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In Vitro – The Potential of Bioinhibition of Yogurt from Etawa Goat's Milk Toward the Growth of *Streptococcus pyogenes* Bacteria

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Abstract

AIM: This study aimed to study the probiotic lactic acid bacteria obtained from fermented Etawa goat's milk (Yogurt) from Kopelma Village, Darussalam District, Banda Aceh City, Aceh Province, Indonesia.

METHODS: This research was conducted in vitro on the growth of Streptococcus pyogenes bacteria on Yogurt using the biomass index test procedure, Ph change, and anti-adhesion test. Data obtained statistically in the form of graphs between S. pyogenes on variations in yogurt concentration and incubation time.

RESULTS: At concentrations of 25%, 50%, and 100% indicated that Yogurt had good inhibition for the growth of S. pyogenes bacterial colonies <300 CFU (0.5 McFarland). The effect of incubation time determines the number of colonies, at incubation time of 24 h, 48 h, and 72 h. Yogurt can give a good response to S. pyogenes, as indicated by the increase in the Yogurt biomass index at a concentration of 100% with an incubation time of 48 h and 72 h. The best biomass index at incubation time of 24 h is at a concentration of 50%.

CONCLUSION: Yogurt pH did not change significantly, both based on incubation time and concentration. Anti-adhesion activity increased according to the incubation time. While the Yogurt concentration did not show a significant difference.

Introduction

Acute respiratory infection or known as ARI is an infection that attacks the upper respiratory tract and can last for 14 days. Globally, the World Health Organization estimates that ARI cases in developing countries are 0.29% as many as 151 million people and in industrialized countries 0.05% as many as 5 million people [1]. Clinical symptoms of chronic tonsillitis are found in the form of local and systemic symptoms such as sore throat or throat infection, bad breath, lethargy, decreased appetite, headache and fever, and often sleepy. This causes unpleasant conditions and interferes with the patient's quality of life [2]. There are several types of bacteria that can infect the throat in humans, the most common being Group A Streptococcus bacteria [3].

Fermented food and beverage products from various ingredients have long been made and known to humans. One of the fermented products is yogurt. Yogurt is a fermented drink made from pure cow's milk. Consuming yogurt regularly will stimulate the growth and activity of friendly bacteria in the intestines [4]. Yogurt products are generally produced from cow's milk, even though it is can be produced by soymilk as a source of vegetable protein [5], but goat's milk can also be an alternative for making yogurt. Compared to cow's milk, goat's milk has complete nutritional content so it is an ideal growth medium for microorganisms [6]. Probiotics in goat's milk yogurt produce antimicrobial compounds including organic acids, diacetyl, and bacteriocins. The antimicrobial compounds produced by lactic acid bacteria (LAB) can inhibit the growth of food spoilage bacteria and pathogenic bacteria [7].

Streptococcus pyogenes is one of the pathogenic bacteria that infect humans [8]. It is estimated that 5–15% of normal individuals have these bacteria and they are usually present in the respiratory tract. *S. pyogenes* bacteria can infect when the body's immune system decreases. These infections can include pharyngitis, tonsillitis, impetigo, and fever. *S. pyogenes* can also cause invasive diseases

such as bone infections, necrotizing fasciitis, muscle inflammation, meningitis, and endocarditis [9].

The large number of antibiotics used to cure infectious diseases in humans can cause side effects, and antibiotic resistance has been a concern [10]. One of the diseases that is often treated by giving antibiotics is a sore throat, such as pharyngitis or oxyillitis [11]. According to research by Schwendicke and Dörfer [12], *Streptococcus* bacteria and pathogens that cause infection can be inhibited using probiotics, one of which is the *Lactobacillus* probiotic found in yogurt. Hasslöf did the same thing [13] regarding inhibiting *Lactobacillus* probiotics against *streptococci* and *candida* bacteria.

Therefore, this research was conducted *in vitro* aims to determine the growth of *S. pyogenes* bacteria after interacting with yogurt, yogurt biomass index test, changes in yogurt pH, and yogurt anti-adhesion test against *S. pyogenes* bacteria. The data obtained were processed statistically in the form of a chart on *S. pyogenese* bacteria after adding several variations in the concentration of yogurt to incubation time.

Materials and Methods

This research is an *in-vitro* study with an experimental research design. This research was conducted from September 2020 to January 2021 at the Research Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia.

Growth of S. pyogenes after interaction with yogurt

As much as 30 μL of yogurt with a concentration (100%, 50% and 25%) interacted with *S. pyogenes*. Then, it was cultured on selective agar media and incubated (24 h, 48 h and 72 h) at 37°C in an aerobic atmosphere. *S. pyogenes* colonies were observed on colony counters. Colonies grown on solid media were calculated to obtain information on the ability of yogurt to inhibit the growth of *S. pyogenes*. The fewer colonies are the better the ability of yogurt to inhibit the growth of *S. pyogenes*. Colony standard assessment was 0.5 (1.5×10^8) or <300 CFU/mL.

Yogurt biomass index test toward S. pyogenes

A total of 1 mL of yogurt with a concentration (100%, 50%, and 25%) was weighed in the test tube and incubated (24 h, 48 h, and 72 h) at 37°C. Weighed again after incubation. 100 μL of *S. pyogenes* was added and weighed. Weight value (g/mL) is an indicator of biomass, both before interaction with *S. pyogenes* and after being given *S. pyogenes*. Calculate the biomass at each concentration using the analytical formula [14].

Changes in yogurt pH after interaction with S. pyogenes

A number of yogurt of each concentration (100%, 50%, and 25%) was put in a glass bottle and added to it 500 μL of *S. pyogenes* bacteria. Before incubation, the mixture of the two was carried out with an initial pH measurement (0 h). Then, the incubation was carried out at 37°C for 24 h, 48 h, and 72 h in an aerobic atmosphere [15].

Yogurt anti-adhesion test toward S. pyogenes

Analysis of anti-adhesion activity of yogurt against S. pyogenes cells was done on the basis of the test microplate with Spectrophotometry at a wavelength of 620 nm. The interaction activity (adhesion) was analyzed based on the working principle of Gram stain. Adhesion interaction process based on incubation time (24 h, 48 h, and 72 h).

Results and Discussion

Growth of S. pyogenes after interaction with yogurt

The results of the incubation of bacteria showed that the growth inhibition was influenced by yogurt. The principle of this test is to evaluate the ability of yogurt to inhibit the growth and development of *S. pyogenes*. The number of *S. pyogenes* colonies determines the intensity of the infection pathogenesis. Based on Figure 1, it is shown that all concentrations show good inhibition against the number of *S. pyogenes* colonies <300 CFU (0.5 McFarland). The effect of the incubation time determines the number of colonies and it is decreases at the 72 h incubation time.

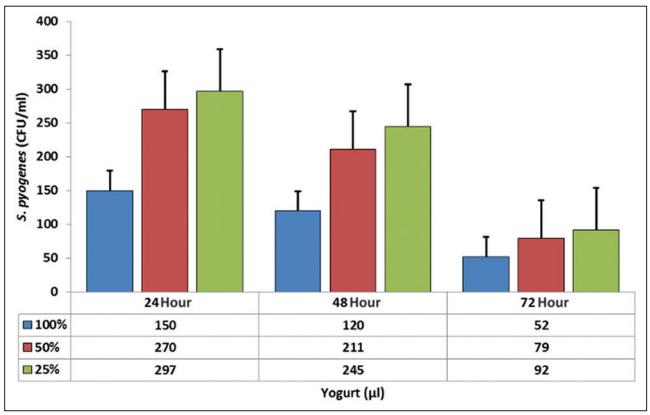

Figure 1: Inhibition of growth of Streptococcus pyogenes on the addition of yogurt

The concentration of yogurt that given has not significantly affect the growth of *S. pyogenes*, but it was influenced by the incubation time. The bacteriocin content in yogurt can facilitate the diffusion process, making it more effective at inhibiting bacterial growth [13]. Therefore, the incubation time can determine the amount of *S. pyogenes* bacteria that can be inhibited by yogurt over time.

Yogurt biomass index test toward S. pyogenes

The principle of this test is to measure the response of the active component (constituent elements) of yogurt to *S. pyogenes* bacteria, which is calculated the biomass profile after the interaction with bacteria based on incubation time. The increase in biomass activity shows that yogurt has a high response to prevent the influence of bacteria based on the breakdown of the active components of yogurt [16].

Figure 2 shows that all yogurt concentrations have a good response to bacterial inhibition, especially at a concentration of 100% with an incubation time of 48 h and 72 h. Whereas at a concentration of 50%, it has an excellent response with an incubation time of 24 h. Therefore, changes in the biomass index were not affected by incubation time. According to research by Fitria, the fermentation activity of carbohydrate and protein *Fusobacterium nucleatum* in the fruit extract of *Sapindus Rarak* DC was not affected by the incubation time. At a concentration of 25%, it has a low biomass index between 50% and 56%, even though it still responds to these bacteria. The higher the biomass index value, the better the yogurt response to prevent bacterial development, and yogurt can play a role in suppressing the growth of *S. pyogenes* in the pathogenesis of infection (Sulastri, 2018).

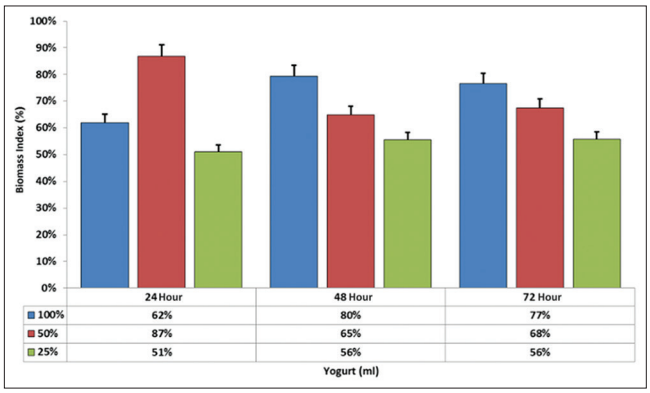

Figure 2: Biomass index of yogurt after interaction with Streptococcus pyogenes bacteria

Changes in yogurt pH after interaction with S. pyogenes

The principle of this test is to measure the response of bacteria to affect the stability of yogurt. The lower the yogurt pH change response, the better yogurt suppresses the growth of *S. pyogenes* bacteria. The results of the analysis based on Figure 3 show that bacteria cannot develop appropriately in yogurt, which is indicated by no significant change in pH, either based on incubation time or based on concentration. This explains that the H_2O_2 compound is one of the metabolic products of LAB in yogurt which can inhibit the growth of pathogenic microbes [13]. According to research conducted by Yani, yogurt's pH tends to be stable after being tested for the growth of *Escherichia coli* and *Salmonella typhi* bacteria.

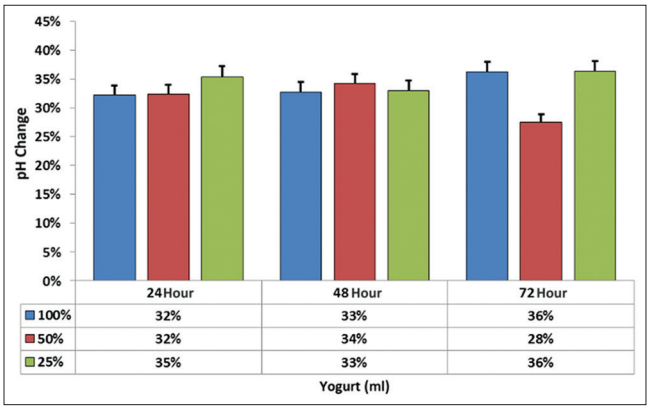

Figure 3: Change in pH of yogurt after interaction with Streptococcus pyogenes

Yogurt anti-adhesion test toward S. pyogenes

The interaction activity (adhesion) was analyzed based on the working principle of Gram stain [17]. The anti-adhesion activity of yogurt toward *S. pyogenes* was measured by spectrophotometry at a wavelength of 620 nm. The anti-adhesion value has a control optical density (OD) value as a calibration. The high OD value indicated that the anti-adhesion of yogurt was good against *S. pyogenes*. Some researchers also stated that *Lactobacillus* has good adherence to the mucosal epithelium due to *lectins* [18], [19]. Figure 4 shows that the anti-adhesion activity has increased based on the incubation time, that are 24 h, 48 h. and 72 h. Meanwhile, the concentration of yogurt did not show a significant difference.

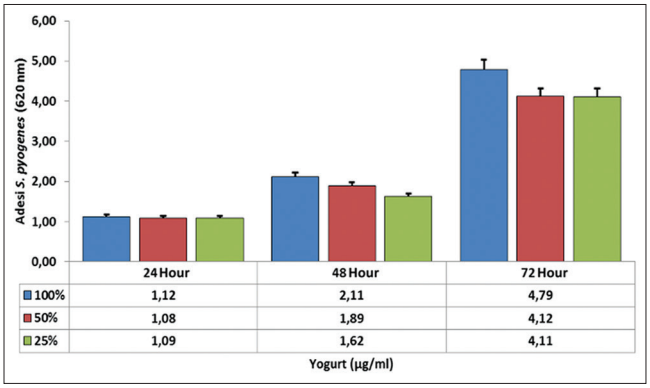

Figure 4: Anti-adhesion of yogurt to Streptococcus pyogenes

Yogurt concentration has relatively the same ability to prevent *S. pyogenes* adhesion. Meanwhile, the quantity of yogurt anti-adhesion power against *S. pyogenes* was influenced by the incubation time. Therefore, the use of yogurt-based on relative time can reduce or prevent adhesion to the host mucosa in the pathogenesis of respiratory tract infections [20].

Conclusion

A number of these tests can be explained that all yogurt concentrations have good inhibition against the growth of *S. pyogenes* bacteria colonies <300 CFU (0.5 McFarland), seen from the decreasing number of colonies at the 72 h incubation time. However, the effect of the pH yogurt did not change significantly, either based on incubation time or based on concentration. Yogurt can give a good response to *S. pyogenes* characterized by increasing yogurt biomass index. At a concentration of 100% with an incubation time of 48 h, the best biomass index showed at 24 h incubation time of 50% concentration. The anti-adhesion activity increased according to the incubation time, namely 24 h, 48 h, and 72 h. Meanwhile, the concentration of yogurt did not show a significant difference. At all concentrations, yogurt had relatively the same ability to prevent the adhesion of *S. pyogenes*. Meanwhile, the incubation time influenced the quantity of yogurt anti-adhesion power against *S. pyogenes*.

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