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The Difference Between the Amount of Glucose as the Product of Metabolism of Glucosyltransferase Enzyme Streptococcus Mutans In Neutral pH and Optimal pH

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ABSTRACT

Background:Caries happens due to the production of organic acid from the sugar metabolism, it cause a decrease in oral pH. The changes of pH affects the formation of glucan as the product of metabolism GTF enzyme S. mutans. Purpose: To investigate whether neutral pH (7) will decrease the amount of glucose compared to pH (5.5, 6.0, and 6,5). Methods: The S. mutans's was cultured in BHI and centrifuged to obtain its supernatants. Six samples for each four group, consisted of 0.9 ml of 0.25 M sucrose in 0.2 M buffer, 0.1 ml of GTF enzyme, and buffer solution (pH 5.5, 6.0, 6.5, and 7) with total volume of 2ml (incubated), were then measured by using HPLC . Result: The amount of glucose (pH 7) are significantly less than samples (pH 5,5 and 6,5). Conclusion: The amount of glucose (pH 7) is less in acid pH, due to the changes of enzyme's charge and denaturation.

INTRODUCTION

Dental caries is a universal infectious disease that is often experienced by human populations. Seventy two percent of Indonesia's population have caries experience, and 46.5% of them are untreated active caries (Depkes RI, 2007). In the process of caries, intra enamel, dentine, and / or cementum crystal's damage occurs with the production of organic acids from glucose metabolism which is fermented by cariogenic bacteria. The large amount of acid in the mouth will cause a decrease in the pH of the oral cavity, so that the enamel and dentine become soluble (Moayad et al., 2015)
Saraf (2006) says that *S. mutans* is a bacterium that colonizes on the teeth since the eruption of first deciduous teeth and has the virulence as a cariogenic microflora (Saraf. S, 2006). This statement is reinforced by Ren et al. (2016) that *S. mutans* is the main pathogen causing caries (Ren *et al*., 2016). *S. mutans* has three virulence factors, they areacidogenic and acid-tolerant properties, the ability to synthesize extracellular polysaccharides using its glucosyltransferase (GTF) enzyme, and the ability to adhere on the teeth surfaces using glucan which is already formed before, they play an important role in the pathogenesis of dental caries (Ren *et al*, 2016), The acidogenic and aciduric properties of S. mutans bacteria cause these bacteria to thrive on acidic Ph (Lynch *et al*., 2013)

Kawarai *et al*. (2015) reported that the GTF enzyme has maximum activity which is limited to pH range of 5.5 to pH 6.5, this statement is in agreement with a study by Puanglek *et al*. (2016) who have conducted research on the different bacteria and stated that the enzyme GTFJ *Escherichia coli* and *Streptococcus salivarius* show higher activity at pH 5-6, while its activity decreased sharply at pH below 5 and above 6, a similar phenomenon also occurred on the activity of the enzyme GTF *S. mutans* (Kawarat *et al*., 2015) (Puanglek *et al,* 2016). The pH value creates the best conditions so that the enzymes can catalyze the reaction to the maximum which is called the optimum pH, thus it can be seen that the optimum pH of the enzyme GTF *S. mutans* is in the range 5.5-6,5 (Bisswanger, 2014).

Mathew *et al*. (2013) stated that the changes in pH have an important role in the field of biochemistry in general and the field of protein chemistry in particular, because it can cause a change effect of the normal form and function. Sometimes a slight change in pH can cause significant changes of the cluster, and resulting in the changes of protein behavior (enzymes) (Matthew *et al*., 2013). Based on Karawai's statement about the optimum pH of the enzyme GTF *S. mutans*, the GTF *S. mutans* enzyme decreased activity at a pH greater than 6.5 but it is not yet known how much the decrease activity from the enzyme GTF *S. mutans*.

According to Scott (2004), a solution or liquid is neutral when it has pH of 7, which indicates that the solution has an equal amount of hydrogen ions and hydroxide ions (Scott & Fong, 2004). To achieve a neutral pH condition in the oral cavity, one of them can be performed by brushing and gargling, so it could neutralize the acidic pH of the oral cavity due to the activity of the enzyme GTF *S. mutans*. The condition of neutral pH can inhibit the demineralization process. Although at the neutral pH the process of caries can be stopped chemically, but whether neutralize the pH condition of the oral cavity has an effect on the activity of GTF *S. mutans* enzyme or not, has not been proven. Based on the theories above, it's selected that pH 5.5, 6.0, 6.5 and 7 are as independent variable in this research which applied with treatment of giving buffer with pH 5.5, 6.0, 6.5, and 7.

MATERIALS AND METHOD

This research was conducted by using samples of GTF *S. mutans* enzyme obtained in *S. mutans* culture supernatant. *S. mutans* bacteria were cultured in a *Brain Heart Infusion (BHI)* medium and were incubated at 37 ° C in an anaerobic jar for 24 hours. Then the culture medium was vibrated with a 150 rpm shaker and centrifuged at 1500 rpm for 30 minutes to get a supernatant containing the enzyme GTF (Adinda Putri, 2013).

Six test tubes were prepared for 4 groups of samples with pH of 5.5, 6.0, 6.5, and 7. Each group was given 0.9 ml of sucrose with 0.25 M concentrate in 0.2 M buffer with pH according to the sample group, add 0.1 ml of GTF enzyme solution and 0.2 M buffer solution according to the previous pH to a total volume of 2 ml. Samples were then incubated at 37 ° C for 2 hours, and glucose levels was measured using HPLC.

Before the sample is loaded into HPLC, measurement of pure glucose level using HPLC was conducted to be the standard glucose value. Thus, glucose levels in samples that have the same detection time as standard glucose values can be ascertained as glucose.

RESULTS AND DISCUSSION

Large amount of glucose concentration measured by HPLC is recorded and analysed in this study. The results of glucose levels obtained from calculations using HPLC are written in a curve form with a large area that reflects the amount of glucose.

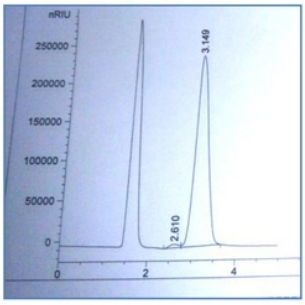

Fig1. Result of glucose levels measured by HPLC. Peak at the minutes 2.610 shows glucose level and peak at minute of 3.149 shows the sucrose level.

Before changing the size of the area to the form of glucose level percentage, the large area of each sample was subtracted by the results of the sample measurement without sucrose for 13311.1, this subtraction was done because of 2% of glucose level on the bacterial culture medium of *S. mutans* in the form of BHI. After subtracting, the large area obtained is converted to a percentage of glucose level by using the following formula:

$$\text{Konsentrasi Glukosa} = \frac{\frac{AC}{As} \times \frac{Vis}{Vic} \times FP}{KS} \times 10$$

Note:
AC = Sample Area
Vic = Example of Injects volume
AS = Standard Area
KS = Standard Concentration
Vis = Standard injects volume
FP = Example of dilution factor

The result of conversion of glucose area on HPLC chromatogram graph obtained by glucose level (%) is shown in Table 1.

Table 1. Average and SD glucose levels (%)

pH	N	$\bar{X}$	SD
5.5	6	4.67	0.73
6	6	4.28	0.90
6.5	6	4.04	0.48
7	6	3.05	0.59

Normality of the data was tested using Kormogorov Smirnov test, with $p > 0.05$. Hence, it is known that the data are normal distributed. Homogeneity test was also conducted to see whether the data are homogeneous and result of $p = 0.513$ ($p > 0.05$), shows that the variant of the four groups is homogeneous.

Later, ANOVA test was performed to see the significant difference in the sample groups by using one-way ANOVA test. In one-way ANOVA test, a value of $p = 0.010$ ($p < 0,05$), was obtained indicating a significant difference result (Ho is rejected). Next, test is continued with *Post Hoc test* which is performed to know whether there is a difference meaning between groups of samples. Post hoc test results is listed in Table 2.

Table 2. Post Hoc test glucose levels at pH 5.5, 6, 6.5, and 7.

	5,5	6	6,5	7
5,5		-	-	*
6	-		-	*
6,5	-	-		-
7	*	*	-	

NOTE:
- : no significant difference
* : there is significant difference

The results of the Post Hoc test in Table 2 shows that glucose levels at pH 5.5, 6, and 6.5 has no significant differences ($p > 0.05$), whereas at glucose level pH 7 has a significant difference with glucose PH 5.5 and 6 ($p < 0.05$). However, there is no significant difference between glucose level of pH 7 and glucose level of pH 6.5 ($p > 0.05$).

DISCUSSION

This research investigated the effect of pH on glucose levels as a metabolism product of GTF *S. mutans* enzyme measured by HPLC to show the difference in high glucose levels in (%). Statistical results obtained show no significant differences in glucose levels among the pH samples of 5.5, 6, and 6.5, but there is a significant difference in the pH 7 sample group against the pH groups 5.5 and 6. Again, there is no significant difference observed with pH 6.5 group.

Glucose levels can reflect the activity of the GTF enzyme, both in breaking up sucrose and in forming glucan, increasing activity of this enzyme will enhance the process of breaking sucrose and the formation of glucan.

Changes in pH have an important role in the field of biochemistry in general and in the field of protein chemistry in particular, this is because changes in pH can cause effects of changes of the normal

form and function of the enzyme (Mathew et al., 2013). Sometimes, slight changes in pH can cause significant changes in the charge of clusters, leading to changes in enzyme behavior while in the environment. This change is caused by the influence of pH that changes the polarity of the enzyme environment, resulting in the change of electrical charge of enzyme ions, and it affects the capacity of the substrate to tie up the enzyme and result in the change of enzyme activity (Salwanee et al., 2013)

The relation of enzyme activity and pH conditions depends on the amino acid chains of the enzyme (Talwar & Srivastava, 2006). Enzymes and oligopeptides that are different have the difference "net charges" at certain pHs, which may cause changes in enzyme activity at a given pH. The charge changes in this enzyme also affect ionic bonds that helps the tertiary and quartz structures of the enzyme, and alter conformation and enzyme activity (Mathew et al., 2013).

From result of Post Hoc test, there is no significant difference between glucose level at pH 5.5, 6, and 6.5, this is because these pH values are the optimum pH range for GTF *S. mutans* enzyme, so the activity of GTF enzyme tends to be high at these pH range. Thus the glucose levels produced by the GTF enzyme also tend to be high, so that each enzyme will work optimally at optimum pH. Condition of pH which is capable of facilitating the enzyme for optimal activity is called the optimum pH, at a point below or above the optimum pH of the enzyme activity may decrease.

Post Hoc test also shows a significant difference between glucose levels at pH 5.5 and 6 with glucose levels at pH 7. This proves that the activity of GTF *S. mutans* enzyme at pH 5.5 and 6 higher than the activity Enzyme GTF *S. mutans*at pH 7. This decreasing activity is caused by the change of charge in the enzyme environment due to the change of ionic charges in the GTF enzyme, thus the interference of the enzyme's capacity to tie up the substrate (Salwanee *et al.*, 2013). Besides being caused by disruption of the enzyme's ability to bind to the substrate, changes in enzyme activity are also caused by pH conditions that also affect the structure of the enzyme. The decrease in GTF enzyme activity is indicated by decreased glucose levels on changes in neutral enzyme pH environment (pH 7) compared to optimum pH conditions (pH 5.5 and 6). This decrease occurs because the enzyme tends to release hydrogen ions into the environment and causes a continuous change of non-covalent bonding to changes in the structure of the enzyme (Alberty, 2007). The presence of hydrogen ions into this environment causes a change in the interaction of non-covalent bonds that retain the enzyme molecular form, resulting in denaturation enzymes and decreased enzyme activity. Denaturation of enzymes is a process of loss of the enzyme structure that causes the enzyme to become inactive. The occurrence of enzyme inactivation due to the loss of the enzyme structure is due to the importance of tertiary structural role in enzyme function and the importance of non-covalent forces that determine the enzyme form (Toole. G & Toole. S, 2004).

The significant decrease of GTF enzyme activity at pH 7 is in accordance with Puanglek *et al* (2016) study that pH can cause changes in the activity of bacterial *S. mutans* GTF enzyme. This statement is reinforced by Kawarai *et al* (2015) in his research that GTF has a maximum activity of pH 5.5 to pH 6.5, 7, 6.

A decrease in enzyme activity GTF to be significant at pH 7 is a proof that by neutralizing the acidic conditions in the oral cavity (mirrored by giving treatment such that pH 7 is achieved) not only resulted in the cessation of demineralization of the tooth, but also resulted in a decrease in enzyme activity GTF which is a virulence main of *S. mutans* bacteria. The decrease in the activity of the enzyme GTF S. mutans can reduce the formation of glucan that accumulates cariogenic bacteria. In accordance with the calculation of the results obtained, indicating that with the effort to neutralize the acidic atmosphere, not only stop the process of caries through chemical mechanisms, but also can inhibit the process of caries from the aspect of caries-causing bacteria.

CONCLUSION

The results showed that in the neutral environment at pH (7), glucose as a metabolic product of *S. mutans* GTF enzyme is lower than the glucose levels of metabolic products of *S. mutans* GTF enzyme at a pH of 5.5 and 6.

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