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The Assessment of Probiotic to Inhibition of Glucosyltransferase Enzyme Of *Streptococcus Mutans* Atcc25175

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Abstract:- ***Streptococcus mutans*** **(*S. mutans*) was reported as the primary agent of dental caries. The Glucosyltransferase (GTF) enzyme produced by *S. mutans* contributes to the virulence factor by converting sucrose into fructose and glucans used in biofilm formation. Probiotics such as yogurt and kefir of bacteria could also affect the oral ecology and potentially inhibit the GTF enzyme produced by *S.mutans*. The assessment of probiotic yogurt and kefir to inhibition of glucosyltransferase enzyme of *Streptococcus mutans*ATCC25175.This research was done in an experimental post-test-only group design using *Microtiter Plate Assay* by measuring the *Optical Density (OD)* of the ELISA reader. Two aspects were analyzed; first, the evaluation of probiotic products concerning their biomass index, biostability, and pH alteration; second, the assessment of GTF enzyme activity and fructose release by *S.mutans*. Both tests aimed to measure probiotics' effectivity in inhibiting the GTF enzyme's movement to glycolate glucose into fructose. Both test materials were analyzed in 25%, 50%, and 100% concentrations within 24 hours, 48 hours, and 72 hours incubation. In evaluating fructose release by the GTF enzyme of *S.mutans*, probiotic yogurt produced a more significant effect than probiotic kefir (p<0,05). This activity was not influenced by incubation periods or probiotic concentrations (p>0,05). The probiotic yogurt and kefir inhibited the GTF enzyme activity and reduced the fructose release by *S.mutans*.**

Keywords:- *Dental Caries, Glucosyltransferase, Probiotic, Streptococcus mutans.*

I. INTRODUCTION

Streptococcus mutans is the leading bacterial cause of dental caries, producing the enzyme Glucosiltransferase (GTF), one of the virulence factors in the etiology of dental caries. This enzyme synthesizes extracellular glucans and contributes significantly to the formation of polysaccharides in the dental plaque matrix (Ren et al., 2016). The GTF enzyme has the role of hydrolyzing sucrose to fructose and glucose and forming glucans, contributing to the cariogenic of dental biofilms so that inhibiting the frequency of GTF enzyme activity can lead to disruption of the virulence of cariogenic biofilms that have the potential to prevent dental caries (Bowen et al., 2018).

The GTF enzyme involvement in the pathogenesis of dental caries has proven that a selective approach to virulence is intuitively more enjoyable than nonspecific antimicrobial agents. This approach can be made by inhibiting the activity of the GTF enzyme (Sadeghinejad et al., 2016). Several studies on the inhibition of GTF enzymes have been conducted. However, the exact mechanism of inhibition of GTF by natural bioactive agents is still being studied (Bowen and Koo, 2011).

The probiotic is one of the recommended bioactive natural agents in caries prevention. Probiotic bacteria are usually added to various foods and commercial dairy products such as yogurt and kefir (Florou-Paneri et al., 2013). Regular consumption of yogurt for two weeks is known to reduce the amount of *S. mutans*in saliva and effectively reduce the plaque index and the number of colonies of Streptococcus sp. in saliva (Ghasemi et al., 2017). Likewise, the right with kefir drinks combined with mouth rinses. Sodium fluoride is known to be effective in reducing the amount of salivary *S. mutans*. However, research on the effectiveness of probiotics in the prevention of dental caries is still limited, so further investigation is needed to determine the antibacterial efficacy of probiotics in preventing dental caries associated with inhibition of GTF *S. mutans*enzyme activity (Patil et al., 2019). On this basis, the purpose of this study was to analyze the potential of inhibition of probiotic yogurt and kefir on the movement

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of the GTF enzyme, which is known to play a significant role in the occurrence of dental caries.

II. MATERIAL AND METHODS

A. Preparation of Yogurt and Kefir Probiotics

Based on the concentration formula, kefir and yogurt probiotic preparations were made for the test material for anti-GTF enzyme activity.Each measuring cup was filled with 25 mL of probiotic kefir and yogurt to get a solution of 50 mL each.The two probiotics were diluted with the addition of aquadest solvent and divided into concentrations of 25%, 50%, and 100%. Then each concentration was incubated for 24, 48, and 72 hours and put in a tube.

B. Culture of Streptococcus mutans

*S. mutans*ATCC 25175 bacteria obtained from the Microbiology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, were then replanted. One *S. mutans*swab oase taken from a stock of glycerol solution was then cultured on TYS20B selective media and incubated in an anaerobic jar at 37ºC 48 h (Gani et al., 2006). The second confirmation test was carried out by gram staining to ensure the correct morphology of S. mutans. The method of gram staining is to make smear preparations (smears) that have been fixed with S. mutans, then drop with crystal violet as the primary dye, wait for 2 minutes, wash with flowing distilled water, drop with 96% ethanol drop by drop until the ethanol is saturated. Clear color, washed with flowing distilled water, dripped with safranin, waited 45 seconds, washed again with flowing distilled water until the preparations were dry, and observed under a microscope to ensure the correct morphology of *S. mutans*, where gram-positive bacteria appeared purple. Colonies growing on solid media were taken in an oasis, incubated at 37ºC for 48 hours, and cultured in 10 mL of BHIB media. The sediment, *S. mutans*ATCC 25175, as compared to the standard McFarland 0.5 (1.5 x 10^8 CFU/mL), then centrifuged at 3000 g for 15 min.Based on the concentration formula, kefir and yogurt probiotic preparations were made for the test material for anti-GTF enzyme activity. 25 mL of probiotic kefir and yogurt, for a total volume of 50 mL. The probiotics were diluted with aquadest solvent and sorted into 25%, 50%, and 100% concentrations.Each concentration was placed in a tube for 24, 48, and 72 hours of incubation.

C. Purification of Glucosyltranferase Enzyme

Enzyme purification begins with enzyme extraction by taking one colony of *S. mutans*bacteria based on their respective concentrations in TYS20B and incubating for 24 h, 48 h, 72 h cultured in BHIB, and the level of turbidity was observed after 48 h. Then the tubes of kefir and yogurt preparations in each concentration and incubation time were centrifuged at 3000 g for 30 min to obtain GTF, develop I, and develop II. Develop I (the supernatant portion of *S. mutans*thatdid not interact with kefir and yogurt (control). Develop II (the supernatant portion of *S. mutans*interacted with kefir and yogurt 25%, 50%, and 100% prepared) then added ethanol and centrifuged 3000 g for 30 minutes, put in the freezer for 1 hour, and added ammonium sulfate, centrifuged again at 2500 g for one h, added with 8 M Urea, in a shaker and left for 15 min, and centrifuged at 3000 g for 30 minutes(Fujiwara et al., 2000).Develop I was produced from the results of centrifugation with the addition of 50% ethanol 5:3, incubation at 37 ^{0}C for 24 h, then 1 h centrifuged again at 5500 g for 30 min, then the supernatant was discarded. The sediment was washed with 5 ml of PBS solution, then centrifuged again, and the supernatant was taken (develop II), which became an enzyme candidate

D. Measured the Glucosiltransferase of Streptococcus mutans

The ELISA approach measured the frequency of the *S. mutans*GTF enzyme (Eto et al., 1999). The GTF enzyme purified and prepared was then used as a test material to obtain the optimal, median, and minimal GTF expression of S. mutans. Kefir, yogurt, and GTF S.mutans solution as a control (-) were added to 100 µl of developing I GTF solution based on concentration and time into 96 well plates, incubated for 10 minutes, washed with 100 µL of PBS, then incubated for 15 min, washed with 100 l of Phosphate-buffered saline (PBS) and in a 100 g shaker for 2 minutes. 100 µL of S.mutans GTF was pipetted to all 96 well plates, incubated for 20 min, pipetted with 100 µL of anti-sera, then incubated for 35 min, washed with 100 l of PBS, shaken at 100 g for 2 minutes, then pipetted to develop II GTF 100 µL, incubated for 20 min, ending with HCL 1 N 50 µL. Then read the results with an Elisa reader based on the OD value at a wavelength of 560 nm.

E. Statistical Analyses

One-way ANOVA, T-Test analyzed data analysis, and Wilcoxon Signed Rank Test with a significance limit of $p<0.05$.

III. RESULTS AND DISCUSSION

They are testing the GTF *S. mutans*enzyme to measure its ability to ferment simple sugars such as fructose as a food source for these bacteria. Probiotics are expected to suppress the activity of the *S. mutans*GTF enzyme using time and concentration variables. Figure 1 shows that the two probiotics have different abilities to stop the GTF *S. mutans*enzyme activity. At 48 hours and 72 hours, both probiotics were powerful in suppressing the production of the GTF *S. mutans*enzyme, especially at concentrations of 100% and 50%. Measurement of the activity of the effect of probiotics by suppressing the movement of the *S. mutans*GTF enzyme using a scale variable <0.1 (strong); 0.1-1 (medium); 1> (weak). The frequency of inhibition of the GTF *S. mutans*enzyme by the average of the three most substantial probiotic concentrations at 72 hours (probiotic kefir) and 48 hours (probiotic yogurt). If seen from the distribution, the incubation time of the two probiotics did not affect the ability to inhibit or release the GTF *S. mutans*enzyme based on the scale ($p>0.05$), as shown in Table 1. It gives the impression that the inhibitory activity by several proteins contained in both probiotics showed that the incubation time-correlated with the concentration of the probiotic ($p<0.05$) when it affected *S. mutans*to

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release the GTF enzyme as a virulent factor causing dental caries.

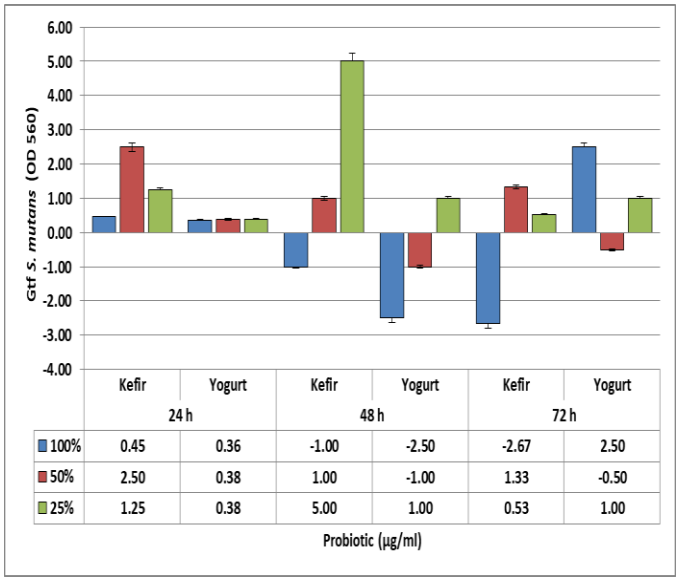

Fig. 1: The inhibition GTF enzymes of *S. mutans*by yogurt and kefir probiotics based on concentration and incubation time

The effect of the two probiotics on the activity of the *S. mutans*GTF enzyme did not differ in preventing the release of the *S. mutans*GTF enzyme. Based on the Independent T-test analysis showed that the two probiotics were not significantly different (p>0.05) with a correlation coefficient (-0.216). The relationship was the opposite, meaning that the two probiotics had other properties when inhibiting the release of the GTF *S. mutans*enzyme. It isrelated to several different Lactic Acid Bacteria (LAB) starters between the two probiotics. Yogurt probiotics inhibited the activity of the GTF *S. mutans*enzyme more strongly than kefir probiotics, meaning that both probiotics still provided an excellent opportunity for the *S. mutans*GTF enzyme to break down carbohydrates in the form of fructose as a food source yogurt probiotics (33%) and kefir probiotics. (54%) it means that as fermented milk drinks, the two test materials hadthe tolerance to the development of *S. mutans*to control the normal flora cycle of the oral cavity.

Table 1 shows a nonparametric statistical analysis and T-test on the inhibitory effect of *S. mutans*GTF enzyme activity.Variable analysis using the OD value of *S. mutans*GTF on the type of probiotic, incubation time, scale, and concentration of probiotics.Based on the analysis using the Wilcoxon Signed Ranks Test, the scale measured the effect of probiotics on the inhibition of GTF *S. mutans*,and the concentration of probiotics both had significant differences (p<0.05), with a correlation coefficient on the scale (0.738) and the concentration (0.467).Meanwhile, the probiotic and incubation time had no significant difference or had no effect (p>0.05) with the correlation coefficient on probiotics (-0.29) and incubation time (0.13).

In this study, a comparison was made of the antibacterial ability of probiotic yogurt and kefir by suppressing the activity of the GTF *S. mutans*enzyme with the release of fructose using time and concentration variables, which were measured using an Elisa reader based on Optical Density (OD) at a wavelength of 590 nm. Probiotic yogurt more strongly inhibited the activity of the enzyme GTF *S. mutans*compared to probiotic kefir. It is could be due to the different starters found in the two probiotics. The level of lactic acid in fermented milk products is influenced by the ability of the starter to form lactic acid, which is determined by the number of starters and the type of starter used (Ayad et al., 2004).

The started bacteria in yogurt consisting of two or three bacterial cultures tend to have differences in pH values, lactic acid, and protein content. This phenomenon was caused by L.acidophilus inhibiting the growth of L.bulgaricus, and S.thermophilus did not show a good interaction with L.bulgaricus. It can be assumed that the more starter contained in yogurt and kefir fermented milk, the more likely it will have an inhibitory effect on the performance of other bacteria used in the starter (Ayad et al., 2004). It is in line with a further study that reported that the Lactobacillus species reduced biofilm formation from *S. mutans*and *L. acidophilus* reduced the expression of gtfB and LuxS genes responsible for biofilm formation and maturation (Wen et al., 2017). The GtfB is accountable for the production of insoluble glucans, which in turn form dental plaque and provide shelter and food for other bacteria that can cause further damage to the oral cavity. The expression of gtfB levels in saliva is directly proportional to the presence of caries (Ahmed et al., 2014).

Variable	StatisticDescription				
	N	Min	Max	Mean	S.Devt
Gtf*S. mutans*	18	-2.67	5.00	0.56	1.80
Probiotic yogurt and kefir	18	1.00	2.00	1.50	0.51
Incubation times	18	1.00	3.00	2.00	0.84
Concentration	18	1.00	3.00	2.00	0.84

Table 1: Statistical analysis of Glucosyltransferase (Gtf) inhibition of *S. mutans*by probiotics based on incubation time and concentration

*Gtf*S. mutans*Vs Probiotic (p=0,058 (>0,05) r= - 0,290; Gtf*S. mutans*Vs. Incubation times (p=0,07 (>0,05) r= -0,13); Gtf*S. mutans*Vs Concentrations (p=0,003 (<0,05) r= -0,467.

Carbohydrates are a substrate for forming energy for the life of microorganisms and extracellular polysaccharides that play an essential role in tooth decay. There are three types of carbohydrates:monosaccharides, oligosaccharides/disaccharides, and polysaccharides (Chen, 2017). Monosaccharides are the most straightforward carbohydrates because they cannot be hydrolyzed into smaller carbohydrate molecules, such as glucose, fructose, mannose, and galactose.

The second group is disaccharides, namely carbohydrates which on hydrolysis give 2-8 monosaccharide molecules, such as sucrose, maltose, and lactose(Tzia et al., 2012). At the same time, the third group is polysaccharides, which are carbohydrates that give hundreds to thousands of monosaccharides on hydrolysis and form large molecules (polymers), for example, starch, cellulose, and glycogen(Hill, 2018). The polysaccharide group has the lowest cariogenic potential, which is relatively harmless. Because the polysaccharide molecules are too large to fuse into the plaque, it takes time to hydrolyze into monosaccharides so that plaque bacteria can metabolize them through anaerobic glycolysis, a mechanism required by many bacteria to obtain chemical energy(Mäkinen, 2010).

The pathogenicity of S. mutansdepends entirely on carbohydrates as a source of energy and survival, using RNA-Sealing (RNA-Seq) technology to compare the transcriptome of S. mutansbacteria grown on glucose, fructose, or sucrose as the sole carbohydrate source. Fructose alone can affect the expression of specific genes as an essential carbohydrate source (Zeng et al., 2016). It was found that fructose can affect the expression of specific genes, in this case, GTF. Why fructose, in particular, elicits this effect over sucrose or glucose is not entirely clear.

This study adds insight into the impact of transport and metabolism of sucrose by S. mutans, where inhibiting the release of fructose by the GTF S. mutansenzyme is one of the efforts to prevent dental caries. The probiotic L. salivarius strains K35 and K43 not only inhibited growth but also decreased the expression of the gtf-coding gene of S. mutansto reduce biofilm formation(Zeng and Burne, 2013).

Due to carbohydrate fermentation, S. mutanscontains a complex glycolytic pathway and can produce lactate, formate, acetate, and ethanol (Takahashi et al., 1987).The exact distribution of the fermented product depends on the growing conditions, with lactate as the main product when glucose is abundant(Banas, 2004).

Lack of lactate dehydrogenase (LDH) strain will cause the cariogenic ability of S. mutansto be reduced.The absence of LDH in the biofilm can kill S. mutans.It is the basis for developing genetic engineering to prevent dental caries(Wang et al., 2018). D-tagatose inhibits the activity of the GTF enzyme, which results in the release of D-fructose from sucrose.D-Fructose (and sucrose) are potent enhancers of gtfB expression.D-fructose induces higher gtfB expression than D-glucose in the early exponential phase(Hasibul et al., 2018).

D-tagatoseinhibits GS-5 mutant S. growth and biofilm formation by interfering with GTF enzyme activity.This effect may be helpful in the prevention of dental caries(Sawada et al., 2015).The interaction of S. mutansbetween the two probiotics can reduce the release of fructose by S. mutansGTF.Fructose release test by the GTF enzyme S. mutansis an indicator of the effect of probiotics on S. mutanswith the expression of the GTF enzyme to release fructose as a reference probiotic as an inhibitor on the development of S. mutans(Nagamine et al., 2020).

Probiotic yogurt more strongly inhibits the release of fructose by the enzyme GTF S. mutans(69%) compared to probiotic kefir (42%),It means that both probiotics are tolerant to the release of the enzyme GTF S. mutansfructose when interacting with fermented milk in the oral cavity. In addition, the two probiotics can maintain the balance of the normal flora of the oral cavity to prevent biological abnormalities of the oral cavity, especially those caused by S. mutansbacteria in dental caries.

Lactic acid bacteria in probiotics produce several antibacterial substances, such as reuterin (bacteriocin), biosurfactants, and H2O2 (Naidu et al., 1999). The reuterins (bacteriocins) are a diverse group of proteins with diverse spectrum and activity, mode of action, molecular weight of encoding genes, and biochemical properties. This reuterin metabolite compound (bacteriocin) can reduce the number of S. mutans, because it can prevent the growth of harmful microbes without affecting the normal flora (Parada et al., 2007).

IV. CONCLUSION

Probiotic yogurt and kefir can inhibit the production of the enzyme glucosyltransferase S. mutans.Yogurt has better performance than kefir based on incubation time and concentration.

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