Inhibitory potensial of probiotic drink against the growth of A. actinomy cetemcomitans bacteria

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Abstract

Objective: To explain the effect of probiotic drinks on the inhibitory growth of Aggregatibacter actinomy cetemcomitans.

Material and Method: This type of research is an experimental laboratory research design with a post-test only control group design. The number of samples in this study were 32. The sample used was a probiotic drink and the population used was A. actinomy cetemcomitans. The antibacterial inhibition test used is the diffusion method of the wells. Then incubated for (1-2) x 24 hours at 37°C temperature. And then the diameter of the inhibition zone formed through the wells is measured using a vernier in millimeters (mm).

Results: The average area of inhibition of probiotic drink content was 0 mm indicating weak inhibition. Based on Mann Whitney non-parametric difference test the value p < 0.05.

Conclusion: Probiotic drinks content did not affect the inhibition of A. actinomy cetemcomitans.

Keywords: Aggregatibacter actinomy cetemcomitans, Antibacterial activities, Lactobacillus casei, Probiotics

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Introduction

Dental and oral diseases have been widespread across Indonesian society. Periodontal disease in Indonesia ranks second which is still a problem. Several surveys state that dental and oral diseases affect 90% of Indonesians and about 86% from that suffer periodontitis. Periodontal disease is a problem of the supporting tissues of the teeth.³ Periodontal disease is an induction to wards inflammation process resulting in periodontal ligament damage, tooth-supporting bone loss and tooth lysis if not treated properly. Periodontitis is an in-formation of the teeth’ supporting tissues, resulting in progressive damage to the periodontal ligament and avascular bone. Anaerobic gram negative bacteria such as Aggregatibacter actinomy cetemcomitans, Porphyromonas gingivalis, and Prevotella intermedia are the three most common microorganisms involved in periodontitis.⁴ One of the gram-negative anaerobic bacteria that plays a role in the formation of subgingival plaque-causing periodontitis is A. actinomy cetemcomitans. A. actinomy cetemcomitans is a Gram-negative, nonmotile, facultatively anaerobic bacterium, short (0.4-1 μm), rod-shaped with rounded ends. These bacteria use epithelial cells as a reservoir for initial attachment and eventually migrate to the tooth surface. Virulent factors possessed by these bacteria are leukotoxin, lipopolysaccharide (LPS), and Cytolytical distending toxin (Cdt). Leukotoxin play a significant role in the pathogenicity of aggressive periodontitis by aggregetibacter actinomy cetemcomitans.⁵ Probiotic drinks containing bacteria such as lactic acid bacteria (LAB) which are beneficial for the digestive tract because they can improve the balance of intestinal microflora and can survive in gastric acidity so that they can occupy the digestive tract intestines in sufficient quite large quantity.⁶ The main species believed to have characteristics as probiotic bacteria are Lactobacillus casei, Lactoba cillus acidophilus and Bifidobacteria. One of the probiotic LAB comes from the genus Lactobacillus and can produce antimicrobial compounds, namely Lactobacillus casei. Lactobacillus casei is classified as a probiotic because it can improve the health of those who consume it by creating an immune system.⁷ According to study that was conducted by Khikmah Nin 2015, probiotic drinks containing Lactobacillus casei Shirota strain were able to inhibit all pathogenic bacteria, namely Salmonella typhi, Escherichia coli, Bacillus cereus and Staphylococcus aureus. The use of probiotics in reducing caries-causing bacteria teeth have paid off a lot. However, there are not so many researches on the use of probiotics in the periodontium. This reflects that there is still a lack of knowledge about how to prevent periodontal disease using probiotics. Therefore, further research is needed.
Material and Methods

The type of research was an experimental laboratory with the post-test only control group design, which is a method by observing and measuring the control group and the treatment group at a predetermined time. The study obtained an ethical clearance 113/KIP/FKGUPDMB/VIII/2019. Bacterial culture and testing were carried out at Balai Bioteknologi Badan Pengkajian dan Penerapan Teknologi (Center for Biotechnology Agency for the Assessment and Application of Technology/BPPT). The research was conducted in August 2019. Starting from the search for research tools and materials, research preparation, then conducting research, analyzing data and making research results. The sample of this study was bacterium A. actinomycetemcomitans obtained from Balai Biotechnologi Badan Pengklajian dan Penerapan Teknologi (Center for Biotechnology Agency for the Assessment and Application of Technology/BPPT). The sample used in this study was a probiotic drink containing Lactobacillus casei. The number of sample replications (r) for each sample group was 16, meaning 16 samples for each group to avoid bias. Thus, the total sample required for this study was 32 samples.

Procedure

Probiotics Preparation.

This study used probiotic drinks containing lactobacillus casei bacterial colonies. Figure 1.

Preparation of A. actinomycetemcomitans bacteria suspension.

The suspension was made by taking several pure culture loops of A. actinomycetemcomitans bacteria and dissolved in 20 ml of BHI liquid media in an Erlenmeyer flask. The suspension was then incubated in an incubator for (1-2) x 24 hours at 37°C. Measurement of the number of cells using the total plate number method. Figure 2.

Inoculation of bacteria on agar media.

The bacterial suspension of A. actinomyctemcomitans was spread evenly on the surface of the MHA agar (MHA plate) in a petri dish using a sterile cotton swab. Next, 4 wells were made using a perforator with a diameter of 6 mm which would be filled with the test solution. Figure 3A.

Antibacterial power test by diffusion method.

Each well in a petri dish was dripped with a probiotic drink containing 20µl of Lactobacillus casei. As a comparison, 20µl of 0.2% chlorhexidine mouthwash was taken as a positive control. Next, the agar medium which had been dripped with the test solution was laid into an anaerobic jar and then incubated at 37°C for (1-2) x 24 hours to allow colony growth to occur. Figure 3B.

Results

The results were obtained after the incubation process was carried out for 24 hours at 37°C by measuring the zone of inhibition, namely the clear area around the well where there was no bacterial colony growth. The inhibition zone formed in each well that was given the test solution was measured in millimetres (mm) and the diameter of the inhibition zone could be measured using a predetermined diameter with a calliper in millimetres (mm) and the diameter of the inhibition zone could be measured using a predetermined formula. Figure 4.

Table 3 shows the value of the Z count and testing were carried out at Balai Bioteknologi Badan Pengkajian dan Penerapan Teknologi (Center for Biotechnology Agency for the Assessment and Application of Technology/BPPT). The sample used in this study was a probiotic drink containing Lactobacillus casei. As a comparison, 20µl of 0.2% chlorhexidine mouthwash was 7.43 ± 0.53 mm power, while the average area of inhibition of 0.2% chlorhexidine mouthwash was 0 mm which accord-
diameter with a calliper in millimetres (mm) and the diameter of the inhibition zone could be measured using the formula. Figure 4.

Data analysis

The analysis used to test the normality of the data is the Shapiro-Wilk method. This test used a computer-based statistical program. If the test results show that the sample is normally distributed, the independent sample test used in this study is the Independent parametric t-test, but if the sample is not normally distributed, the independent sample test that will be used in this study is the Mann Whitney non-parametric test.

The purpose of this study is basically to determine the effect of probiotic drinks Yoghurt on the inhibitory power of A. actinomycetemcomitans bacteria. This research was conducted in August 2019 at the Balai Bioteknologi Badan Pengkajian dan Penerapan Teknologi (Center for Biotechnology Agency for the Assessment and Application of Technology/BPPT) as many as 32 samples consisting of 16 samples as a treatment group using a probiotic drink and 16 samples as a positive control group using 0.2% chlorhexidine mouthwash. The research method used is the well diffusion method which is then incubated for (1-2) x 24 hours at 37°C. The inhibition zone formed in each well which was given the test solution was measured for its diameter with a calliper in millimetres (mm) and the diameter of the inhibition zone could be measured using a predetermined formula. Figure 5.13

In this study, the average area of inhibition of probiotic drinks Yoghurt was 0 mm which according to Davis and Stout (1971) had a weak inhibitory power, while the average area of inhibition of 0.2% chlorhexidine mouthwash was 7.43 ± 0.53 mm which according to Davis and Stout (1971) has moderate inhibitory strength, can be seen in table 1.14

The normality test in all data groups used the Shapiro-Wilk test because the number of samples was less than 50. The p-value in probiotic drinks containing L. casei was less than 0.05 indicating an abnormal distribution, while in chlorhexidine mouthwash of 0.2% the p-value was more than 0.05 which indicates a normal distribution. Therefore, the independent sample test used is the Mann Whitney non-parametric test which can be seen in table 2.

Table 3 shows the value of the Z count which is -5.156. The p-value is 0.000 < 0.05. If the p-value is less than the critical limit of 0.05 then there is a significant difference between the two groups.

Discussion

According to the results of this study, it was found that in 1 positive control petri dish indicated the formation of an inhibition zone, while in probiotic drinks containing Lactobacillus casei it did not show the formation of an inhibition zone. The results of the Mann Whitney non-parametric test, it can be concluded that probiotic drinks containing Lactobacillus casei do not affect A. actinomycetemcomitans bacteria, while a study conducted by Dhony et al. in 2014 said that the antibacterial effect of probiotics was able to inhibit the growth of Aggregatibacter actinomycetemcomitans bacteria. This is because Lactobacillus casei is a Gram-positive bacterium with a bacterial cell wall consisting of several layers of peptidoglycan which forms a thick and rigid structure and contains a cell wall substance called teichoic acid, while A. actinomycetemcomitans is a Gram-negative bacterium with a cell wall consisting
The purpose of this study is basically to determine which non-parametric test will be used in this study is the Mann-Whitney test. Independent parametric t-test, but if the sample is shown that the sample is normally distributed, the normality test is used to test the normality of the data. According to Davis and Stout (1971) had a weak inhibitory effect of clove lower extract against streptococcus formalin levels in tofu boiled and soaked in hot water.

Table 1. The average area of inhibition of probiotic drinks and chlorhexidine mouthwash of 0.2% 

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Area of Inhibition (mean ± standard deviation) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic Drinks Containing L.casei</td>
<td>0</td>
</tr>
<tr>
<td>Chlorhexidine Mouthwash of 0.2%</td>
<td>7.43 ± 0.53</td>
</tr>
</tbody>
</table>

Table 2. Shapiro-Wilk normality test results 

<table>
<thead>
<tr>
<th>Group</th>
<th>Statistics</th>
<th>df</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic Drinks Containing L.casei</td>
<td>-</td>
<td>16</td>
<td>0.00</td>
</tr>
<tr>
<td>Chlorhexidine Mouthwash of 0.2%</td>
<td>0.916</td>
<td>16</td>
<td>0.148</td>
</tr>
</tbody>
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Table 3. Different test of area of inhibition with non-parametric mann whitney 

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of on one or more thin layers of peptidoglycan, so that the cell walls of Gram-negative bacteria are more susceptible to physical shocks, such as administration of antibiotics or other antibacterial agents. The decrease in the number or the death of lactic acid bacteria can also be caused by many factors including limiting factors such as reduced nutrients in the substrate or medium, the formation of acids, metabolites or compounds produced by lactic acid bacteria themselves, the use of probiotic bacteria, starter involution level, incubation temperature, and storage period. In addition, the low pH is an important factor that can inhibit the survival of lactic acid bacteria. The mechanism of cell death due to the accumulation of organic acids is the entry of Hyaluronic Acid (HA) into the cytoplasm of lactic acid bacteria which will eventually break down into H+ and A- ions in the cell. The build-up of H+ ions in the cells will cause an electrolyte imbalance in the bacterial cells so that the bacteria will try to release H+ which results in the bacteria releasing large amounts of ATP and causing cell death. One of the gram-negative anaerobic bacteria that plays a role in the formation of subgingival plaque that causes periodontitis is A. actinomycetemcomitans. Subgingival plaque cannot be removed easily mechanically; therefore, it needs to be combined with the use of antimicrobial and antibiotic agents. One of the antimicrobial agents that can be used is a probiotic drink containing Lactobacillus casei. The results of this study, it turns out that probiotic drinks containing Lactobacillus casei have no significant effect to help inhibit the growth of the bacteria A. actinomycetemcomitans which is one of the bacteria that causes aggressive periodontitis, while according to research, probiotic drinks containing Lactobacillus casei Shirota strain can inhibit all pathogenic bacteria, such as Salmonella thyphii, Escherichia coli, Bacillus cereus and Staphylococcus aureus.

Conclusion

Based on the results and discussion of this research, the following conclusions are obtained: Probiotic drinks are not effective in inhibiting the growth of A. actinomycetemcomitans bacteria. The results of the study using probiotic drinks did not give a significant effect.

Acknowledgment

None.

Conflict of Interest

The authors report no conflict of interest

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