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THE EFFECT OF JICAMA (*Pachyrhizus erosus*) CONCENTRATION ON THE GROWTH OF *Staphylococcus aureus* BACTERIA IN SOLID MEDIA

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Abstract

Background*: Synthetic media for microbial growth are expensive, thus opening the way for making bacterial growth media using local raw materials at low prices. Jicama is one of the local raw materials that can be used as natural media for the growth of microorganisms. It contains a source of carbohydrates needed for the development of microorganisms.* ***Aims****: This study aims to determine the effect of variations in the concentration of Jicama, which has the potential to grow Staphylococcus aureus bacteria.* ***Methods****: This study used experimental research with the Static Group Comparison method. In this study, the experimental group Staphylococcus aureus was instilled in Jicama solid media with various concentrations (2%, 3%, 4%, 5%, 6%) with five repetitions.* ***The results****: The results showed that the average number of Staphylococcus aureus bacteria colonies in jicama flour with a concentration of 2% was 0, a concentration of 3% was 0, a concentration of 4% was 0.8, a concentration of 5% was 1.4, and at a concentration of 6% was 2.2. The results of the Kruskal-Wallis test with a degree of confidence of 0.05 obtained a p-value = 0.000 where the p-value <0.05 so that it can be concluded that there is a significant effect of variations in the concentration of jicama flour on the growth of Staphylococcus aureus bacteria.* ***Conclusions****: For future researchers, it is necessary to conduct further research using higher concentrations of jicama flour and by adding other nutrients, such as animal protein, so that the growth of Staphylococcus aureus bacteria is more optimal.*

Keywords: Jicama natural media (*Pachyrhizus erosus*), *Staphylococcus aureus*

1. Introduction

Bacteria need adequate space and nutrients to grow and develop. Within the scope of the microbiology laboratory, bacteria can be grown on a growth medium (1). One of the bacteria that is often used for microbiological research is *Staphylococcus aureus*. According to (2), these bacteria are included in the normal flora in the oral cavity. They are Gram-positive bacteria that are round in clusters resembling grapes, produce a yellow pigment, produce spores, and generally grow with a diameter of around 0.8-1.0 µm.

Research by (3) said that *Staphylococcus aureus* bacteria quickly grow in most laboratory media.

Based on the constituent materials, the media is divided into two types, namely synthetic media, and natural media. Synthetic media, namely media consisting of ingredients whose composition is known, such as Nutrient Agar media. Natural media, namely media consisting of natural ingredients such as potato extract, carrot juice and tubers (4). The Nutrient Agar medium contains peptone, yeast and beef extract, which function as nitrogen and carbon sources, vitamins, and several other compounds to support bacterial growth. But for now, instant media is quite expensive, ranging from IDR 500,000 to IDR 1,500,000 for every 500 per gram so that the composition of the growth media can be manipulated for the purpose of isolating the identification of certain bacteria according to their respective goals (5).

The optimum growth medium for *Staphylococcus aureus* bacteria requires amino acids. These bacteria cannot grow on media that do not contain amino acids or proteins. Apart from amino acids or protein, *Staphylococcus aureus* also requires growing vitamins (6). Jicama (*Pachyrhizus erosus*) is a tuber that contains starch (7). Based on (8), Jicama contains various vitamins, including A, B and C. Jicama tubers in 100 grams contain 90.07 grams of water, 38 kcal of energy, 0.72 grams of protein, total fat 0.09 grams, 8.82 grams of carbohydrates, 4.9 grams of fibre, 1.8 grams of total sugar.

Jicama contains nutrients needed for the growth of microorganisms, some of which are protein, vitamins, energy, total fat, carbohydrate, calcium, phosphorus, and iron. The microorganism that want's to be cultured on Jicama media is *Staphylococcus aureus*, which is a Gram-positive coccus bacterium. This study aims to determine whether Jicama can be a natural growth medium for *Staphylococcus aureus* bacteria.

There have been many studies on alternative media for bacterial growth, one of which was a thesis entitled "Tofu dregs flour as a growth medium for Serratia marcescens bacteria" which is the author's reference. The difference in this study lies in the type of natural media used, the concentration and the bacteria tested. In previous studies using alternative media tofu dregs flour with concentrations of 6% w/v, 7% w/v, 8% w/v, 9% w/v, and 10% w/v using Serratia marcescens bacteria. This study uses natural media from yam flour with a concentration of 2%, 3%, 4%, 5%, and 6% using *Staphylococcus aureus* bacteria (9).

2. Research Methods

This study uses a true experimental research type, namely a research method used to find the effect of certain treatments

on others under controlled conditions (10). In this study, the data obtained will be presented in the form of tables, pictures, and descriptive explanations. The tools used in this research were knives, analytical scales, ovens, pH meters, vortexes, tube needles, dropper pipettes, autoclaves, Bunsens, incubators, Petri dishes, 100-mesh sieves, hotplates, stirring rods, Erlenmeyers, and measuring cups. The materials used in this research were fresh jicama, agar, distilled water, nutrient agar, and physiological NaCl. The microbe used is a pure bacterial culture of *Staphylococcus aureus*.

The first stage of work is making jicama flour by washing it thoroughly, peeling the skin, cutting it thinly, and drying it in the oven at 100 ^{O}C degrees until dry. Then, the dried jicama is ground with a blender to produce flour and sifted with a 100-mesh sieve. The second stage is making jicama flour media by sterilizing the equipment first at a temperature of 100 – 160^{O}C, weighing 2 grams, 3 grams, 4 grams, 5 grams, and 6 grams of jicama flour and adding 2 grams to the Erlenmeyer flask, then dissolving it with 100 mL of distilled water on a hot plate and checked for pH, then the media was sterilized using an autoclave at a temperature of 121^{O}C, 2 atm pressure for 15 minutes, then five media were made for each concentration and 15 - 20 mL of the media was poured into Petri dishes. The third stage is inoculating *Staphylococcus aureus* bacteria using the scratch method, which involves scratching as many lines as possible on the media using a loop needle. Incubation is carried out at a temperature of 37 ^{O}C for 2 x 24 hours.

Table 1. Description of the growth of *Staphylococcus aureus* bacteria at various concentrations of yam bean flour media

Repetition Sample	**Number of Bacterial Colonies Growing at Concentration of Jicama Flour**				
	2%	3%	4%	5%	6%
1	0	0	1	1	2
2	0	0	1	1	2
3	0	0	0	1	2
4	0	0	1	2	3
5	0	0	1	2	2
Total	0	0	4	7	11
Averege	0	0	0.8	1.4	2.2

Source: Primary Data (2022)

Table 2. Shapiro-Wilk Data Normality Test Results

	Variable	p-value
Staphylococcus aureus	2%	-
	3%	-
	4%	.000
	5%	.006
	6%	.000

Source: Primary Data (2022)

Table 3. Kruskal-Wallis Non-Parametric Test Results

Variable	Concentration of Purple Sweet PotatoFlour	N	Mean Ranking	p-value
Staphylococcus aureus bacteria colonies on Jicama media	2%	5	6.00	0.000
	3%	5	6.00	
	4%	5	13.20	
	5%	5	17.60	
	6%	5	22.20	
	Total	25		

Source: Primary Data (2022)

3. Results and Discussions

Table 1 shows that *Staphylococcus aureus* bacteria colonies did not experience growth in Jicama flour media at concentrations of 2% and 3%, while at concentrations of 4%, 5%, and 6%, there was growth of *Staphylococcus aureus* bacteria. Repetition of samples five times showed differences in the number of *Staphylococcus aureus* bacteria colonies that were not too far away at concentrations of 4%, 5%, and 6%. It can be seen at concentrations of 2% and 3%, where no *Staphylococcus aureus* bacteria colonies grew, and this could be due to the lack of vegetable protein at concentrations of 2% and 3% so that nutrition was not sufficient, which resulted in *Staphylococcus aureus* bacteria not growing at these concentrations. At a concentration of 4%, the average bacterial colonies grew by 0.8, at a concentration of 5% by 1.4, and at a concentration of 6% by 2.2. This was due to vegetable protein at these concentrations being greater so that *Staphylococcus aureus* bacteria could grow even though not optimal. The protein content in the Nutrient Agar media is as much as 98%. Besides that, the type of protein in Nutrient Agar is animal protein, while the type of protein in Jicama is vegetable protein.

Growth that is not optimal can be caused by the presence of growth-inhibiting substances contained in the media. This is in accordance with (11), namely several conditions for the media to be regarded as a medium for the growth of microorganisms, one of which is that the media does not contain substances that inhibit the growth of microorganisms. In addition, research conducted by (12) showed that Jicama contains vitamin C, B1, protein and phenolic compounds, which can function as antibacterials. Based on (13), the phytochemical test of yam ethanol extract was positive for containing lots of flavonoids, alkaloids, terpenoids, tannins and saponins. (14) said that flavonoids, saponins, terpenoids and alkaloids are compounds that have potential as antibacterials. The mechanism of action of flavonoids damages cell membranes and denatures bacterial cell proteins irreparably. Saponins damage the permeability of cell membranes so that essential components in bacterial cells will come out in the form of nucleic acids, proteins, and nucleotide acids. Terpenoids interfere with the formation of membranes and bacterial cell walls so that the two organs are not formed properly. Based on the theory that has been mentioned, the antibacterial compounds contained in Jicama also play a role in the process of inhibiting *Staphylococcus aureus* bacteria so that the number of colonies that grow is small and small in size.

Other factors that affect bacterial growth include nutritional factors, temperature, pH and osmotic pressure (15). Nutrition affects the development of *Staphylococcus aureus* bacteria. Nutrients can be used as a source of energy, carbon, sulfur, nitrogen, phosphorus, minerals, and vitamins. Yam tubers contain lots of fibre, vitamin C, and minerals such as calcium, potassium, and phosphorus (16). According to the Indonesian Food Composition Table 2017, the nutrient content in 100 grams of Jicama is energy of 59 Kcal, protein 1.4 g, fat 0.2 g, carbohydrates 12.8 g, calcium 15 g, phosphorus 18 mg, vitamin B1 0.4 mg, 20 mg vitamin C, 0.5 IU vitamin A, 0.6 mg iron, 85.1 g water (17). Based on research conducted, the growth of *Staphylococcus aureus* bacteria on Jicama flour media is round, small in size, yellowish white in colour and tends to have a slow growth rate, and this is due to the inadequate nutritional content of Jicama flour, one of which is protein. The protein contained in Jicama is vegetable protein.

According to (18), temperature determines the activity of enzymes involved in chemical activity. At the optimal growth temperature, there will be an optimal growth rate, and the maximum number of cells will be produced. The temperature for growing *Staphylococcus aureus* is 28-38^0

C, but *Staphylococcus aureus* grows optimally at 37ºC (19). The best growth of *Staphylococcus aureus* is in an aerobic atmosphere, which is facultatively anaerobic, and the optimum pH for growth is 7.4 (15). The growth of bacteria will be hampered at a low pH because bacteria need a neutral environment with a pH of 7.0 (20). In the manufacture of yam flour media, the pH was 7 with an incubator temperature of 37ºC so that the media preparation was in accordance with the literature, so it can be seen that temperature and pH are not factors that affect growth in this study. The media must be sterile before use so that microorganisms can grow properly. In the laboratory, sterilize the media using an autoclave at 121ºC with a pressure of 1 atm for 15 minutes (11). The media sterility test was carried out by incubation at room temperature for 2x24 hours, and all concentration variations did not show the presence of bacterial growth or other contaminating agents, which indicated that the yam bean flour media was sterile.

General description of the results of research conducted to grow *Staphylococcus aureus* bacteria with yam flour media at concentrations of 2%, 3%, 4%, 5%, and 6%. The inoculation method used was a streak plate with an incubation time of 2x24 hours at room temperature (37ºC). The research results are shown in Table 1.

In accordance with the research objective to determine the effect of the concentration of yam flour on the growth of *Staphylococcus aureus* bacteria, the data obtained from the calculation of the number of colonies at each concentration was processed and statistically tested. The data obtained was tested for the normality of the data and was used to find out whether the data was normally distributed or not. The data normality test used the Shapiro-Wilk test because the number of samples was < 50. Based on the results of the Shapiro-Wilk test (Table 2), the p-value was <0.05, which means that the data distribution was abnormal, so the statistical test used was the Kruskal-Wallis test.

Based on Table 3, the results of the Kruskal-Wallis non-parametric test for the research data were 0.000 (p <0.05). Thus, it can be stated that there is a significant effect of the concentration of yam flour on the growth of *Staphylococcus aureus* bacteria.

The results of this study were processed and analyzed using a non-parametric statistical test, namely Kruskal-Wallis obtained a significance value of 0,000 with a 95% confidence level. This indicates that there is a significant effect of variations in the concentration of yam bean flour on the growth of *Staphylococcus aureus* bacteria. The greater the concentration of jicama flour, the more

nutrients such as vegetable protein, carbohydrates, minerals, and others, thus making the growth of *Staphylococcus aureus* bacteria better.

4. Conclusions

From the results of research on making bacterial growth media using yam flour with different concentration variations have been carried out, the conclusions are as follows:

a. The average number of *Staphylococcus aureus* bacteria colonies in Jicama media with concentrations of 2%, 3%, 4%, 5%, and 6% were 0, 0, 0.8, 1.4, and 2.2.

b. *Staphylococcus aureus* bacteria colonies, as indicated by the value of p = 0.000 ($p < 0.05$).

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