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Morphological Analysis and Biochemical Properties of Staphylococcus aureus Cultures Grown on Alternative Media Peanut Flour (Arachis Hypogaea L.)

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ABSTRACT

Sources of nutrition especially sufficient protein, is needed for the growth of Staphylococcus aureus bacteria. Synthetic media is the most common medium for growing Staphylococcus aureus. Peanut is a biological source in nature with a higher protein content than synthetic materials which can be used as an alternative. Objective to determine the morphology and biochemical properties of Staphylococcus aureus bacteria grown on alternative media of peanut flour (Arachis hypogaea L.) as a medium for bacterial growth. This is a True-Experiment study with a completely randomized design. The experimental unit consists of five variations in the concentration of peanut alternative media. The sampling technique was done by simple random sampling. Data analysis used Descriptive. Staphylococcus aureus bacteria can grow at all concentrations of alternative media of peanut flour. The colony size at concentration of 2% was very small with weakly fermented mannitol. Meanwhile a concentration of 3% to 5% produces moderately fermented mannitol. The concentration of 6% is the concentration with the most widely fermented mannitol. The biochemical tests of all concentrations yielded positive catalase and coagulase, and the sugar test yielded positive reactions. An alternative medium of peanut flour can be used as a medium for the growth of Staphylococcus aureus bacteria with the most effective concentration of 6%.

INTRODUCTION

*Staphylococcus aureus*It is a commensal and pathogenic bacteria in humans. About 30% of the human population is infected with Staphylococcus aureus. Generally, these bacteria are found on the skin, respiratory tract and digestive tract without causing health problems (Zeller & Golub, 2011). There were approximately 18,650 cases of infection by Staphylococcus aureus which resulted in death from the 94,000 cases of infection that occurred overall in America. The prevalence of Staphylococcus aureus infection is also quite high in Asia, reaching 70% in 2007. Meanwhile in Indonesia in 2006 the prevalence reached 23.5%. In the 2016 study of germ patterns and resistance to antibiotics at NTB Hospital, 37

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bacteria were identified, 5 of which were Staphylococcus (Diyantika et al., 2017; Pristianingrum et al., 2021).

Identification of Staphylococcus aureus bacteria is carried out by culture, namely by growing the bacteria on a special media. Testing growth properties, calculating the number of microbes, and the process of isolating microorganisms requires suitable media as nutrients for their growth and reproduction (Thohari et al., 2019; Rezekika & Harianto, 2019). Several previous studies have succeeded in making alternative media for the growth of microorganisms from legumes, namely green beans, red beans, cowpeas, and soybeans (Thohari et al., 2019; Nuryati & Sujono, 2017; Suhartati et al., 2018 ; Hijriyanti, 2020).

Another type of legume that has a fairly high protein content is peanuts. Peanut (Arachis hypogaea L.) is the second most important legume commodity after soybeans in Indonesia. Peanuts are rich in fat, high protein, iron, vitamin E, vitamin B complex, phosphorus, vitamin A, vitamin K, lecithin. , choline, and calcium. The protein content of peanut seeds is a parameter that determines the nutritional quality of the seeds. Peanut seeds contain 40–48% oil, 25% protein, and 18% carbohydrates and complex B vitamins (Zulchi & Puad, 2017).

Based on this description, the authors are interested in conducting research on the analysis of the morphology and biochemical properties of Staphylococcus aureus culture when grown on alternative media peanut flour (Arachis hypogaea L) as a substitute for beef extract and bacto peptone on MSA media.

MATERIALS / METHODS

This research is a True Experiment research which aims to determine a symptom or effect that arises as a result of the treatment. The research design used was a Completely Randomized Design (CRD) with 5 different treatments of peanut flour as an alternative medium for the growth of Staphylococcus aureus. The sampling technique in this study was Simple Random Sampling with the sample criteria namely ripe peanut seeds, undamaged peanut seeds, and freshly made peanut flour used. The data in this study used primary data obtained from microscopic and macroscopic examinations to determine the colony morphology and morphology of growing bacteria based on the results of planting at each alternative media concentration. While the biochemical properties of bacteria were obtained from the catalase test, coagulase test and sugar test which included glucose, maltose and lactose tests. Research data were analyzed descriptively.

RESULTS AND DISCUSSION

Based on the research that has been done, macroscopic and microscopic observations of colonies were obtained. Microscopic observations were made by making Gram stains to see the morphology of the bacteria which were then observed under a microscope with an objective magnification of 100x shown in table 1 below.

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Table 1. Macroscopic and Microscopic Observations of Colony Morphology of Staphylococcus aureus on Alternative Media and Control Media

Variation	Observation of Bacterial Colony Morphology	
	Macroscopic	Microscopy
Control	Round shape Small size Color: Muddy Yellow (mannitol broad fermenter) Edge : Flat Elevation : Convex Surface : Smooth Consistency : Soft	Round shape Purple Arrangement: Clustered Gram properties: Coccus Grams Positive
2%	Round shape Size : Very Small Color: Muddy Yellow (weak fermenter mannitol) Edge : Flat Elevation : Convex Surface : Smooth Consistency : Soft	Round shape Purple Arrangement: Clustered Gram properties: Coccus Grams Positive
3%	Round shape Small size Color: Muddy Yellow (moderate fermenter mannitol) Edge : Even Elevation : Convex Surface : Smooth Consistency : Soft	Round shape Purple Arrangement: Clustered Gram properties: Coccus Grams Positive
4%	Round shape Small size Color: Muddy Yellow (moderate fermenter mannitol) Edge : Flat Elevation : Convex Surface : Smooth Consistency : Soft	Round shape Purple Arrangement: Clustered Gram properties: Coccus Grams Positive
5%	Round shape Small size Color: Muddy Yellow (moderate fermenter mannitol) Edge : Flat Elevation : Convex Surface : Smooth Consistency : Soft	Round shape Purple Arrangement: Clustered Gram properties: Coccus Grams Positive

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Variation	Observation of Bacterial Colony Morphology	
	Macroscopic	Microscopy
6%	Round shape Small size Color: Muddy Yellow (mannitol broad fermenter) Edge : Flat Elevation : Convex Surface : Smooth Consistency : Soft	Round shape Purple Arrangement: Clustered Gram properties: Coccus Grams Positive

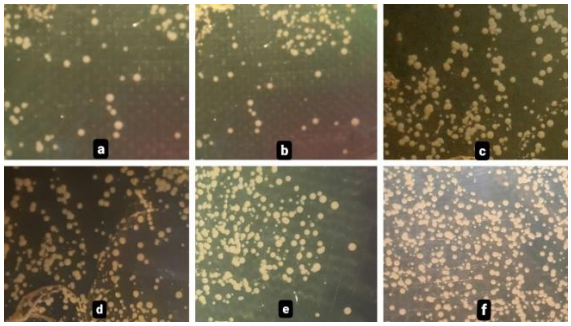

Figure 1. Macroscopic Staphylococcus Colony aureus in Alternative Media and Control Media

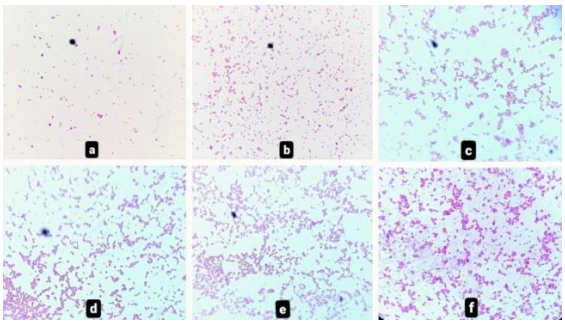

Figure 2. Macroscopic Staphylococcus Colony aureus in Alternative Media and Control Media

Information:
(a) MSA media made from 2% peanut flour
(b) MSA media made from 3% peanut flour
(c) MSA media made from 4% peanut flour
(d) MSA media made from 5% peanut flour
(e) MSA media made from 6% peanut flour
(f) Control MSA media.

Based on table 1 on the observation of colony morphology, it shows that at a concentration of 2% Staphylococcus aureus bacteria can grow but the distribution is uneven and the size is very small and the mannitol fermenter zone is weak. Whereas at a concentration of 6% the size of the small bacterial colonies was almost the same as the growth of the colonies on the control media and had the widest fermenter zone. The results of Gram staining showed that the Staphylococcus aureus bacteria were purple in color and round in shape like grape strands.

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Table 2. Results of Observation of Biochemical Properties of Staphylococcus aureus Bacteria in Alternative Media and Control Media.

Variation	Biochemical Properties Test				
	Affirmation Test		Sugar Test		
	Catalase	Coagulase	Glucose	Maltose	Lactose
Control	Positive	Positive (agglutination occurs)	+	+	+
2%	Positive	Positive (agglutination occurs)	+	+	+
3%	Positive	Positive (agglutination occurs)	+	+	+
4%	Positive	Positive (agglutination occurs)	+	+	+
5%	Positive	Positive (agglutination occurs)	+	+	+

Note: (+) = positive, fermentation occurs because the color changes from purple to yellow

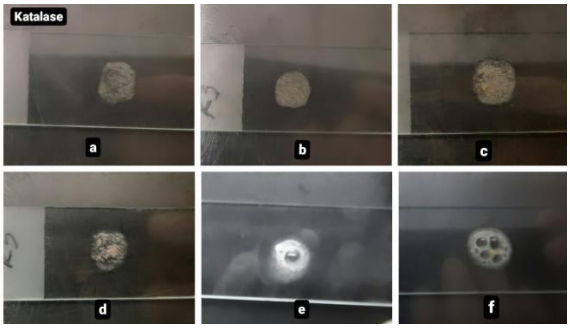

Figure 3. Catalase Test in Media Colonies Colonies Alternative and Control Media

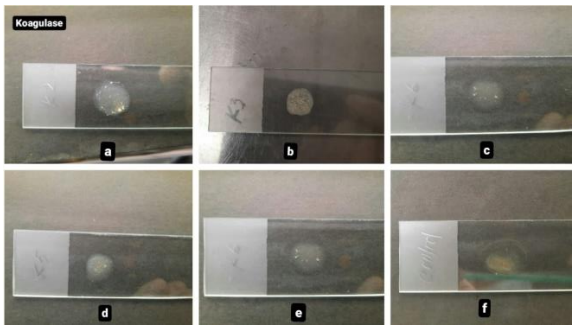

Figure 3. Catalase Test in Media Alternative and Control Media

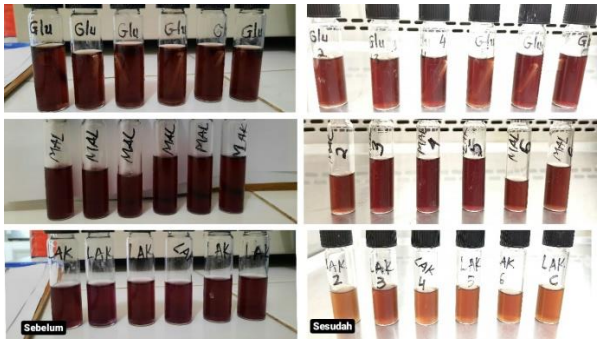

Figure 5. Sugar Test on Alternative Media Colonies and Control Media

Information:
(a) MSA media made from 2% peanut flour
(b) MSA media made from 3% peanut flour
(c) MSA media made from 4% peanut flour
(d) MSA media made from 5% peanut flour
(e) MSA media made from 6% peanut flour
(f) Control MSA media.

Table 2 shows positive coagulase test results which are characterized by the formation of agglutinations such as fine sand lumps after being homogenized with citrate plasma. All sugar tests showed a positive reaction which was indicated by a change in the color of the medium from purple to yellow and the formation of gas in the Durham tube which was placed upside down in the sugar medium.

Macroscopically the growth and development of Staphylococcus aureus bacteria looks optimal and is not much different from standard media. This can be assessed based on the size, shape of the colony, color, elevation, surface margins and the nature of the mannitol fermenter of the colonies growing on the media. The results on the MSA control medium with the morphological characteristics of the colonies are round, small in size, convex elevation, smooth surface with flat edges. The results of Gram staining revealed the morphological characteristics of the purple colored bacteria, which means they are members of Gram positive bacteria and have a clustered coccus shape. The purple color is caused by the bacteria retaining the first color, which is crystal violet.

The bacterial colonies produced from the control MSA media were small, most of which were of equal size. Colonies from alternative media at concentrations of 2%, 3%, 4% and 5% had smaller colony sizes and a small number of colonies that were similar in size to the size of the colonies on control MSA media. Whereas at a concentration of 6% the size of the colonies resembled the colonies of MSA media with the widest mannitol fermenter zone. This is because the alternative media concentration of 6% has more protein content than other concentrations. The size of the colonies produced at all concentrations of the alternative media was not evenly distributed, there were still smaller colonies with a significant number. This can be caused because the nutrients in alternative media are not optimal for the growth of Staphylococcus aureus bacteria.

Peanuts have a relatively high protein and carbohydrate content, peanuts also contain essential amino acids, one of which is lysine so that bacteria can grow in alternative media made from peanuts (Zamilah et al., 2020). The duration of heating when processing flour into peanut extract, namely by heating 100oC for 5 minutes, is considered insufficient to dissolve the nutrients in peanut flour. The nutrients in peanut flour that have not dissolved are also filtered out in the filtering process. During the heating process, protein heating occurs in peanuts which can cause denaturation reactions, loss of enzyme activity, changes in solubility, changes in color, amino acid residues, and breaking of peptide bonds. This reaction is affected by temperature and heating time. This reaction also causes protein levels to decrease (Sundari et al., 2015). As a result, if the nutritional content of peanuts decreases, it can slow down the growth of bacteria. Because if the nutrients needed by the bacteria are sufficient, it will accelerate the growth of bacteria, and vice versa if the nutrients needed are not sufficient, the bacteria must adapt to the environment and the formation of enzymes to break down the substrate which takes a longer time (Zamilah et al., 2020).

This research is in line with previous research conducted by Suhartati et al (2018) regarding the use of soybean powder as an ingredient for making mannitol salt agar media

for the growth of Staphylococcus bacteria. The results showed that the most suitable concentration for the growth of Staphylococcus bacteria was a concentration of 6%. In addition, another study by Novitasari et al (2019) regarding the potential of anchovy as an alternative medium for the growth of Staphylococcus aureus bacteria showed that the most suitable concentration for the growth of Staphylococcus bacteria was a concentration of 6%.

Some of the weaknesses of this study were seen in the filtering process of peanut flour extract which was less effective resulting in alternative media that were made to appear more turbid and appear not clear, making it difficult to observe the bacterial colonies macroscopically. In addition, alternative media for peanuts also appear reddish-orange in color. This is because the turbidity produced from the concentrated peanut flour extract causes the phenol red added to the media to appear less red and produce an orange color.

CONCLUSIONS

Based on the results of the study it can be concluded that alternative media for peanut flour can be used as a growth medium for Staphylococcus aureus bacteria with the most effective concentration of 6%.

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