In vitro antimicrobial activity of Anchovy substrate on Mutans streptococci

S. Mangundjaja*., M. Juniastuti*., A. Sofjan**
*Department of Oral Biology, **Department of Dental Material, Faculty of Dentistry Universitas Indonesia

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 on mutans streptococci. **Method:** Anchovy substrate of *Stelophorus commersonii* 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: *Streptococcus mutans* LM7, *Streptococcus mutans* JC2, *Streptococcus mutans* KPSK2, *Streptococcus mutans* Ing Britt and *Streptococcus sobrinus* B13. The statistical analysis was done by in a descriptive. **Results:** The MIC value 10%/ml, average of inhibition zone 2.90 mm for *Streptococcus mutans* LM7; The MIC value 10%/ml, average of inhibition zone 2.75 mm for *Streptococcus mutans* JC2; The MIC value 10%/ml, average of inhibition zone 2.60 mm for *Streptococcus mutans* KPSK2; The MIC value 10%/ml, average of inhibition zone 4.75 mm for *Streptococcus mutans* Ing Britt; The MIC value 10%/ml, average of inhibition zone 2.50 mm for *Streptococcus sobrinus* B13 and their MBC value was the same with MIC. This study shows that Anchovy substrate of *Stelophorus commersonii* has antimicrobial activity against mutans streptococci.

Key words: Anchovy substrate, Mutans streptococci

Introduction

*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 hygiene\(^3\). 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 *Stelophorus commersonii*, 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 35 ppm, thereby anticipating that oral health is maintained by regularly consuming Anchovy fish\(^5\).

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

**Materials and Methods**

The material used in this study is a substrate substance from *Stelophorus commersonii*. The bacteria used as analysis unit were strains *Streptococcus mutansLM7*, *Streptococcus mutansJC2*, *Streptococcus mutansIng Britt*, *Streptococcus mutansKPSK2* and *Streptococcus sobrinus B13*. 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\(^0\) Celsius degree for 3 X 24 hours.

**Working method Sensitivity test to antibiotic can be done in two ways**\(^7\):

**I. Drug serial dilution method:**

a. Making the bacteria culture medium a.l. 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\(^0\) Celsius degree for 2 X 24 hours.

a.2. After 2 days, compare the turbidity of bacteria media culture of BHI with Brown III standard solution.

a.3 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.
a.4. 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 $9 \times 10^8$ bacteria/ml.

b. Bacteria dilution

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

b.1. Prepare 7 tubes of each 9 ml containing physiologic saline solution, and also prepare 1 tube containing 5 ml physiologic saline solution.

b.2. Take 1 ml bacteria from item a.4, the 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.

b.3. Take 5 ml of bacteria from the seventh tube and put them into the eighth tube and shake it thoroughly.

b.4. The number of bacteria is estimated at + 50 cells/ml each tube shall be labeled.

c. Sensitivity test of bacteria to Anchovy substrate.

c.1. Prepare 5 sets of test tubes, each tube is filled with 9 ml BHI and is labeled 1 - 5 respectively.

c.2. Put 1 gram of Anchovy substrate as much with 1 : 1 concentration into first tube then stir it well.

c.3. From the first tube, take 1 ml of solution and put into the second tube, do the same thing through the fifth tube.

C.4. After dilution finished, then put 1 ml of diluted *Streptococcus mutans* from item bA in those five test tube. All test tubes put in anaerobic jar at 37° Celsius degree for 2 X 24 hours.

c.5. After 72 hours, macroscopically we can see in which tube, the bacteria can not growth. Record the result to determine the Minimum Inhibition Concentration (M IC)

II. Method using disk with drug in solid media

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

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

II.3. Those petri dishes are incubated in anaerobic jar at 37° Celsius degree for 3 X 24 hours.

II.4. 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**

Table 1 showed that the Minimum Inhibitory Concentration MIC is $10^{-1}$/ml and MBC $10^{-1}$/ml for *Streptococcus mutans* LM7, *Streptococcus mutans* JC2, *Streptococcus mutans* KPSK2, *Streptococcus mutans* IngBritt and *Streptococcus sobrinus* B13.

Table 1. Results of serial dilution test on *mutans* of *Streptococcus mutans* to Anchovy substrate.

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>The concentration of Anchovy substrate (/ml)</th>
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<tbody>
<tr>
<td></td>
<td>01 (I)</td>
</tr>
<tr>
<td><em>S. mutans</em> LM7</td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em> JC2</td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em> KPSK2</td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em> Ing Brit</td>
<td>-</td>
</tr>
<tr>
<td><em>S. sobrinus</em> B13</td>
<td>-</td>
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</tbody>
</table>

+ growing - not 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 mutans* LM7 is 2.90 mm; 2.75 mm for *Streptococcus mutans* JC2; 2.60 mm for *Streptococcus mutans* KPSK2; 4.75 mm for *Streptococcus mutans* Ing-Britt; 2.50 mm for *Streptococcus sobrinus* B13.
Table 2. The result on inhibitory zone measurement in bacterial growth of mutans of *Streptococcus mutans* on DST agar media.

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Inhibitory zone in mm</th>
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<tbody>
<tr>
<td><em>Streptococcus mutans LM7</em></td>
<td>2.90</td>
</tr>
<tr>
<td><em>Streptococcus mutans JC2</em></td>
<td>2.75</td>
</tr>
<tr>
<td><em>Streptococcus. mutans KPSK2</em></td>
<td>2.60</td>
</tr>
<tr>
<td><em>Streptococcus. mutans Ing-Brit</em></td>
<td>4.75</td>
</tr>
<tr>
<td><em>Streptococcus sobrinus B13</em></td>
<td>2.50</td>
</tr>
</tbody>
</table>

Average of inhibitory zone of standard strain of mutans of *Streptococcus mutans* 3.10 mm

**Discussion**

The results showed that all of standard strains of *Streptococcus mutans LM7*, *Streptococcus mutans JC2*, *Streptococcus mutans KPSK2*, *Streptococcus mutans IngBrit* and *Streptococcus sobrinus B 13* are sensitive to concentration 10⁻¹/ml Anchovy substrate.

Inhibitory zone of *Streptococcus mutans* LM7 is 2.90 mm; 2.75 for *Streptococcus mutans JC2*: 2.60 mm for *Streptococcus mutans KPSK2*; 4.75 mm for *Streptococcus mutans Ing-Brit*; 2.50 mm for *Streptococcus sobrinus B13*.

Another study has proven that Anchovy substrate of *Stelophorus commersonii* has antimicrobial activity against local strains of mutans of *Streptococcus mutans* isolated from humans harbouring species in Bangka Island Indonesia. 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 *Stelophorus commersonii*, that has fluoride property about 5 - 35 ppm.
Conclusion and suggestion

The research showed that commersons anchovy substrate has bactericide activity on mutans of Streptococcus mutans with minimum inhibition concentration (MIC) 10%/ml.

Commersons anchovy of Stelophorus commersonii is traditional food for the population along the coast of Indonesia.

Because Commersons anchovy substrate contains high fluoride ion, it also can be used as a topical application, so early risk of caries can be anticipated.

References

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Presented at The International Meeting Dentistry for The Nano-Informatic-Genomic-Technology Era In Conjunction with The 7th Thai Dental Faculties Board Scientific Meeting Sofitel Raja Orchid Hotel Khon Kaen Thailand 2-4 February 2005