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Submission date: 10-Jul-2020 08:56AM (UTC+0800)
Submission ID: 1355585734
File name: 8722.pdf (8.06M)
Word count: 2680
Character count: 13397

The forming of bacteria biofilm from *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* as a marker for early detection in dental caries and periodontitis

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Abstract

Background: This is an initial study of the biofilm of *Streptococcus mutans (S.mutans)* and *Aggregatibacter actinomycetemcomitans (A.a)*. *S. mutans and A.a*are bacteria that cause infection diseases in the oral cavity. These bacteria have the ability to form biofilms. The study of bacterial biofilm proteins was used as an alternative to early prevention for oral infections. It would be used for the purpose of creating a marker for Infection Detection Kit in the oral cavity.

Objective: To easily detect caries or Periodontitis with the biofilms of *S. mutans* and *A.a* at the early stage. The forming of biofilm proteins from *S.mutans* and *A.a* induced with 5% glucose, 5% lactose, 5% soy protein, and 5% iron will be use as a marker for early detection to Dental caries and Periodontitis.

Methods: SDS-PAGE electrophoresis technique was used in the study to measure the molecular weight of *S. mutans* and *A.a* biofilms induced with 5% glucose, 5% lactose, 5% soy protein, and 5% iron.

Results: Biofilm bands of *S. mutans* and *A.a* were formed with the various numbers depending on the induction used. These results are early chararterization of biofilm that will beused as a marker for early detection of infectious diseases in oral cavity (Dental Caries and Periodontitis).

Conclusions: S. mutans bacteria induced with 5% glucose had one band of biofilm protein, with 5% lactose had four bands of biofilm proteins, and with soy protein had seven bands of biofilm protein, but with 5% iron did not produce any protein bands and neither did *A.a.*

Introduction

Streptococcus mutans (*S. mutans*) and *Aggregatibacter actinomycetemcomitans* (*A.a*) are bacteria that can cause tissue infections in the oral cavity.

S. mutans will infect the hard tissues of the teeth by fermentation and result in acid products.[1] *S. mutans* have some characteristics such as the ability to attach to the enamel surface, produce metabolicity, and the ability to form biofilms producing extracellular polysaccharides substabce (EPS) and these properties support the occurrence of dental caries.[2]

A.a bacteria are the cause of periodontal tissue infections. *A.a* produces some products that can cause some damages to the periodontal ligaments and alveolar bone and form pockets and gingival recession (Periodontal disease). *A.a* are bacteria mostly found in aggressive periodontitis with a frequency of around 90%, and in chronic periodontitis with a frequency of ± 21%.[3]

Biofilms are layers formed by colonies of microbial cells that attach to the surface, and are in a state of static (silence), slimy, and not easily released.[4] Another definition of biofilm proposed by Krzyściak *et al.* is a collection of microorganisms that attach to the surface and are enveloped by an extra cellular matrix as a defense mechanism from the external factors.[5]

The development and formation of a biofilm in the oral cavity are affected by some changes of the environmental conditions. One form of the change of the environmental conditions in the oral cavity is the presence of the exposure to food intake. Some examples of food intake consumed daily are glucose and lactose as a source of carbohydrates, soy protein, and also iron as one of the minerals needed by the body. Various kinds of food ingredients can induce the formation of *S. mutans* and *A.a* biofilms in the oral cavity. High glucose concentrations can increase the bacterial metabolism and can form EPS layers. The EPS helps the bacteria to adhere to the surface of the teeth and form the biofilm and self defense matrix.[6]

Bacteria in a biofilm environment have different properties from the planktonic form. In the microenvironment they will express in the formation of biofilms with the characters that are influenced by the presence of nutrients around them. Biofilms formed by individual bacterial cells are controlled by certain genes expressing the biofilm formation. The biofilms formed have different amino acid sequences according to the inducer. [5]

Jamal *et al.* also stated the same thing that biofilm-forming bacteria activate several genes to express stress genes that can change the resistant phenotype due to the induction from certain conditions, for example: cell density, nutrition or temperature, pH and osmolarity.[7-10]

In this study, *S. mutans* and *A.a* were induced to produce biofilms that were compatible with the inducers, namely: 5% glucose, 5% lactose, 5% soy protein, and 5% iron. The above inducing agents represent the diet ofthe daily food and can be used for the metabolism of our body as well as for microbes.[7] The biofilm formed was tested for its protein molecular weight by using the SDS-PAGE electrophoresis procedure.

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Key words: Biofilm *Streptococcus mutans*, biofilm *Aggregatibacter actinomycetemcomitans*, early detection in dental caries, early detection in periodontitis

Contributions: ID and T instructor laboratorium. PN, TB: correcting paper. PNB and IAP: collecting samples.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: The work was support by PDUPT grand, Rector of Universitas Airlangga and the Dean of Dental Medicine Faculty.

Acknowledgements: I would like to extend my special gratitude to the Ministry of Research and Technology of Republic of Indonesia for funding this research. I am also truly grateful to the Rector of Universitas Airlangga and the Dean of Faculty of Dental Medicine.

Conference presentation: Part of this paper was presented at INSBIOMM conference, 2019 27-28th August.

Received for publication:
Accepted for publication:

Infectious Disease Reports 2020; 12(s1):8722
doi:10.4081/idr.2020.8722

Materials and Methods

The biofilms of *S. mutans* and *A.a*bacteria were grown on BHIB media. As many as 11.1 BHI powder (OXOID) was added to 300 ml distilled water or aquades. The mixture was divided into 4 other erlenmeyer tubes, and each was filled with 50 ml and each Erlenmeyer was added by 5% glucose inducer (SIGMA), 5% lactose (SIGMA), and 5% iron (Choice Chem Ltd.) by 2, 5 gr. *S. mutans* and *A.a* biofilms were specifically grown with the induction of 5% soybean protein (OXOID), and TSB medium (= Trypticase Soy Broth) was used (9 gr TSB powder was added with 50 ml aquades). The culture (*S. mutans* and *A.a*) was made with an equivalent density of Mc Farland 8 to obtain the protein used in the SDS-PAGE (Thermo) work. *S. mutans* and *A.a* biofilm isolation was carried out and formed the results of induction. The biofilms formed at the base of the Erlenmeyer tubes were added with PBS+Tween (SIGMA) 0.05% and transferred to eppendorf. Then centrifugation (Fisher Scientific) was carried out at a speed of 12,000 rpm x 10 minutes. The supernatant was transferred to eppendorf and precipitated with alcohol and incubated overnight. The protein concentration was calculated by using Nanodrop.

Results

Based on the SDS-PAGE procedure, each protein band that appears can be calculated for its molecular weight in units of kDa. The following is an illustration of the *S. mutans* and *A.a* protein bands that appear after being induced with 5% glucose, 5% lactose, 5% soy protein and 5% iron.

From Figures 1 and 2 it can be seen that:

a. *S. mutans* biofilms which have been induced by 5% mucosa produced 1 protein band (61.7 kDa), and so did *A.a*with one protein band (37.5 kDa)
b. *S. mutans* biofilms which have been induced with lactose 5% produced 4 protein bands (180 kDa, 153.9 kDa, 43.9 kDa, and 37.5 kDa), whereas *A.a*had 5 proteins (77.9 kDa, 52.6 kDa, 46.8 kDa, 36.6 kDa, 28.5 kDa)
c. *S. mutans* biofilms which have been induced with 5% soy protein produced 7 protein bands (157.9 kDa, 86.6 kDa, 66.5 kDa, 50.1 kDa, 37.9 kDa, 32.3 kDa, and 29, 4 kDa), and so did A.a with 7 protein bands (77.9 kDa, 71.3 kDa, 47.4 kDa, 40.4 kDa, 37.2 kDa, 28.8 kDa, and 11.8 kDa)
d. Both*S. mutans* and *A.a* biofilms induced with iron 5% produced no protein band.

Discussion

This research is a first step to make a Kit Detection. The result cannot be applied directly for Kit Detection of oral infectious diseases, but it supports its development. For this reason, further research must be carried out to realize the application of a Kit Detection for oral infectious diseases by doing sequences of selected amino acid of molecular weight matched with the saliva of the patient. (Previously the researcher has done a research on the molecular weight of biofilm of *S.mutan* and *A.a*).

The analysis of *S. mutans* and *A.a* biofilm proteins with SDS-PAGE was to determine the molecular weight of *S. mutans* and *A.a* biofilm proteins induced with 5% glucose, 5% lactose, 5% soy protein, and 5% iron. used by Jena Bioscience Blueray was used for the marker and Coomasie Blue was for coloring.

At 5% glucose induction in both *S. mutans* and *A.a* bacteria, only one different protein band appeared (*S. mutans* = 61.7 kDa; *A.a* = 37.5 kDa). It means that it could be interpreted that the formation of the biofilms for *S. mutans* and *A.a* induced with 5% glucose were specific, namely 61.7 kDa (*S. mutans*) and 37.5 kDa (*Aa*). *A.a* protein 37.5 kDa was identified as a protein exoplyphosphatase (Svensater, 2001).

A-5% lactose induction in *S. mutans* and *A.a* bacteria resulted in 4 different protein bands each (*S. mutans* = 180 kDa, 153,9 kDa, 43,9 kDa, dan 37,5 kDa and *A.a* = 77,9 kDa, 52,6 kDa, 46,8 kDa, 36,6 kDa, 28,5 kDa). At 5% lactose induction the biofilm formed more than one protein bands. It means that there was more than one ingredient (4 ingredients) in 5% lactose which could express the formation of protein bands in each bacterium. It is necessary to carry out some further tests (amino acid sequences) of each protein band formed. For *S. mutans* bacteria, the bands of biofilm protein candidateswere 43.9 kDa and 37.5 kDa, while for *A.a* 46.8 kDa; 36.6 kDa and 28.5 kDa. The selection of candidates was based on Karatan & Watnick's provisions stating that *biofilm-associated protein* (Bap) has a size of more

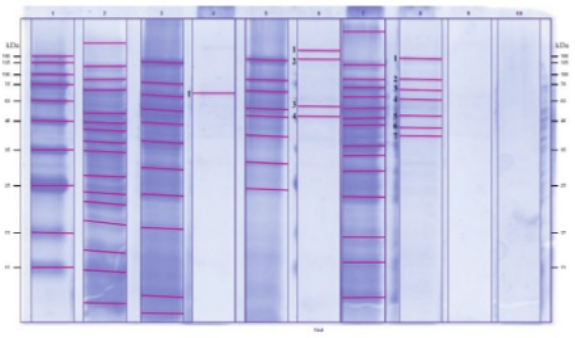

Figure 1. Results of electrophoresis. KDa = weight of molecules in kilo dalton, lane 1 = marker, lane 2 = standard (planktonic), lane 3 = glucose-induced pellet, lane 4 = glucose-induced biofilm, lane 5 = lactose-induced pellet, lane 6 = lactose-induced biofilm, lane 7 = soy protein-induced pellets, lane 8 = soy protein-induced biofilms, lane 9 = substance-induced pellets, lane 10 = iron-induced biofilms.

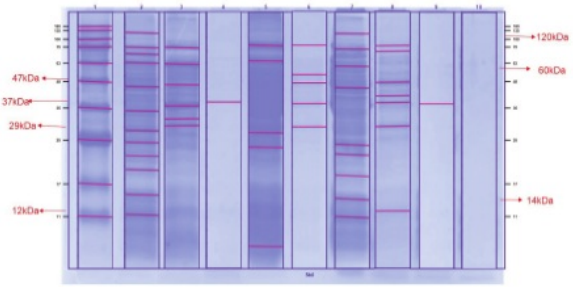

Figure 2. Results of electrophoresis. kDa = molecular weight in kilo dalton, lane 1 = marker, lane 2 = standard (planktonic), lane 3 = glucose-induced pellet, lane 4 = glucose-induced biofilm, lane 5 = lactose-induced pellet, lane 6 = lactose-induced biofilm, lane 7 = soy protein-induced pellet, lane 8 = soy protein-induced biofilm, lane 9 = iron-induced pellet, lane 10 = iron-induced biofilm.

than 1,800 amino acids and as many as 8,800 amino acids which are a group of *multi domain proteins* that have structural similarities and functions to assist the formation of biofilm in a number of bacterial species.It was suspected that most of these proteins were to anchor on the cell surface, interact loosely with the cell surface, or be secreted into the medium. Therefore, the Bap group was thought to hold cells in a biofilm by interacting with similar proteins on the surface or around the cells.[11] The induction of 5% soy protein in *S. mutans* and *A.a.* produced 7 protein bands (they were:*S. mutans* = 157.9 kDa, 86.6 kDa, 66.5 kDa, 50.1 kDa, 37 , 9 kDa, 32.3 kDa, and 29.4 kDa; *A.a* = 77.9 kDa, 71.3 kDa, 47.4 kDa, 40.4 kDa, 37.2 kDa, 28.8 kDa, and 11, 8 kDa). The candidates for *S. mutans* and *A.a*biofilms induced by 5% soy protein were as follows: *S.mutan:* 50.1 kDa, 37.9 kDa, 32.3 kDa, and 29.4 kDa, and *A.a*: 47.4 kDa, 40.4 kDa, 37.2 kDa, 28.8 kDa, and 11.8 kDa.

The bands did not appear at all in the induction of 5% iron in both *S. mutans* and *A.a* bacteria. It was expected, as Fe (iron) at certain concentrations can reduce the number of bacteria in biofilms formed in the oral cavity. It is consistent with the statement of Pecharki *et al.* In their in situ study they stated that iron at a concentration of 100µg/mL was able to reduce the number of *S. mutans* cells present in the dental biofilms.[9] Iron has an anti-bacterial effect that can kill *S. mutans* or interfere with the ability of these bacteria to form biofilms. It has the ability to inhibit F-ATPase of *S. mutans* so it can affect the acidogenicity and asidurisitas of *S. mutans*. It can also interfere with the metabolism of sucrose, and reduce the production of extracellular polysaccharides (EPS).[9]

In biofilms, microorganisms can develop different patterns of gene expression with cells in planktonic conditions. It can be seen from the results of research that many protein bands in biofilms were missing so that the protein expression was less than that of planktonic. The decrease in protein expression is due to the biofilm formation having metabolic activity, biosynthesis (biosynthesis of amino acids, coenzymes, cofactors or fatty acids), and the nutrient transport tends to be low. According to Palacios *et al.,* there were often significant differences in the growth of bacterial biofilms characterized by *down-regulation* in protein expression. The results of the analysis of the differences in protein expression when the bacteria form the biofilm with planktonics, they said that the mature biofilms tended to decrease the metabolic activities.[9]

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