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The Difference in Biofilm Molecular Weight in *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* Induced by Sucrose and Soy Protein (Glycine Soja)

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Abstract

Context: Biofilms consist of microbial cells and extracellular polymeric substance (EPS). *Streptococcus mutans* and *Aggregatibacter actinomycetemcomtans* are bacteria that can form biofilms and generate EPS. Biofilm formation can be induced by specific substances such as sucrose and protein. **Aims:** To identify the molecular weight that determines biofilm protein profile expression of *S. mutans and A. actinomycetemcomitans* induced by sucrose (carbohydrate) and soy protein (glycine soja). **Settings and Design:** Experimental laboratory study. **Materials and Methods:** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular weight. **Statistical Analysis Used:** Nil. **Results:** The results of analysis of protein SDS-PAGE showed the presence of 28 protein bands on *A. actinomycetemcomitans* biofilm in the media trypticase soy broth (TSB), 20 protein bands on biofilms of *S. mutans* in the media TSB, 29 protein bands on biofilm *A. actinomycetemcomitans* in the media brain heart infusion (BHI) + sucrose 2%, and 13 protein bands on biofilms of *S. mutans* in the media BHI + sucrose 2%. **Conclusion:** There are differences in biofilm protein profile expression that determine the molecular weight of *S. mutans* biofilm and *A. actinomycetemcomitans* induced by sucrose (carbohydrate) and soy protein (glycine soja).

Keywords: *Biofilm, molecular weight, protein*

Introduction

In human oral cavity, there are various kinds of microorganisms, either normal or pathogenic flora. Bacteria in the oral cavity develop in a complex composition and species structure to maintain their attachments on the surface. Each bacterium has a self-defense ability for survival in an environmental change, such as chemical exposure, physical pressure, and radiation. The example of self-defense in bacteria is the formation of bacterial biofilms.[1]

Biofilm is an aggregation of microorganism cells, particularly bacteria, which are inherent in a biological or inanimate surface and covered with patches of carbohydrates produced by the bacteria. Biofilm is a nutrient trap for the growth of microorganisms and helps prevent cells' detachment from the surface of living or inanimate objects. Surface attachment biofilm is an important habitat for microorganisms because nutrients can be trapped on the surface so that the nutrient content can be higher than in the liquid.[2]

Biofilms consist of microbial cells and extracellular polymeric substance (EPS). EPS can include 50%–90% of total organic carbon biofilm and can be considered as the primary biofilm matrix material. During its growth, biofilm cells will produce EPS which will help bacteria to attach on the surface and with one another to form microcolonies. If the cells continue to grow and form a thicker layer, the microbes that are attached to the inner surface layer will lack nutrients and accumulation of toxic products of bacterial biofilms will happen. The easiest biofilm example in the oral cavity is plaque.[3,4]

Streptococcus mutans and *Aggregatibacter actinomycetemcomitans* are bacteria that can form biofilms and produce EPS.[5] In this case, *S. mutans* is the main cause of caries and *A. actinomycetemcomitans* is the main cause of periodontitis. Both the diseases are the major problems that occur in the oral cavity.[6,7]

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Biofilm formation can be induced by several factors, one of which is the presence of chemicals (as inducer), for example, sucrose and protein. Sucrose is a type of carbohydrate that can help biofilm formation and bacterial adhesion to tooth surface. Several protein have been shown to have high-affinity adhesion and play a central role in the initiation of biofilm formation. The latest genomic and proteomic studies have identified many gene expression and differential gene products influential in biofilm formation.[8]

The composition of the biofilm are proteins, while proteins in biofilm are composed of fractions or operon proteins that can express the physical character of certain bacteria. Fraction or protein operon is controlled by certain genes of the bacteria. Measurement of molecular weight fractions or specific operon is expressed in physical character of certain bacteria.[9] This study on bacterial biofilms induced by both sucrose and soy was expected to determine whether biofilm proteins induced by different materials express different protein molecular weights in bacteria either of the same or different species.

Materials and Methods

The type of this research was laboratory experimental research. This research was conducted in the Laboratory of Microbiology and Biomedicine, Faculty of Medicine, Brawijaya University, Malang. The procedure in this study included bacterial culture, the growth of bacterial biofilms, bacterial protein isolation, and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

A. actinomycetemcomitans culture method was performed on specific media, the Luria berthani, incubated in an incubator containing 5% CO_2 at 37°C for 2–3 days.[10] *S. mutans* culture from the stock that acquired 1 (one) author to clarify was inserted in the Brain Heart Infusion Broth tube and then incubated for 24 h in anaerobic atmosphere at 37°C. *S. Mutans* was grown in tryptone yeast cysteine medium. The bacteria were flattened using a spreader, and then inserted into the exicator or an anaerobic jar for 2 × 24 h.[11]

S. mutans and *A. actinomycetemcomitans* biofilm formation was divided into three main groups: group I [protein induction with media trypticase soy broth (TSB)], group II (induced by sucrose 2%), and group III (control).

In group I, the following method was conducted: each bacterial *A. actinomycetemcomitans* and *S. mutans* was placed into the Durham tube with 200 mL of TSB equipped with 1% glucose at 37°C in CO_2 10%, incubated overnight to grow a biofilm.[12] In group II, we performed the method as follows: each bacterium *S. mutans* and *A. Actinomycetemcomitans* was placed into the tubes with 200 mL of brain heart infusion (BHI) equipped with 2% sucrose and incubated at 36.6°C for 18–24 h; while in the control group, *S. mutans* and *A. actinomycetemcomitans*, respectively, each within a tube, were placed into the tubes with 200 mL of BHI and incubated at 36.6°C for 18–24 Hours.[13]

Protein isolation was done by centrifugation at a speed of 6000 rpm for 15 min. The supernatant was discarded. Meanwhile, a pellet was added to phosphate buffered saline 0.05% Tween (PBST) and 0.01742 g of phenyl methyl sulfonyl fluoride (PMSF) in 10 mL of dimethyl sulfoxide as much as 5 × volume (1 cm^3 pellets: 5 cm^3 PBST–PMSF). The mixture was vortexed for 10 min and centrifugation was continued for 15 min at a speed of 6000 rpm at a temperature of 25°C. The pellet was discarded. Absolute ethanol was added in a ratio of 1:1, then allowed to stand for 24 h, and then centrifuged for 15 min at a speed of 10,000 rpm. The precipitate that formed at the bottom falcon was dried in air until the odor of ethanol evaporated, and then it was added with Tris–HCl buffer with in a ratio of 1:1. This precipitate was a protein isolate.[14]

SDS-PAGE analysis was performed on protein isolate, separating gel with 12% and 3% stacking gel electrophoresis. Protein isolate was added to 10 mL of reducing sample buffer, and then simmered for 5 min at 100°C. Electrophoresis running was done at 130 V, 30 mA to form tracking dye at 0.5 cm above the bottom of the gel. Gel results of running were immersed in the staining solution, and the gel was shaken for 30 min. The gel was soaked in a destaining solution to remove the dye, added with strain paper, and shaken until the gel became limpid. Thereafter, the gel was scanned to observe the protein fractions.[14]

Results

The electrophoresis procedure that has been performed obtained a description of some protein fractions *A. actinomycetemcomitans* and *S. mutans* biofilm [Figures 1 and 2]. The results of analysis of protein SDS-PAGE showed the presence of 28 protein bands on *A. actinomycetemcomitans* biofilm in the media TSB, 20 protein bands on biofilms of *S. mutans* in the media TSB, 29 protein bands on biofilm *A. actinomycetemcomitans* in the media BHI + sucrose 2%, and 13 protein bands on biofilms of *S. mutans* in the media BHI + 2% sucrose; whereas in the control group *A. Actinomycetemcomitans* expressed 12 protein bands and *S. Mutans* expressed 11 protein bands [Table 1].

Discussion

The results of this study indicated that from the electrophoresis procedure, several analysis of biofilm protein could be performed. First, analysis of glycine soja–induced *S. mutans* biofilm protein. In comparison to control, there were seven different proteins based on a wide molecular weight range of 231.8, 68.9, 64.4, 50.8, 44.5, 36.5, and 13.7 kDa. Second, the analysis of sucrose-induced *S. mutans* biofilm protein, which, in comparison to the

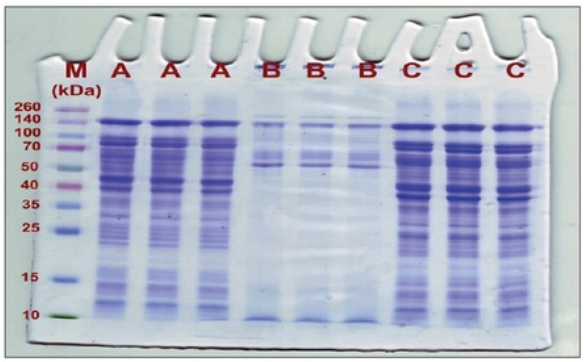

Figure 1: The results of SDS - PAGE biofilm protein. the molecular weight of the unit kilodalton = kDa, M = Marker protein, A = Biofilm Aggregatibacter actinomycetemcomitans with media Trypticase Soy Broth (TSB), B = Biofilm of Streptococcus mutans with media Trypticase Soy Broth (TSB), and C = Aggregatibacter actinomycetemcomitans biofilm with Brain Heart Infusion medium (BHI) + 2% sucrose

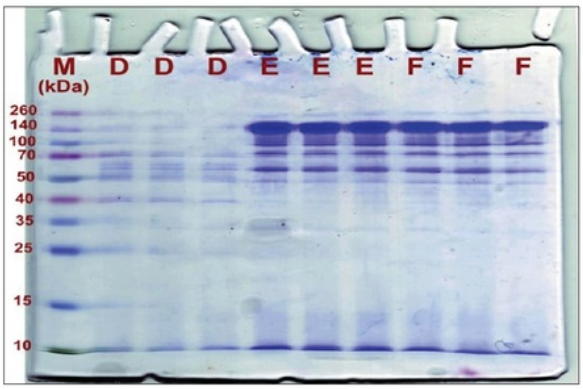

Figure 2: The results of SDS - PAGE protein biofilm. the molecular weight of the unit kilodalton = kDa, M = Marker protein, D = biofilm of Streptococcus mutans with media Brain Heart Infusion (BHI) + Sucrose 2%, E = biofilm in the control group Aggregatibacter actinomycetemcomitans, and F = the control group of Streptococcus mutans

control, showed seven different proteins of 139.6, 103.4, 72.6, 63, 59, 51.7, and 15.1 kDa. Analysis of *S. mutans* biofilm protein induced by sucrose and glycine soja showed differences in three protein bands formed, the two bands of 231.8 and 44.5 kDa in protein-induced biofilm protein and one band of 139.6 kDa in sucrose-induced *S. mutans* biofilm protein. However, these three bands were not found either in comparison or in control.

The literature shows that in 45-kDa band (44.5 kDa), there is a dlt (d-alanine-activating-enzyme) gene on the accumulation of intracellular polysaccharides, which suggests the presence of oral pathogens. This can cause dental caries due to growth phases, high carbohydrate diets, and other factors present in dental plaque. The gene is found only in protein-induced *S. mutans*.[15] The band 135 kDa (139.6 kDa) expresses the gene glucosyltransferase-I (GTF-I), which is in gtfC, which causes *S. mutans* to bind strongly to tooth surface. The gene is present only in sucrose-induced *S. mutans*.[16] In the literature, the highest band is found in 220 kDa band (231.8 kDa) that expresses ferritin, the iron that is present in every living creature.[17]

The analysis of glycine soja–induced *A. actinomycetemcomitans* biofilm protein compared with control showed 14 different proteins based on a wide molecular weight range, that is, 157, 100, 82, 68.9, 62.4, 51.6, 36.1, 21.7, 21, 18.6, 16.6, 15.6, 13.9, and 13.1 kDa. Analysis of sucrose-induced *A. actinomycetemcomitans* biofilm protein compared with control showed 15 different proteins, that is, 260, 67.9, 60.1, 51.6, 36.2, 35.4, 34.6, 26.9, 21,7, 20.1, 18.3, 16.4, 15.6, 13.9, 12.9, and 12.9 kDa.

Based on the comparison to control, the biofilm protein of *A. actinomycetemcomitans* induced by sucrose and gycine soja showed differences in three protein bands, two bands of 157 and 100 kDa in protein-induced *A. actinomycetemcomitans* biofilm protein and one 260 kDa

Table 1: Protein molecular weight

No	Protein molecular weight (kDa)					
	A.a in TSB	S.m in TSB	A.a in BHI	S.m in BHI	Control A.a	Control S.m
1.	157	231.8	260	158.9	239.3	260
2.	100	150	146.6	139.6	144	153.8
3.	93.6	113.9	94.9	103.4	109.7	110.3
4.	82	97.4	79.9	72.6	97.8	97
5.	68.9	75.8	67.9	63	76.4	75.7
6.	62.4	68.9	60.1	59	72.3	56.8
7.	59.6	64.4	54.8	51.7	57.6	48.7
8.	55.2	55.7	51.6	49.1	49.5	39.5
9.	51.6	50.8	49.2	39.9	39.8	32.3
10.	48.4	48.1	43.2	33	32.9	23.3
11.	42.3	44.5	39.2	24.9	24.6	10.2
12.	38.8	41.8	36.2	15.1	10.3	-
13.	36.1	39.1	35.4	10.1	-	-
14.	31.9	38	34.6	-	-	-
15.	30.1	36.5	30.3	-	-	-
16.	25.5	31.5	26.9	-	-	-
17.	24.4	23.7	25.8	-	-	-
18.	23.1	22.9	24.4	-	-	-
19.	21.7	13.7	23.1	-	-	-
20	21	10	21.7	-	-	-
21.	18.6	-	20.1	-	-	-
22.	16.6	-	18.3	-	-	-
23.	15.6	-	16.4	-	-	-
24.	13.9	-	15.6	-	-	-
25.	13.1	-	13.9	-	-	-
26.	11.8	-	12.9	-	-	-
27.	10.9	-	11.5	-	-	-
28.	10	-	10.5	-	-	-
29.	-	-	10	-	-	-

band on sucrose-induced *A. actinomycetemcomitans* biofilm protein. However, they were not found in comparison or control. The literature shows that 116 kDa band expresses leukotoxin which can damage neutrophils and monocytes in humans.[18] At a higher molecular weight of 116 kDa band, the expression of the formed gene has not yet been described, or perhaps the protein has not been completely split so that the molecular weight becomes high.

Analysis of the protein biofilm of *S. mutans* and protein-induced *A. actinomycetemcomitans* showed a different band after comparison, either by comparison or by control, that is, 231.8 kDa band on protein-induced *S. mutans* biofilm protein. However, there is also a similar molecular weight in the control of *A. actinomycetemcomitans*, that is, the 239.3-kDa band. As in the above explanation, the highest molecular weight in *S. mutans* was 220 kDa, which expressed ferritin. Therefore, the expression of the 239.3-kDa band on the control of *A. actinomycetemcomitans* was also possible to express ferritin, or the band may express the cell wall in the bacterium *A. actinomycetemcomitans*.

Biofilm protein analysis of *S. mutans* and *A. actinomycetemcomitans* induced by sucrose showed two different bands after comparison, both with comparator and control, that is, 260 kDa bands on sucrose-induced *A. actinomycetemcomitans* biofilm protein and 139.6 kDa band on sucrose-induced *S. mutans* biofilm protein, but both were not present in control or comparison. The presence of the 260-kDa band on sucrose-induced *A. actinomycetemcomitans* may be because the protein has not been completely split so that the protein has a high molecular weight. On the other hand, the 139.6-kDa band on sucrose-induced *S. mutans*, according to the above discussion, may show that the adhesion to tooth surfaces in *S. mutans* is stronger than that of *A. actinomycetemcomitans* adhesion.

Based on the molecular weight results, many molecular weights were almost the same or only differed from 0.1 to 0.9 kDa, but it was possible that the expressed genes were actually the same. This small molecular weight difference could be due to molecular charge density that differed between the pH of the media and the pH of the molecule. Buffers could also affect protein charge density and consequently affect the level and direction of movement. The shape and size of the molecule could also affect the results.

Different fractions of the biofilm protein of *S. mutans* and *A. actinomycetemcomitans*, each induced or not induced by protein and sucrose, may indicate that different expressions of biofilm proteins induced with different materials may express different proteins, either in the same or different bacterial species.

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Conflicts of interest

There are no conflicts of interest.

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