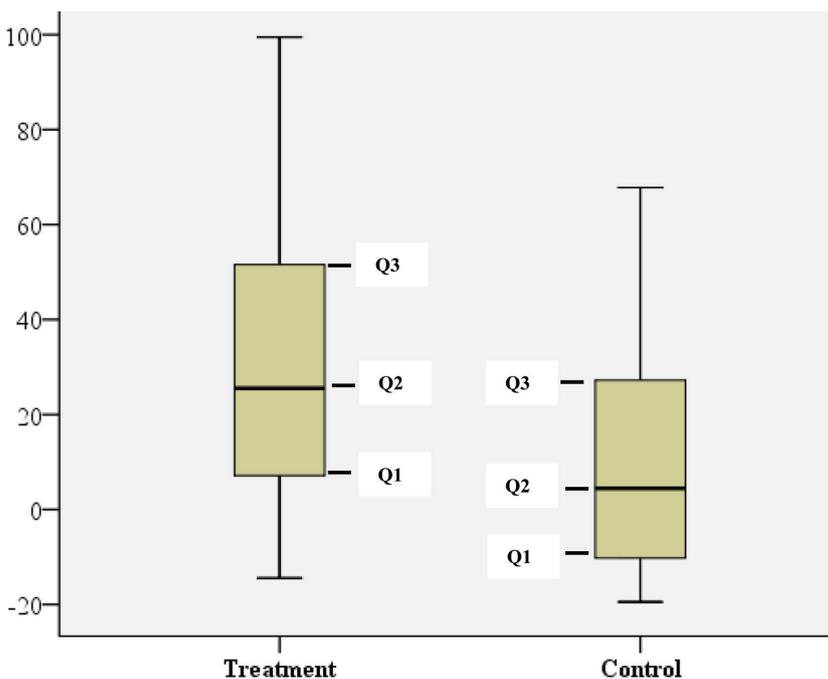


Figure 2 Box Plot of SIgA rate Treatment Group : Q1 = 8.325, Q2 (Median) = 26.668, Q3 = 53.315, Max = 257.411, Min = -14.460. Control Group: Q1 = -9.633, Q2 (Median) = 4.463, Q3 = 26.667, Max = 67.796, Min = -19.481

Table 1 Standard measurement table

Standard	Absorbance	Concentration (ng/ml)
C1	0.013	0.0
C2	0.111	31.2
C3	0.216	62.5
C4	0.445	125
C5	0.913	250
C6	1.548	500
C7	2.534	1000

Table 2 SIGA levels in the saliva of experimental animals

	Mean	SD	Q3	Median (Q2)	Q1	Max	Min
Treatment	43.188	62.225	53.315	26.668	8.324	257.411	-14.460
Control	11.457	26.929	26.667	4.463	-9.633	67.796	-19.481

Min: minimum value, Q1: the lowest quartile, Q2: median, Q3: the highest quartile, Max: maximum value

Table 3 Tests of data normality

Group	Shapiro-Wilk		
	Statistic	n	p-value
Treatment Control	0.266	18	0.000
	0.902	18	0.062*

*normal data distribution ($p > 0.05$)

Table 4 Mann whitney test on SigA level

Variable	Mean \pm SD	p-value
Treatment Group	43.1 \pm 62.22	0.033
Control Group	11.4 \pm 26.92	

*significant ($p < 0.05$)

Another strain of *Lactobacillus* found in the probiotic supplement is *Lactobacillus casei*. These bacteria are beneficial bacteria that are naturally found in the oral cavity and human intestines. *Lactobacillus casei* is a Gram-positive, anaerobic, rod-shaped bacterium, living at 15° C and belonging to the hetero-fermentative group.¹⁶ The use of *Lactobacillus casei* in this prevention of caries is one of the attempts to control dental plaque, which can be done by inhibiting the growth of pathogenic bacteria *Streptococcus mutans* by utilizing the mechanism of action of probiotic bacteria. By increasing SIgA in saliva, in addition to SIgA function as barrier by strengthening the oral mucosal epithelium, SIgA will bind to the epitope of the antigen I/II part of *Streptococcus mutans*, so that the andesine cannot bind to the salivary particle, then *Streptococcus mutans* cannot colonize on the tooth surface. Inhibition of plaque matrix formation and

inhibition of bacterial aggregation initiation may prevent early colonization of bacteria. Attempts to inhibit initial colonization are expected to prevent the formation of late colonization, thus preventing the formation of dental plaque.^{3,17}

The purpose of this study was to determine effect of probiotics *Lactobacillus casei* supplement on SIgA saliva level of wistar rats

MATERIAL AND METHODS

The research objects used 36 male wistar rats aged 8-12 weeks, obtained from animal research laboratory, University of Padjadjaran Bandung. Inclusion criteria were male sex rats, age 8-12 weeks, weight 200-250 grams and healthy. The exclusion criteria were rats with salivary gland disease, weighing more than 10% during adaptation of the research process and rats receiving high-carbohydrate supplements (80%) higher than standard feeding. The type of this study was pure experimental (prospective intervention). The sampling technique used was simple random sampling technique. The independent variable is the provision of probiotic supplement while the dependent variable is SIgA level.

The material used in this study was *Lactobacillus casei* probiotic supplement with a dose of 1.17ml/200g BW, Spuit 3cc, 1cc syringe, gloves, mask, mouse cage, saliva box, oral sonde. The instrument used for the inspection of SIgA were ELISA kit IgA, blue and yellow tip, 450 \pm nm filter reader, multichannel pipette and disposable tip, Eppendorf tube for diluting sample, distilled water, container wash solution, precision pipette (2 μ L to 200 μ L) and timer. To increase the amount of saliva, rats in pilocarpine were injection with a dose of 0.144mg /200gBW. The rat's hyperactivity during salivary uptake was overcome by injection of 1.35 mg/200g mouse ketamine.

The number of repetitions in this study used the formula of Federer: $(n-1) (t-1) \geq 15$, obtained $n \geq 16$, added with 10% dropout criteria, to anticipate dead and sick rats during the study, therefore there were 18 rats in each group. In this study there are two groups, then the total number of rats used in this study was 36.

Here is how it works, at first before the research, rats were adapted in the laboratory for 7 days given standard food and drink (HI-GRO medicated 551.2) Rats fulfilling inclusion criteria were divided into two groups: control group given only standard feeding and drinking and treatment group given probiotic supplement *Lactobacillus casei* per oral 1.17ml / 200gBW, given 2 times daily at 09.00 and 15.00 for 14 days. 3) On the 14th-day

salivary intake was initiated with peritoneal injection of ketamine 1.17mg/ 200g BW, to reduce rat hyperactivity. Rats injected peritoneal pilocarpine 0.144 mg / 200g BW to stimulate saliva, after 20 minutes of saliva taken with aspiration devices around the salivary gland region in the mouth. Saliva was taken 2-3 cc then transferred into a sterile tube and inserted into a cooler containing ice. Sampling was done in the same way and at the same time. After the research was completed the rats are sacrificed by giving the lethal dose of anesthesia of 400 mg/kg BW. 7) Measurement of SIgA levels was done using ELISA reader kit, with procedures such as: equilibrate all ingredients and prepare all reagents at room temperature before use. Standard Pipet 100 μ L, including a zero control into wells that have been prepared. Pipet 100 μ L sample into selected wells. Incubate the plate at room temperature for 30 minutes (30 ± 2), cover the surface during incubation. After incubation, fluid aspiration is present in the wells. Insert into each wash well buffer with 1x dilution and aspiration. Repeat this process until 3x (total 4x washing). Pipet 100 μ L 1x enzyme-antibody conjugate on each well. Incubate at room temperature for 30 minutes, plate closed and in the dark during incubation. Wash and dry with suction paper each. Pipet 100 μ L TMB substrate solution into each well. Incubation in the dark at room temperature for 10 minutes. After 10 minutes add 100 μ L stop solution to each well. Then immediately running microplate reader at 450 nm.

Data were analyzed using SPSS version 22, test of normality was analyzed with Shapiro-Wilk test. The Mann Whitney test was used to determine the difference between the treatment group and control group.

RESULT

Before the SIg A examination is conducted on the sample, the standard determination is done first. The standard measurement results are represented by a linear curve as shown in [Table 1](#) and [Figure 1](#). From the standard curve showing the linear form, we found an equation of $y = 386.1(x) - 37.63$ which will be used to determine the calculated content SIgA in the sample that has been known the absorbance value (as coofsien \times), as seen in [Table 2](#). Box Plot SIgA level of experimental animal as seen in [Figure 2](#).

The data normality test using Saphiro-Wilk test showed abnormal data distribution ($p < 0.05$), as seen in [Table 3](#), Mann-Whitney analysis was performed in both groups which showed significant difference with $p = 0.033(p < 0.05)$, it can be concluded that administering probiotic Lactobacillus

casei 1.17 ml/200gBW 2 times daily for 14 days can increase SIgA levels in saliva of wistar rats.

DISCUSSION

Dental caries is an infectious disease of hard tooth tissue; the disease is caused by pathogenic bacteria in the oral cavity. Streptococcus mutans are said to be the main cause of caries that begins with the formation of plaque on the tooth surface. Various studies have been done to suppress the number of Streptococcus mutans in the oral cavity, such as studies using probiotics to increase SIgA in saliva and oral mucosa.^{4,18}

Administration of probiotics containing Lactobacillus casei in this study is expected to modulate the immune system by increasing levels of SIgA saliva of wistar rats. In this study, there were significant differences in SIgA levels in both groups of experimental animals, where the SIgA group treatment was higher than the control group. This suggests that administering Lactobacillus casei probiotics twice daily for 14 days may increase SIgA levels in the saliva of wistar rats.

Probiotic bacteria can affect both local and humoral immune systems. In cellular immune response it is said that probiotics will increase splenocytes proliferation as a result of mitogen in T-cells and B-cells. Lactobacillus rhamnosus can increase the number of SIgA-producing cells and other immunoglobulin-producing cells, and stimulate the release of local interferons that facilitate antigen processing.¹⁹

Increased levels of SIgA saliva in this study, allegedly because the probiotic benefits of Lactobacillus casei in improving the body's immune system is through the ability of probiotics induce SIgA formation. This is in line with the study of Wresdiyati et al. (2013) whereas probiotics are present in enhancing the body's immune system through probiotic capability inducing SIgA formation, macrophage activation, pro-inflammatory cytokines, producing vitamins and lactic acid that act as antioxidants.¹⁰ Other studies also suggest that SIgA is a major class antibody present in secretion of body fluids such as saliva, tears or gastrointestinal mucous. SIgA is dominant in the immune system of the mucous membrane which is the first defense against the dangers of environmental factors. As we know, SIgA saliva is a predominant protein in humoral mucosal response, and plays a role in neutralizing toxins, viruses and exotoxins and eliminating pathogenic microorganisms.^{16,18}

Probiotics also contribute to increased cytokine production, for example the Streptococcus thermophilus strain will increase the production

of cytokines TNF and IL-6 via macrophage cells. *Lactobacillus bulgaricus* strain, *Bifidobacterium culoescenti*, and *Bifidobacterium bifidum* will increase the production of IL-6 through T-helper cells. The role of probiotics in non-specific immunity is the ability to increase the effects of phagocytosis against pathogens and can reduce hypersensitive reactions.¹⁹

Probiotic bacterial protective function of *Lactobacillus casei* is suspected to inhibit pathogenic bacteria in the oral cavity, in the presence of colonization of probiotic bacteria in the oral cavity, there will be nutritional competition and competition where attachment between probiotic bacteria and pathogen bacteria.⁹ SIgA secretion can cause agglutination of pathogenic bacteria and inhibit attachment of bacteria to the epithelial cells of the mucous membrane. In addition, another study suggests that the role of SIgA in inhibiting the attachment of pathogenic bacteria *Streptococcus mutans* as a cause of dental plaque is that SIgA binds to the epitope of the surface protein possessed by *Streptococcus mutans* i.e. antigen I / II, so that the epitope cannot bind to the salivary particle this will cause *Streptococcus mutans* to not colonize the tooth surface.^{1,3,18}

Lactobacillus casei can thrive at a very acidic pH that is at a pH below 3, even at pH 2 can survive despite significant colonic degradation.¹⁶ Thus this bacterium can compete with pathogenic bacteria *Streptococcus mutans* in acidic atmosphere in the oral cavity. In line with Meurman et al. who also demonstrated the activity of *Lactobacillus rhamnosus* GG against *Streptococcus mutans* at low pH and the bacteria easily prevent the cariogenic effect of the *Streptococcus mutans*.¹³

CONCLUSION

There is an increase of SIgA level in saliva of wistar rats receiving probiotic supplementation of *Lactobacillus casei* in preventing dental caries. Administration of probiotic supplementation *Lactobacillus casei* can be recommended as adjuvant in preventive therapy of dental plaque formation, so it can reduce the number of dental caries in the community, after a dose test and clinical trials.

CONFLICT OF INTEREST

Author has no conflict of interest regarding all element on this study.

REFERENCES

1. Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. The virulence of *Streptococcus mutans* and the ability to form biofilms. *European Journal of Clinical Microbiology and Infectious Diseases*. 2014;33:499-515.
2. Ahn SJ, Ahn SJ, Wen ZT, Brady J, Burne RA. Characteristics of biofilm formation by *Streptococcus mutans* in the presence of saliva. *Infect Immun*. 2008;76(9):4259-68.
3. Yuliati A. The identification of *Streptococcus mutans* epitopes to secretory. *Dent J*. 2005;38:103-7.
4. Brandtzaeg P. Secretory IgA: Designed for anti-microbial defense. *Front Immunol*. 2013;4:1-17.
5. Brandtzaeg P. Secretory immunity with special reference to the oral cavity. *J Oral Microbiol*. 2013;5(20401):1-24.
6. Ericson D, Hamberg K, Bratthall G, Sinkiewicz-Enggren G, Ljunggren L. Salivary IgA response to probiotic bacteria and *Streptococcus mutans* after the use of chewing gum containing *Lactobacillus reuteri*. *Pathog Dis*. 2013;68(3):82-7.
7. Utami ER. Antibiotics, resistance, and rational therapy. *Saintis*. 2011;1(4):191-8.
8. Krismariono. A. Systemic Antibiotics on Periodontal Treatment. *Periodontic Journal*. 2009;1(1):15-19.
9. Soeharsono. Probiotics (scientific aspect and practical use). *Bacterial Scientific* 2010.1(2):1-20.
10. Wresdiyati T. Local probiotics increase IgA secretory from small intestine in wistar rats infected with *E.coli*. *Veterinary Journal*. 2013;7(2): 109-115.
11. Wasfi R, Abd El-Rahman OA, Zafer MM, Ashour HM. Probiotic *Lactobacillus* sp. inhibit growth, biofilm formation and gene expression of caries-inducing *Streptococcus mutans*. *J Cell Mol Med*. 2018;22(1):1-12.
12. Handy, M.P. The effect of probiotics drink on in vitro inhibition of *S.mutans*. *Scientific Journal of Dentistry Muhhamadiyah*. 2014;1(1):1-14.
13. Tahmourespour A, Kermanshahi RK. The effect of a probiotic strain (*Lactobacillus acidophilus*) on the plaque formation of oral streptococci. *Bosn J Basic Med Sci*. 2011;11(1):37-40.
14. Ossowski I von, Satokari R, Reunanen J, Lebeer S, De Keersmaecker SCJ, Vanderleyden J, et al. Functional characterization of a mucus-specific LPXTG surface adhesin from probiotic *Lactobacillus rhamnosus* GG. *Appl Environ Microbiol*. 2011;77(13):4465-72.
15. Haukioja A. Probiotics and Oral Health. *European Journal of Dentistry*. 2010; 4(3):348-355.
16. Sunaryanto. *Lactobacillus casei* test as a probiotics. *J Biotechnol dan Biosains Indonesia*. 2014;1:9-15.
17. Terai T. Screening of Probiotic Candidates in Human Oral Bacteria for the Prevention of Dental Disease. *J. Plos One*. 2015;10(6):1-20.
18. Trochimiak T, Hübner-Woźniak E. Effect of exercise on the level of immunoglobulin a in saliva. *Biol Sport*. 2012;29(4):255-61.
19. Jindal G, Pandey RK, Singh RK, Pandey N. Can early exposure to probiotics in children prevent dental caries? A current perspective. *J Oral Biol Craniofacial Res [Internet]*. Elsevier Inc; 2012;2(2):110-5.



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