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Alternative Media For Bacterial Growth Staphylococcus aureus: Sweet Potato Infusa (Ipomoea batatas), Soybeans (Glycine Max (L) Merrill) and Yeast Extract

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Abstract

Background: *Staphylococcus aureus needs nourishment for its growth. The needed nutrients of carbohydrates, proteins, nitrogen, vitamins, and minerals from the media. The media commonly used for bacterial growth is the nutrient medium for high prices. Alternative media from natural local foodstuffs are obtained easily and cheaply instead of instant media. This study aims to identify alternative media mixed with infuse sweet potato (ipomoea batatas), soybeans (glycine Max (l) Merrill), and yeast extract that can be used to grow the staphylococcus aureus bacteria.* ***Methode****: this type of research is pure experimental research, and the design used is the posttest-only control design group. Staphylococcus aureus suspension was grown on alternative media and nutrient agar media as a comparison. measurements of colony diameter and number of colonies were compared between alternative media and nutrient agar.* ***Result*** *The results Research has provided an average number of staphylococcus aureus bacteria that grow on alternative media 78.00 x 10^4 CFU /ml and nutrient media for 94.19 x 10^4 CFU /ml. Whereas the colony's diameter of alternative media 2.56 mm, and the media nutrient increased by 2.66 mm.* ***Conclusion:*** *Alternative media from a blend of yam, soy infuse, and yeast extract can be used for the growth of the staphylococcus aureus bacteria*

Keywords*: Staphylococcus aureus; Alternative Media; Potato Infusa; soy infuse; soy infuse*

1. Introduction

Staphylococcus aureus is a highly invasive bacteria based on a coagulase enzyme (oxalate plasma) that is characterized by deflating red blood cells, producing coagulase, creating pigments, and reducing manitol to acid [1]. Staphylococcus aureus causes many diseases, including atopic dermatitis (AD), psoriasis, pulmonary cystic fibrosis (CF), allergic asthma, pneumonia, food poisoning, chronic granulomatous disease (CGD), osteomyelitis, diabetic foot infections (DFIs) [2]. Bacteria need nutrition

for their growth, so adding nutrients is needed to grow and to learn their properties by considering environmental harmony [3].

A good bacteriologic media, which has a corresponding growth zone, the diet requires moisture content and the exchange of substances or metabolism, as well as a source of carbon, minerals, vitamins, and gases; osmosis pressures of isotonic, acid degrees (pH) are usually neutral, but alkaline is also available, with appropriate temperatures and sterile [4]. The media commonly used for bacterial growth in the laboratory is Nutrient Agar media made from beef extract, peptone, and agar [5]. Beef extracts contain water levels, salt, creatinine, ash, and dissolved percentage of organic material. Staphylococcus aureus needs an extract protein from beef extract for its growth [6]. Nutrient Media To requires a lot of expenditure in its manufacture. This is an obstacle in the science of microbiology, so it is necessary to have innovations currently being developed, namely using natural resources to provide nutrients for growth media at low cost and easily obtained. Natural resources that can be used are sweet potatoes, soybeans, and yeast extract. According to the World Health Organization (WHO), the calcium content in sweet potatoes is higher than in rice, corn, wheat, or sorghum [7]. Soybeans include leguminous plants as basic food ingredients (soy sauce, tofu, and tempeh) and are an important source of vegetable protein in Indonesia [8] Yeast extract as a nitrogen source in bacterial growth media requires a yeast extract product that meets standards [9].

Several studies show alternative media can grow bacteria. Alternative media from sweet potato flour (*Ipomoea batatas*) and soybean flour obtained effective results in growing *Escherichia coli* and *Staphylococcus aureus* bacteria [10]. The results of other research using alternative media mixed with sweet potato infusion (*Ipomoea batatas*), soybean infusion (*Glycine max (L) Merrill*), and yeast extract have a very effective level of effectiveness for the growth and reproduction of *Escherichia coli* bacteria. [11]. This research aims to compare alternative media for the growth of *Staphylococcus aureus* bacteria from sweet potato (*Ipomoea batatas*) infusion, soy beans (*Glycine Max (L) Merrill*), and yeast extract with nutrient agar media.

2. Research Methods

This research is true experimental research. The design used in this research is the posttest only control group design. This research was repeated 16 times, based on Federer's calculation formula as follows: $(t-1)(r-1) \geq 15$ where t = number of treatments and r = number of replications. t=2 Based on calculations, r = 16 [12]. This research used subjects in the form of Gram-Positive *Staphylococcus aureus* bacteria obtained from the Yogyakarta Health and Calibration Laboratory Center which had previously

been cultured on Blood Agar Plate media and then made a bacterial suspension to a suspension density of $1x10^4$ CFU/ml. This research was carried out from February 2023 to March 2023. Research material was the compotition of alternative media are Yeast extract 6.0 gr, Meat extract 3.0 ml, Potato infusion 10% 10.0 ml, Soybean infusion 10% 10.0 ml, Sugar 10.0 gr, Agar 12.0 gr Nutrient Agar media as a control medium, and 0.85% NaCl are needed. McFarland standard 0.5, distilled water, and *Staphylococcus aureus.* The determination test for sweet potatoes and soybeans was carried out at the Department of Pharmaceutical Biology Unit II, Gadjah Mada University. The flour is made at the PAU UGM Nutrition and Food Engineering Laboratory. The scheme for making alternative media Figure 1.

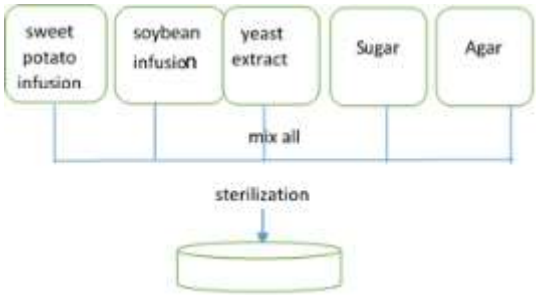


Figure 1. Schema for making alternative media

Rejuvenation of the *Staphylococcus aureus* bacteria was continued by making a bacterial suspension by equalizing the turbidity level to the McFarland standard of 0.5. The bacterial suspension that has been made is grown in alternative media and NA media in the Bacteriology Laboratory, Medical Laboratory Technology Department, Health Polytechnic, Ministry of Health, Yogyakarta. *Staphylococcus aureus* bacteria were rejuvenated by inoculating them on Blood Agar Plate media with an incubation temperature of 37 °C for 24 hours. After 24 hours of incubation, a bacterial suspension was made by equalizing the turbidity to a McFarland standard of 0.5. Then, 50 µl of the suspension was pipetted onto the media, then inoculated using a spread cup technique aseptically using a sterile tube bent into the shape of the letter L. The media that had been implanted was incubated at a temperature of 37 °C for 2 x 24 hours. After that, the number of colonies was counted using a counter and the diameter of the bacterial colonies using a caliper. The percentage of effectiveness is calculated using the formula:

$$\text{Percentage of effectiveness} = \frac{\text{Average number or diameter on alternative media}}{\text{Average number or diameter on NA media}} x100\%$$

The percentage of effectiveness is grouped based on the size of the percentage of effectiveness grouped as follows Percentage Criteria 100% Very Effective; 90-100% Effective; 80-90% Quite Effective; 60-80% Less Effective; <60% Not Effective [13].

3. Results and Discussions

The results of the identification test for *Staphylococcus aureus* with the characteristics of β-Hemolysis, coagulase (+), catalase (+), and DNAse (+), are

presented in Figure 2. These characteristics correspond to the identification characteristics of *Staphylococcus aureus*.

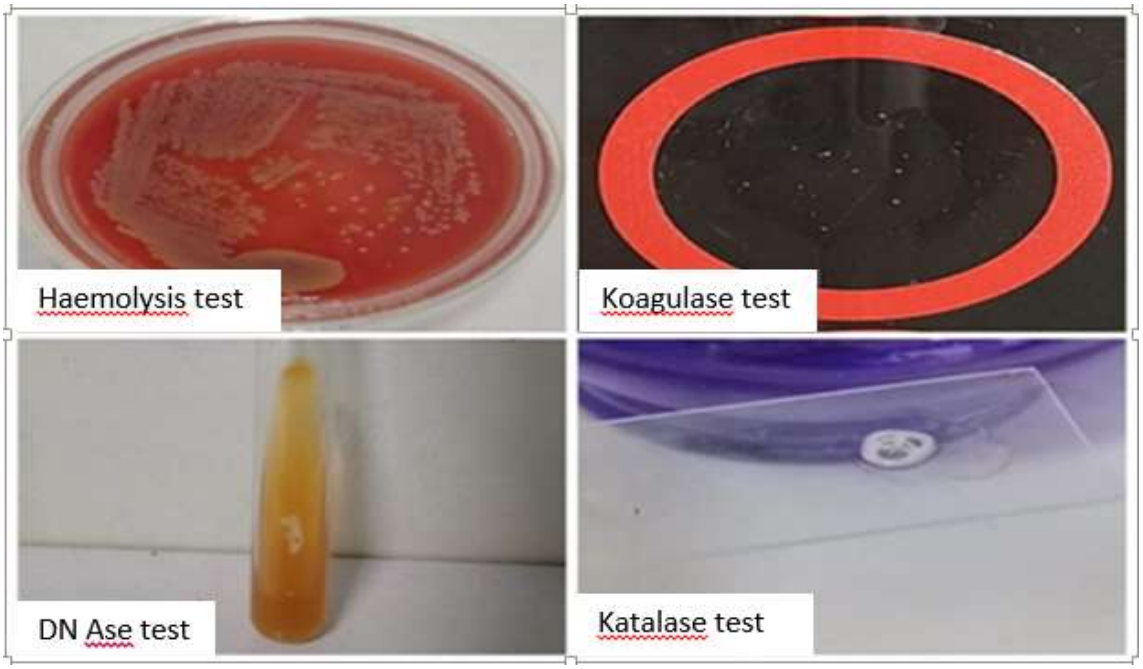
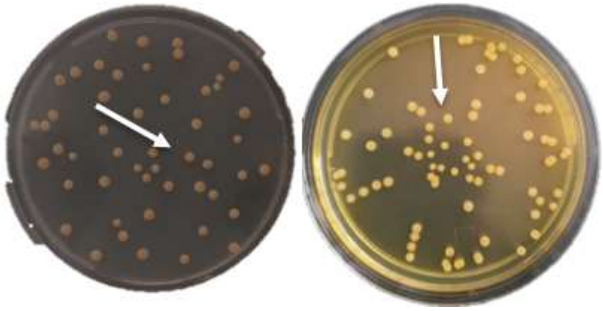


Figure 2. Biochemical test of *Staphylococcus aureus*.



A B

Figure 3. The result of a colony of *Staphylococcus aureus* bacteria: A. alternative B. Agar nutrients

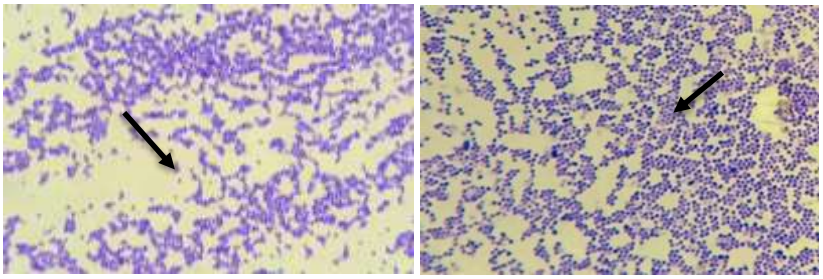


Figure 4. Morphology of *Staphylococcus aureus* Bacterial Cells Growing on Media: (A) Alternative (B) Nutrient Agar

Table 1. Calculation of the Number of Colonies and Measurement of the Colony Diameter of *Staphylococcus aureus* Bacteria on Alternative Media and Nutrient Agar Media

	Alternatif media		*Nutrient Agar Media*	
	Number of colonies (x10^4 CFU/ml)	Colony diameter (mm)	Number of colonies (x10^4 CFU/ml)	Colony diameter (mm)
Minimum	65	2,45	82	2,55
Maximum	88	2,72	115	2,79
Average	78,00	2,56	94,19	2,66

Source: Primary data (2021)

Table 2. Percentage of the effectiveness of growth number of colonies and the diameter of the staphylococcus aureus bacteria colony

	Percentage of effectiveness	Criteria
Number of colonies	82,81%	Quite effective (80-90%)
Colony diameter	96,47%	Effective (90-100%)

Source: Primary data

Observation of the morphology of *Staphylococcus aureus* bacterial colonies was carried out by comparing colony growth on alternative media and nutrient agar media macroscopically and microscopically. The results of macroscopic observations of the morphology of *Staphylococcus aureus* bacterial colonies are shown in Figure 3. Meanwhile, the results of microscopic observations of the morphology of *Staphylococcus aureus* bacterial colonies are shown in Figure 3.

Table 1 shows that the number of colonies and colony diameter on alternative media is less than on Nutrient Agar media, but the difference is not too much. Table 2 shows that the results of the percentage of effectiveness on the alternative media for the number of colonies were quite effective; however, for the percentage of colony diameter, the results were effective. This can be seen from the size of the colony diameter on the alternative media, which is not much different from the size of the colonies growing on NA media. A good media contains water to maintain humidity and exchange of substances or metabolism and contains a source of carbon, minerals, vitamins, and gas. And osmotic pressure is isotonic, and the degree of acidity (pH) is usually neutral but also available alkaline, with an appropriate temperature, and is sterile [14]. The media commonly used for bacterial growth in the laboratory is Nutrient Agar (NA) media which is made from beef extract, peptone and yeast [3]. The content of this medium is replaced with a mixture of sweet potato flour, soybean flour, and yeast extract. Sweet potatoes contain carbohydrates, fat, protein, and minerals but have a high calcium content [15]. Soybeans have a high protein content. Yeast extract has a high nitrogen content. The content in the replacement media is able to support bacterial growth because the nutrients the bacteria need are already contained in the

media. Bacteria require the bare minimum of resources to develop, including water, a carbon source, a nitrogen source, and certain mineral salts. Water is essential for the solubilization, transportation, and maintenance of hydrolysis reactions of nutrients. For certain bacteria to flourish, free water is necessary. In bacteria, carbon is the most prevalent component element. The production of carbon compounds by bacteria, including proteins, lipids, carbohydrates, and nucleic acids, is vital. Regarding sources of nitrogen, many of them are present in many of the substances that go into making a culture medium. It can be discovered in the organic form, which corresponds to protein hydrolysates; in particular, tryptone or protease-peptone in the case of hydrolysate, but nitrates in the inorganic form. Bacteria need nitrogen to synthesize their proteins [16]. Alternative media are highly effective in promoting the growth of Staphylococcus, as indicated by the number of colonies and their respective diameters.

This study obtained the average number of *Staphylococcus aureus* bacterial colonies 78,00 x 10^4 CFU/ml and the average colony diameter of 2,56 mm. This result is from previous research, namely using sweet potato flour and soybeans as alternative media for Escherichia coli and *Staphylococcus aureus* bacteria growth. The results showed that the average number of colonies was 213.31 x 10^5 CFU/ml for Staphylococcus aureus bacteria and 177.37 x 10^5 CFU/ml on Escherichia coli bacteria [10] This research has similarities: the ingredients used are sweet potatoes and soybeans, but the only difference is the dilution of the suspension and the addition of yeast extract. The results obtained are from previous research on a mixture of sweet potato infusion (*Ipomoea batatas*), soybean infusion (*Glycine max (L.) Merrill*), and yeast extract as an alternative media for the growth of Escherichia coli bacteria. Soy toxin (SBTX) is a protein isolated from soybeans and composed of two polypeptide subunits (17 and 27 kDa). SBTX had in vitro activity Phytopathogenic against the fungus. Test results of previous study soybean toxin has not affected the *Staphylococcus aureus* [17]. The research showed that the average number of colonies was 110 x 10^4 CFU/ ml, and the average colony diameter was 3.57 mm. The comparison of the results obtained is not much different because the growth and colony size of Escherichia coli bacteria is greater than that of *Staphylococcus aureus* bacteria [11]. The results of previous research on making alternative media using white sweet potatoes on *Staphylococcus aureus* showed the number of bacterial colonies was 125 x 10^6 CFU/ml. The number of colonies on alternative media with white sweet potatoes was greater than with NA. White sweet potatoes contain a type of raffinose carbohydrate, which is composed

of fructose, glucose, and galactose and can be used as an energy source by bacteria [18]. Past research shows the growth of colonies with a round shape, and medium size, the growth of *Staphylococcus aureus* bacteria is fertile, the color of the colonies is milky white, the colonies are clearly visible and easy to observe [3]. The results of this study showed the same thing, but the colony size and color were better. The size obtained was medium to large, and the color of the colonies obtained was yellowish-white after 2 x 24-hour observations. This result occurred because the materials used were more complex than previous research.

4. Conclusions

Alternative media from a blend of yam, soy infuse, and yeast extract are effectively used for the growth of the *Staphylococcus aureus* bacteria with the colony's diameter of alternative media 2.56 mm and an average number of *Staphylococcus aureus* bacteria that grow on alternative media 78.00 x 10^4 CFU /ml.

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