

Effect of Anchovy Substrate of *Stolephorus baganensis* on Mutans Streptococci Isolated from Human Harboursing Species

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Abstract

Anchovy fish is traditional food, which is beneficial for health, one of its benefits is capability to prevent dental caries because it has active substance of fluoride. **Objectives:** The aim of the study was to determine the sensitivity of Anchovy substrate of *Stolephorus baganensis* on mutans of *Streptococcus mutans*. **Methods:** Anchovy substrate of *Stolephorus baganensis* was examined in vitro to inhibit the bacterial growth by determining the inhibition zone (agar diffusion method), minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The microorganisms tested were local strains of mutans of *Streptococcus mutans* isolated from human harbouring species in Untung Jawa Island Indonesia labeled as: *Streptococcus mutans1*, *Streptococcus mutans2*, *Streptococcus mutans3*, *Streptococcus mutans4*, *Streptococcus mutans5*, *Streptococcus mutans6*. The statistical analysis was done by in a descriptive. **Results:** The MIC value 10%/ml, average of inhibition zone 1.90 mm for *Streptococcus mutans1*; The MIC value 10%/ml, average of inhibition zone 2.00 mm for *Streptococcus mutans2*; The MIC value 10%/ml, average of inhibition zone 2.10 mm for *Streptococcus mutans3*; The MIC value 10%/ml, average of inhibition zone 3.50 mm for *Streptococcus mutans4*; The MIC value 10%/ml, average of inhibition zone 2.60 mm for *Streptococcus mutans5*; The MIC value 10%/ml, average of inhibition zone 1.50 mm for *Streptococcus mutans6* and their MBC value was the same with MIC. **Conclusion:** This study shows that Anchovy substrate of *Stolephorus baganensis*, has antimicrobial activity against local strains of *Streptococcus mutans* isolated from human harbouring species.

Key words: Anchovy substrate, *Stolephorus baganensis*, Mutans Streptococci

Streptococcus mutans harbored in the dental plaque is thought to be the main agent to caries prevalence. Acidogenicity and aciduricity are important biochemical characteristics for cariogenicity of microorganisms.

The mutans streptococci have both of these properties and considered the most cariogenic group within the oral micro flora.^{1,2}

For this reason, early prevention is needed to maintain the oral health by rinsing through using mouthwash and tooth brushing. It is the most widely used and socially accepted form of oral hygiene³. Mouth rinsing and tooth brushing are the principal way for mechanical removal of plaque and to prevent *Streptococcus mutans* colonized in teeth^{3,4}.

Anchovy fish of *Stolephorus baganensis*, contains nutritional contents of carbohydrates, proteins, fats, vitamins and minerals. One of the important nutritional elements in Anchovy fish is the fluoride. The fluoride level in this fish is quite high ranging from 5 to 18 ppm, thereby anticipating that oral health is maintained by regularly consuming Anchovy fish⁵.

This research is expected to contribute significantly to the world of science that Anchovy substrate of *Stolephorus baganensis*, can inhibit the bacterial growth of mutans of *Streptococci mutans*, therefore in a long term of consuming Anchovy fish of *Stolephorus baganensis*, caries can be prevented.

Materials and Methods

The material used in this study is a substrate substance from *Stolephorus baganensis*. The microorganisms tested were local strains of mutans of *Streptococcus mutans* isolated from human harbouring species in Untung Jawa Island Indonesia labeled as: *Streptococcus mutans1*, *Streptococcus mutans2*, *Streptococcus mutans3*, *Streptococcus mutans4*, *Streptococcus mutans5*, *Streptococcus mutans6*.

Mutans of *Streptococcus mutans* are cultivated in Tryptose-Yeast Sucrose with Bacitracin (TYS20B)⁶. Brain Heart Infusion Broth (BHI), and Diagnostic Sensitivity Test (DST) was performed. Those specimen are incubated in anaerobic jar at 37° Celsius degree for 3 X 24 hours.

Sensitivity test to antibiotic can be done in two ways⁷:

I. Drug serial dilution method

From the cultivated mutans streptococci in TYS20B, take one loop of bacteria and cultivate them in liquid culture medium BHI, then incubated it in anaerobic jar at 37° Celsius degree for 2 X 24 hours. After 2 days, compare the turbidity of bacteria media culture of BHI with Brown III standard solution.

As it was found that bacteria culture in BHI media is more turbid, add sterile saline solution, little by little until the turbidity is equal

to Brown III standard solution. When the turbidity of bacteria culture medium was equal to Brown III standard solution, the number of bacteria cell/ml in bacteria culture medium was counted, their number being 9×10^8 bacteria/ml.

If equalization process has been done, culture of mutans of *Streptococcus mutans* bacteria shall be diluted as follows:

Prepare 7 tubes of each 9 ml containing physiologic saline solution, and also prepare 1 tube containing 5 ml physiologic saline solution. Take 1 ml bacteria then put into the first tube, shake them thoroughly, from the first tube take 1 ml bacteria culture and put into the second tube, and do the same thing through the seventh tube.

Take 5 ml of bacteria from the seventh tube and put them into the eighth tube and shake it thoroughly. The number of bacteria is estimated at ± 50 cells/ml each tube shall be labeled.

Sensitivity test of bacteria to Anchovy substrate of *Stolephorus baganensis*.

Prepare 5 sets of test tubes, each tube is filled with 9 ml BHI and is labeled 1 - 5 respectively. Put 1 gram of Anchovy substrate as much with 1 : 1 concentration into first tube then stir it well.

From the first tube, take 1 ml of solution and put into the second tube, do the same thing through the fifth tube. After dilution finished, then put 1 ml of diluted *Streptococcus mutans* in those five test tube. All test tubes put in anaerobic jar at 37° Celsius degree for 2 X 24 hours.

After 72 hours, macroscopically we can see in which tube, the bacteria can not growth.

Record the result to determine the Minimum Inhibition Concentration (MIC)

II. Method using disk with drug in solid media

Determining bacterial sensitivity by the diffusion method, using disc of antibiotic impregnated filter paper.

Diluted 1 ml of mutans streptococci in the agar DST petri disc, the bacteria suspension wet the DST agar thoroughly. Then put dilution of Anchovy substrate on a disk and put it on the surface of DST agar.

Those petri disks are incubated in anaerobic jar at 37° Celsius degree for 3 X 24 hours. Inhibition zone will show around the disk and measuring the diameter of the isolated zone around the samples.

Data obtained is analyzed in a descriptive.

Results

The results of sensitivity test of *Streptococcus mutans1*, *Streptococcus mutans2*, *Streptococcus mutans3*, *Streptococcus mutans4*, *Streptococcus mutans5* and *Streptococcus mutans6* to Anchovy substrate with serial dilution method can be seen on table 1 and the inhibition zone can be seen on table 2.

Table1. Showed The Minimum Inhibitory Concentration (MIC) is 10^1 /ml and MBC 10^0 /ml for *Streptococcus mutans1*, *Streptococcus mutans2*, *Streptococcus mutans3*, *Streptococcus mutans4*, *Streptococcus mutans5* and *Streptococcus mutans6*.

Table1. Results of serial dilution test on mutans of *Streptococcus mutans* to Anchovy substrate of *Stolephorus baganensis*.

Type of bacteria	The concentration of Anchovy substrate (/ml)						C(+)	C(-)
	01 (I)	0.01 (II)	0.001 (III)	0.0001 (IV)	0.00001 (V)			
<i>S.mutans1</i>	-	+	+	+	+	+	-	
<i>S.mutans2</i>	-	+	+	+	+	+	-	
<i>S.mutans3</i>	-	+	+	+	+	+	-	
<i>S.mutans4</i>	-	+	+	+	+	+	-	
<i>S.mutans5</i>	-	+	+	+	+	+	-	
<i>S.mutans6</i>	-	+	+	+	+	+	-	

- (not growing) + (growing)

C(+) Positive control (without Anchovy substrate)

C(-) Negative control (with Anchovy substrate)

Table 2. The measurement of inhibitory zone which is carried out from the border of disk to zone with bacterial growth showed that Inhibitory zone of *Streptococcus mutans1* is 1.90 mm; 2.00 mm for *Streptococcus mutans2*;

2.10 mm for *Streptococcus mutans3*; 3.50 mm for *Streptococcus mutans4*; 2.60 mm for *Streptococcus mutans5*; 1.50 mm for *Streptococcus mutans6*

Table 2. The result on inhibitory zone measurement in bacterial growth of mutans of *Streptococcus mutans* on DST agar media.

Type of Bacteria	Inhibitory zone in mm
<i>Streptococcus mutans1</i>	1.90
<i>Streptococcus mutans2</i>	2.00
<i>Streptococcus mutans3</i>	2.10
<i>Streptococcus mutans4</i>	3.50
<i>Streptococcus mutans5</i>	2.60
<i>Streptococcus mutans6</i>	1.50

Average of inhibitory zone of local strains of *Streptococcus mutans* 2.43 mm

Discussion

The results showed that all of local strains of mutans of *Streptococcus mutans1*, *Streptococcus mutans2*, *Streptococcus mutans3*, *Streptococcus mutans4*, *Streptococcus mutans5*, *Streptococcus mutans6* are sensitive to concentration 10^{-1} /ml Anchovy substrate.

Inhibitory zone of *Streptococcus mutans1* 1.90 mm; 2.00 for *Streptococcus mutans2*, 2.10 mm for *Streptococcus mutans3*; 3.50 mm for *Streptococcus mutans4*; 2.60 mm for *Streptococcus mutans5*, 1.50 mm for *Streptococcus mutans6*.

Another study has proven that the member of genus *Stolephorus* of *Stolephorus commersonii* has antimicrobial activity against local strains of mutans of *Streptococcus mutans* isolated from humans harbouring species in Bangka Island Indonesia^{8,9}.

In Indonesia, dental caries is still a big problem in the dentistry, although efforts to overcome it have been made, such as by using fluoridation method, which can be systemic and topical application.

However considering the very small need of systemic fluoride by human beings, i.e about 1.7 - 3.3 ppm daily, it is very difficult to determine the concentration of ionic fluoride which should be given to the people, because of the harmful side effect of systemic fluoride administration, so this method is already left out.

So we carried out another method of topical fluoridation among others by chewing food, before it swallowed, which is rich in fluoride, i.e anchovy *Stolephorus baganensis*, that has fluoride property about 5 - 18 ppm⁵.

Conclusion and suggestion

The research showed that anchovy substrate has bactericide activity on mutans of *Streptococcus mutans* with minimum inhibition concentration (MIC) 10^{-1} /ml.

Anchovy of *Stolephorus baganensis* is traditional food for the population along the coast of Indonesia. Because anchovy substrate contains high fluoride ion, it also can

be used as a topical application, so early risk of caries can be anticipated.

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