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Utilization Of Coconut Dregs As An Alternative Medium For The Growth Of Bacillus Subtilis And Escherchia Coli

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ABSTRACT

Coconut dregs are waste produced after processing coconuts into coconut oil. Coconut pulp has a high nutritional content which can be used for bacterial growth. The nutritional content of coconut dregs is 38.1% carbohydrates, 5.6% crude protein, 16.3% crude fat, 31.6% crude fiber, 2.6% ash content and 5.5% water content. The research used coconut dregs as an alternative medium for the growth of gram-negative and gram-positive bacteria. In the trials, B. subtilis and E. coli bacteria were used. The type of research used was a true experiment with a completely randomized design. The results of the research showed that the highest average number of colonies on the coconut dregs alternative media was in treatment A1, namely the average of B.subtilis colonies was 50.6 x 10^2 CFU/mL and E.coli 36 .6 x 10^2 CFU/mL while the control media averaged B.subtilis colonies 75 x 10^2 CFU/mL and E.coli 86.3 x 10^2 CFU/mL. The results of statistical tests using anova $p<0.05$ with a sig value of 0.001 show that there is an influence of differences in the concentration of coconut dregs and agar as an alternative medium for bacterial growth. The post hoc test compares the concentration between treatment A1 and control with a sig p value of >0.05 (0.250), namely There is no significant difference, while the concentration between treatments A2, A3 and A4 with control has a sig p value <0.05, namely 0.000, meaning there is a significant difference. So treatment A1 is the best treatment for the growth of B.subtilis and E.coli bacteria.

INTRODUCTION

Growth media is a material or substance that can be used for the breeding of microorganisms. As a place to grow and develop, the availability and needs of microbes must be guaranteed in order to grow and develop. The substrate must contain nutrients and oxygen necessary for bacteria. Nutrients necessary for the growth of microorganisms require metallic elements such as sodium, potassium, calcium, magnesium, manganese, iron, zinc, copper, phosphorus, cobalt, hydrogen, oxygen, and sulfur (Thohari et al., 2019)

Encouraging researchers to develop bacterial growth media made from natural ingredients at a more affordable cost is an important initiative. The ingredients chosen must contain nutrients essential for bacterial growth, such as abundant

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carbohydrates and proteins. This is because carbohydrates are a major component in bacterial metabolism, and almost half of the dry weight of bacteria is carbon, which is usually present in carbohydrate compounds. Therefore, carbohydrates play an important role in supporting bacterial growth. In addition, various protein sources have also been proven to be successfully used as alternative growth media for microorganisms (Juriah &; Sari, 2018)

Coconut pulp is the rest of the coconut processing process or coconut oil production. In general, this waste is often not used efficiently by the community and is usually only used as animal feed. However, further research has proven that coconut pulp actually contains very high nutritional value. Its composition includes 38.1% carbohydrates, 5.6% crude protein, 16.3% crude fat, 31.6% crude fiber, 2.6% ash content, and 5.5% water content. This nutrient content has great importance in supporting bacterial growth (Azis &; Akolo, 2018)

Some researchers have succeeded in applying microorganism growth media derived from available natural resources (Suhartati &; Nuraini, 2018). One example of this is the use of legumes, especially soybeans, which have a fairly high protein content, as an alternative medium to support bacterial growth. Based on research conducted by Anisah in 2015, it is known that bacteria such as S.aureus and E.coli can be cultured successfully using media made from gembili tuber extract, arrowroot tuber, and canna tuber. In addition, the bacterium Bacillus sp. can also grow well on media made from tofu liquid waste (Juriah &; Sari, 2018)

The purpose of this study is to utilize coconut pulp as an alternative medium for the growth of bacteria Bacillus subtilis and *Escherchia coli* and to determine the difference in the growth rate of *Bacillus subtilis* and *E.Coli* in alternative media coconut pulp with nutrient agar media (control)

MATERIALS/METHOD

This study is a true experiment with a complete randomized design conducted at the Maharani Malang Stikes Laboratory in the period July 3-13, 2023. The research sample was selected by simple random sampling method.The data collected are primary data obtained from the implementation of treatment of samples. Data analysis was performed using ANOVA test using SPSS software version 25

RESULTS AND DISCUSSION

This study began with a preliminary trial phase involving variations in the concentration of coconut pulp and agar as an alternative growth medium for *Bacillus subtilis* and *Escherichia coli*. The results of this preliminary test are represented in the following table.

Table 1.Preliminary Test Results of Making Alternative Media for Coconut Pulp for Bacterial Growth

Treatment	Media Test Results	Bacterial Growth				Nutrients
		B.Subtilis		*E.Coli*		
		24 Jam	48 Jam	24 Jam	48 Jam	

A1	Solid Slightly Soft	+	+	+	+	Carbohydrates ,protein and have a neutral pH.
A2	Solid Slightly Soft	-	+	+	+	
A3	Dense	-	+	-	+	
A4	Dense	-	+	-	+	
A5	Dense	-	-	-	-	
A6	Dense	-	-	-	-	

Information:
A1: coconut pulp flour 2.27gram + agar 1.5gram + aquades 200mL
A2: coconut pulp flour 3.27gram + agar 2.5gram + aquades 200mL
A2: coconut pulp flour 4.27gram + agar 3.5gram + aquades 200mL
A4: coconut pulp flour 5.27gram + agar 4.5gram + aquades 200mL
A5: coconut pulp flour 6.27gram + agar 5.5gram + aquades 200mL
A6: coconut pulp flour 7.27gram + agar 6.5gram + aquades 200mL

Based on table 1 preliminary,test result the concentrationof media thatis good for bacterial growth is in the treatment of A1,A2,A3 and A4

Table 2.Growth of *Bacillus subtilis* colonies in alternative medium of coconut pulp

Treatment	Number of colonies and repetitions			Average number of colonies (10^2 CFU/mL)
	I	II	III	
A1	63	52	37	50,6 x 10^2 CFU/mL
A2	48	36	18	34 x 10^2 CFU/mL
A3	12	7	6	8,3 x 10^2 CFU/mL
A4	5	3	2	3,3 x10^2 CFU/mL
Control (NA)	104	64	57	75 x 10^2 CFU/mL

Based on table 2 above, it shows that each treatment has a difference in the number of colonies of *bacillus subtilis* bacteria in alternative media of coconut pulp and also in control media (NA) which has the highest average number of colonies of 75x 10^2 CFU / mL, while in alternative media the highest average value in A1 treatment with an average of 50.6 x10^{-2} CFU / mL.

Table 3.Growth of *Escherichia coli* colonies on alternative media of coconut pulp

Treatment	Number of colonies and repetitions			Average number of colonies (10^2 CFU/mL)
	I	II	III	
A1	40	36	34	36,6 x 10^2 CFU/mL
A2	28	15	15	19,3 x 10^2 CFU/mL
A3	11	6	5	7,3 x 10^2 CFU/mL
A4	4	5	1	3,3 x 10^2 CFU/mL
Control (NA)	117	76	66	86,3 x 10^2 CFU/mL

Based on table 3 above,it shows that in each treatment there are differences in the number of colonies of escherchia coli bacteria in alternative media of coconut pulp and also in control media (NA) which has the highest average number of colonies of 86.3x10²CFU/mL,while in alternative media the highest average is found in A1 treatment with an average of 36.6x 10²CFU/mL

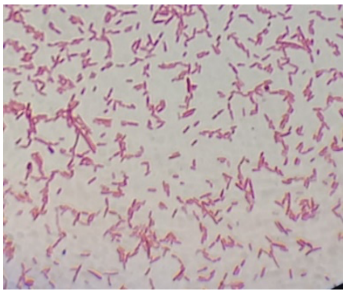

Figure 1. *Escherichia coli* rod-shaped morphology is red

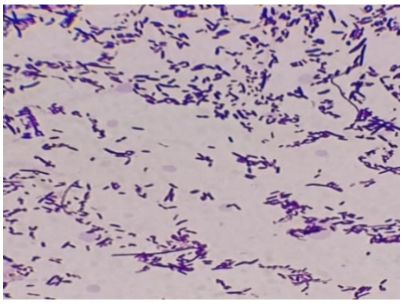

Figure 2. Bacillus *subtilis* bacteria purple rod-shaped morphology

Tests of Normality

		Shapiro-Wilk		
	Coconut pulp flour mass	Statistic	df	Itself.
Number of bacterial colonies	2,27gram	.939	3	.525
	3,27gram	.995	3	.863
	4,27gram	.840	3	.214
	5,27gram	.776	3	.058
	Check(s)	.847	3	.234

a. Lilliefors Significance Correction

Table 4.Test of *Normality* of Number of Colonies of *Bacillus subtilis* and *Escherichia coli* on Alternative Media of Coconut Dregs

Table 5. Test of homogeneity of colonies of *bacteria Bacillus subtilis* and *Escherichia coli*

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Itself.
Number of bacterial colonies	Based on Mean	1.887	2	7	.221
	Based on Median	.293	2	7	.755
	Based on Median and with adjusted df	.293	2	3.739	.762
	Based on trimmed mean	1.668	2	7	.256

Table *6.ANOVA* test of *Bacillus subtilis* and *Escherichia coli* on alternative media of coconut pulp

ANOVA

Media concentration

	Sum of Squares	df	Mean Square	F	Itself.
Between Groups	78770700.000	4	19692675.000	36.415	.001
Within Groups	2703900.000	5	540780.000		
Total	81474600.000	9			

Table 7.Uji post hoc multiple comparison

Multiple Comparisons

Dependent Variable: Media concentration

Bonferroni

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Itself.	95% Confidence Interval	
					Lower Bound	Upper Bound
A1	A2	23.333*	7.120	.031	1.42	45.25
	A3	35.833*	7.120	.000	13.92	57.75
	A4	40.333*	7.120	.000	18.42	62.25
	Control	-37.000*	7.120	.250	-58.92	-15.08
A2	A1	-23.333*	7.120	.031	-45.25	-1.42
	A3	12.500	7.120	.914	-9.42	34.42
	A4	17.000	7.120	.248	-4.92	38.92
	Control	-60.333*	7.120	.000	-82.25	-38.42

A3	A1	-35.833*	7.120	.000	-57.75	-13.92
	A2	-12.500	7.120	.914	-34.42	9.42
	A4	4.500	7.120	1.000	-17.42	26.42
	Control	-72.833*	7.120	.000	-94.75	-50.92
A4	A1	-40.333*	7.120	.000	-62.25	-18.42
	A2	-17.000	7.120	.248	-38.92	4.92
	A3	-4.500	7.120	1.000	-26.42	17.42
	Control	-77.333*	7.120	.000	-99.25	-55.42
control	A1	37.000*	7.120	.250	15.08	58.92
	A2	60.333*	7.120	.000	38.42	82.25
	A3	72.833*	7.120	.000	50.92	94.75
	A4	77.333*	7.120	.000	55.42	99.25

*. The mean difference is significant at the 0.05 level.

Based on table 1.preliminary test results of media concentration in 6 treatments and incubated for 48 hours, good media concentrations were found in treatments A1, A2, A3, and A4. During this treatment, test bacteria, *namely Bacillus subtilis* and *Escherchia coli* were shown to be able to grow on alternative media of coconut pulp. The nutritional content contained in alternative media of coconut pulp is carbohydrates, proteins, and neutral pH. Then the best treatment from the initial test is repeated to ensure the alternative medium of coconut pulp does not change at that concentration or *Bacillus subtilis* and *Escherichia coli* can still grow, incubated for 2x24 hours at 37°C.

The growth medium must contain the elements needed for the growth of microorganisms, namely carbohydrates and proteins. Carbohydrates and proteins are used in the synthesis process. Coconut pulp contains quite a lot of protein and carbohydrates. According to (Karina et al., 2019) coconut pulp contains 23% protein and 74.69% carbohydrates, so it can be ascertained that coconut pulp has the potential for bacterial growth media.

Nutrient agar media is a universal media whose composition has been tested, but the price is relatively expensive.so that the manufacture of alternative media with available materials, cheap and containing nutrients that can encourage bacterial growth. Alternative media from young coconut pulp is obtained at low prices and contains nutrients such as carbohydrates, proteins, fats, crude fiber, vitamin A, vitamin D, and vitamin E, as well as how to make media that is quite simple and can grow bacteria.bacteria really need carbohydrates because carbohydrates are the most important substrate in bacterial metabolism. Almost half of the dry weight of bacteria is elemental carbon, carbon is present in carbohydrate compounds. In addition to carbohydrates, protein also plays an important role in bacterial growth, because protein is a source of nutrients needed by cells as a structural component of cells or in the process of cell formation (Saragih &; Ndruma, 2020).

In table 2. The growth of *colonies of Bacillus subtilis* bacteria in alternative coconut pulp media showed the highest average number of colonies found in A1 treatment, which was 50.6x10^2 CFU/mL, while in control media the average number of colonies was $75x10^2$ CFU/mL and in table 3. The growth of *Escherichia coli* bacterial colonies in alternative coconut pulp media shows the highest average number of colonies found in A1 treatment, which is $36.6x10^2$ CFU / mL while in media control of average number of colonies was $86.3x10^2$ CFU/mL. From the two tables, the highest average number of colonies in alternative coconut pulp media is found in the A1 treatment, meaning that the concentration of A1 is better than the concentration of A2, A3, and A4 because in the A1 treatment the number of colonies is closest to the control media (NA).

A2, A3, and A4 treatments resulted in lower colony counts when compared to A1 treatments. In the alternative medium, namely coconut pulp, the number of colonies grown in the A2, A3, and A4 treatments was also less than that observed in the control media (NA) and A1 treatment. Despite this, the number of these colonies can still be observed and calculated. The decrease in colony growth in A2, A3, and A4 treatment is due to the higher protein content in NA media when compared to the protein content in alternative coconut pulp media (Karina et al., 2019)

The number of colonies in the A2, A3 and A4 treatments is less than in the A1 treatment while the nutrients are larger, this is because the alternative media of coconut pulp contains more complex nutritional compounds so that the growth is less than optimal complex compounds such as carbohydrates, will be broken down into glucose (Saragih &; Ndruma, 2020).

Research conducted by Juriah & Sari, (2018) shows that the growth rate of bacteria in alternative media derived from natural materials tends to be slower than bacterial growth in production media, as seen in research related to Bacillus sp. by using tofu liquid waste media which shows slower growth compared to Bacillus sp. bred in agar nutrient media. Therefore, observation of bacterial growth needs to be carried out after incubation for 48 hours. The difference in the rate of bacterial growth in the two types of media can be explained by the factor of acclimatization of bacteria in tofu liquid waste media which takes longer due to lack of available nutrients.while in alternative media coconut pulp the growth rate of bacteria is slower than the control media because coconut pulp contains complex nutritional compounds, so bacteria take longer to break down these compounds into a simple form that can be taken and used by bacterial cells for cellular synthesis and energy production, according to the findings in research by (Kusmiati, Meti et al., 2022)

Bacterial growth is influenced by a number of factors, including nutrition, acidity (pH), temperature, and osmotic pressure. Nutrients are essential elements used by bacteria as an energy source, including sulfur, carbon, nitrogen, phosphorus, minerals, and vitamins. In addition to these factors, when bacteria move to a new environment, they undergo an adaptation stage where this process involves the formation of new enzymes needed to adapt to the new growth environment (kurniati et al., 2020)

To support optimal growth and reproduction of bacteria in a medium, there must be a number of conditions met, including the availability of appropriate nutrients for the bacteria, adequate humidity levels, appropriate pH levels, the presence of sufficient amounts of oxygen, the media used for bacterial growth must be sterile and free from the presence of other microorganisms, and the media must be incubated at a certain temperature (Laila, 2020).

In table 4, the results of the normality test data show that each concentration of coconut pulp and the control group has a normal distribution, with a significance value of >0.05. While in Table 5, the results of the data homogeneity test show a significance value of >0.05, which is 0.221, which indicates that the data is homogeneous. Data that meet the criteria of normal distribution and homogeneity were then analyzed using ANOVA, as stated in Table 6. The result of ANOVA shows a significance value of 0.001 ($p < 0.05$), which means that (Ho) is rejected and (Hi) is accepted. This indicates that there is a significant influence due to differences in the concentration of coconut pulp and agar as an alternative medium on the growth of *bacteria Bacillus subtilis* and *Escherichia coli*. The next step is to conduct a post hoc multiple comparison test.

Based on table 7.post hoc results obtained a comparison of A1 concentration with A2, A3, A4 concentration, and control of significant p value <0.05, showing a significant difference.ie A1 concentration with A2 p value <0.05 ie sig 0.031 shows a significant difference between A1 coconut pulp concentration with A2 coconut pulp concentration. While the concentration comparison between A3 with A2 and A4 significant value p >0.05 shows no significant difference. While the concentration comparison between controls with A2, A3 and A4 significant values of p<0.05 showed a significant difference. While the comparison of A1 with the control sig value of p >0.05 which is 0.250 shows no significant difference. Thus, the conclusion is that the lower the concentration used, the higher the growth rate *of Bacillus subtilis* and *Escherichia coli* bacteria.

In this study, gram staining was carried out to distinguish gram-positive and negative bacteria that were added to alternative media of coconut pulp and agar nutrients. The results of microscope observations were obtained at a magnification of 1000x *Bacillus subtilis* bacteria purple clustered rod-shaped colonies, while *Escherichia coli* bacteria were red rod-shaped colonies. Bacterial colonies grown on alternative coconut pulp media are smaller than on control media (NA).

Bacillus subtilis is a type of saprophytic bacteria and soil bacteria that has an important role in the nutrient cycle because of its ability to produce a variety of enzymes. These bacteria have been utilized in industry to produce various substances such as proteases, amylase, antibiotics, and chemicals. *Bacillus subtilis* belongs to the category of Gram positive bacteria and has the shape of a bacillus (rod) with the ability to form oval-shaped endospores in the center of its cells. When grown on agar media, colonies of *Bacillus subtilis* are usually spherical with irregular edges, non-glossy surfaces, and brownish in color. The size of the bacterium *Bacillus subtilis* ranges from 2-3 μm long and 0.7-0.8 μm wide These bacteria can live both in aerobic (with oxygen) and facultative anaerobic (without oxygen) conditions. *Bacillus subtilis is* also able to grow at an incubation temperature of around 37 ° C and at a pH level of about 7 (Inherni Marti Abna, 2018)

Escherichia coli is a type of Gram negative bacteria that has a morphology in the form of a straight short rod, or often called cocobacilli. These bacteria do not have capsules or spores. *E. coli* belongs to the Enterobacteriaceae family and has facultative anaerobic properties, which means it can grow both in conditions with or without oxygen. *E. coli* also has the ability to move by using peritric flagella, so it is referred to as a motile bacterium that has flagella all over its surface. The growth rate of *E. coli* ranges from 10-40°C, but the optimum temperature for its growth is around 37°C. In addition, the optimal pH for E. *coli* growth is in the range of 7 to 7.5. (Khaerunnisa et al., 2019)

CONCLUSIONS

Based on the results of research on the use of coconut pulp as a growth medium for *bacteria Bacillus subtilis* and *Escherichia coli* can be concluded as follows:

1. The best concentration of coconut pulp flour for alternative media for the growth of *bacteria Bacillus subtilis* and *Escherichia coli* is at the concentration of A1, where at that concentration the number of colonies is closest to the control media (NA).
2. In the one way anova test, a significant value of $p < 0.05$ was obtained, which is 0.001, which means that there is an influence on the concentration of coconut pulp and agar on the growth of the number of *colonies of Bacillus subtilis* and *escherchia coli.*
3. Post hoc multiple comparison test comparison between A1 concentration with control significant value $p > 0.05$ which means there is no significant difference.while in control concentration with A2, A3 and A4 significant value $p < 0.05$ which means there is a significant difference, so that A1 treatment is the best treatment for the growth of *bacteria Bacillus subtilis* and *Escherichia coli* because at concentration A1 there is no difference with the control media.

It is expected that future studies will use smaller concentrations of media with different methods and types of bacteria.

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