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RESEARCH ARTICLE
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Sorgum Agar (Sorghum bicolor) as Substitute Nutrient Agar Media for Cultivation *Escherichia coli*

Pestariati[1(CA)], Mufidah[2]
[1(CA)]Department of Technology Medical Laboratory, Poltekkes Kemenkes Surabaya, Indonesia;
pestariati@gmail.com (Corresponding Author)
[2]PT Primed Sumber Berkah Utama, Indonesia; chrstkartika@gmail.com

ABSTRACT

Media (nutrient agar) is a medium that is often used for bacteriological examinations and the price is relatively more expensive, Sorghum bicolor materials that are easy to get and cheap. Sorghum contains carbohydrates and protein which are suitable for manufacturing agar nutrients. This research was an experimental quantitative analysis laboratory. The research used Sorghum bicolor flour as carbohydrates and proteins in nutrient agar media. This study used variations in the mass of sorghum flour 2.50 grams, 3.75 grams, 5.00 grams, and 6.25 grams. The average number of bacterial colonies at mass variations was 2.50 grams, 3.75 grams, 5.00 grams, and 6.25 grams, namely 70.75 × 1013 CFU / mL, 60.75 × 1013 CFU / mL, 49, 25 × 1013 CFU / mL, and 42 × 1013 CFU / mL with significant differences in the value of $p = 0.000$ or $<\alpha = 0.05$. 5.00 gram sorghum flour yields almost the same colony count as Nutrie Media for manufacturing as the gold standard. Conclusion 5 grams of sorghum flour can be used as an alternative media for nutrition and protein in Nutrient agar for Manufacturing.

Keywords: sorghum bicolor l; moenchnutrient agar media; *Escherichia coli*

INTRODUCTION

Diagnosis of bacteria requires growth media, Bacteria need nutrient sources of carbon, nitrogen, sulfur, phosphorus, inorganic salts (potassium, magnesium, sodium, calcium, and iron) [(1)] and a number of micronutrients (zinc, copper, manganese, selenium, and tungsten) [(2)]. These nutrients are then broken down into small molecules and assembled by bacteria to make up cell components [(3)]. Media requires moisture, isotonic osmose pressure, pH, appropriate temperature and is sterile [(4)]. Nutrient agar is the medium most often used for bacterial growth [(5)]. Nutrient media so that the dehydrate medium is an expensive factory-made medium, so research is needed to find a substitute medium from easily available and inexpensive materials. Other researchers examined substitutes for carbohydrates and protein in the media nutrient agar, among others [(6)]. There is also research that used boiled water for yellow and purple tubers as an alternative growth medium for *Escherichia coli* and *Staphylococcus* [(6)], used soy bean as an alternative medium for the growth of *Pseudomonas aeruginosa* [(7)], used *Pachyrrhizuserosus* and bean sprouts (Viga radiate) as an alternative medium for the growth of *Escherichia coli* and *Staphylococcus aureus* [(8)], used sorghum as an alternative medium for the growth of *Candida albicans*.

Sorghum *(Sorghum bicolor)* is a grain crop in hot and dry climates [(9)]. Sorghum comes from the region around the Niger River in Africa [(10)]. Sorghum contains protein which has potential as a substitute medium for NA (nutrient agar), the protein contained in sogum has the highest value when compared to other serelia plants [(11)]. Therefore, it is necessary research to prove sorghum as a substitute medium for NA media (nutrient agar) plant on the growth of *Escherichia coli.* Therefore, the purpose of this research is to develop a more affordable media to grow *Escherichia coli*.

METHODS

Design

This type of laboratory experimental research was carried out at the Bacteriology Laboratory of the Health Analyst Department of the Ministry of Health, Surabaya, from October 2019 to April 2020. Research Materials, Sorghum flour *(Sorghum bicolor)* with various mass variations namely 2.50 grams, 3.75 grams, 5.00 grams, and 6.25 grams with replication 4 times, NaCl, bacteriological agar, distilled water, culture pure *Escherichia coli* ATCC 25922, PZ 0.9% sterile, standard Mc Farland 0.5 and NA media (nutrient agar) as a positive control.

Procedure

Sterilization of tools, some tools and media for research must be sterilized by autoclaving 121° C for 15 minutes. For making NA (Nutrient Agar) media manufacturers (gold Standard), the researcher dissolved 2.8 grams of dehydrate media (nutrient agar) in 100 mL of distilled water then heated until completely dissolved, measured the pH of 7.4 ± 2. And sterilized in autoclave 121° C for 15 minutes [(12),(13)]. Making Sorghum Agar *(Sorghum bicolor)* Substitute Media / alternative media ,. Researchers weighed sorghum flour with several mass variations dissolved in 100 mL of distilled water, then added 0.5 grams of NaCl, and 1.5 grams of bacteriological agar. heated until completely dissolved, measured pH 7.4 ± 2. And sterilized in an autoclave of 121° C for 15 minutes. Nutrient media for manufacturers and alternatives were poured into a petridisk and cooled at room temperature until solidified.

Dilution of *Escherichia coli*

Researchers dissolved the pure culture of *Escherichia coli* into 9 mL of sterile 0.9% PZ. Then equalized with the Mc Farland standard 0.5 (0.5 mL $BaCl_2$ 1.175% + 99.5 mL H_2SO_4 1%). until turbidity is proportional to the Mc Farland turbidity of 0.5 [(14)]. Then the suspense is diluted to 1013 (according to the preliminary test results).

Planted 1 mL of suspension 1013 on a plate of sorghum agar *(Sorghum bicolor)* in each mass variation and manufacturer's NA (nutrient agar) media as a positive control.

Calculation of Bacteria using the Total Plate Number Method

Researchers planted *Escherichia coli* on nutrient agar and 37° C alternatives for 24 hours. Researchers observed and counted the number of colonies that grew using a colony counter [(15)].

Data Analysis Technique

Data were analyzed using the Anova test.

RESULTS

Normality Test

The results of the normality test showed that the significant value obtained was > α = 0.05 data for each variation of the mass of sorghum flour on sorghum agar (*Sorghum bicolor*) media and positive control was normally distributed.

Homogeneity Test

Results show that the significant value obtained was 0.750. Based on guidelines in decision making if the value of Asymp. Sign (2- tailed) > α = 0.05, the data was homogeneous.

One Way Anova Test

The results show that the value was significant. In this test was 0.000. Based on the guidelines for decision making, if the Asymp.Sig value < α = 0.05, there is a difference in the average number of *Escherichia coli* colonies in each mass variation of sorghum flour (Sorghum bicolor).

Table 1. Data on preliminary test results determination of *Escherichia coli* suspension concentration

Concentration bacterial suspension	Number of colonies	Colony characteristics
10^8	>300	No single colony was formed
10^9	>300	No single colony was formed
10^{10}	>300	No single colony was formed
10^{11}	>300	Some were formed single colonies
10^{12}	97	Most of them formed a single colony
10^{13}	50	A single colony was formed
10^{14}	4	A single colony was formed

In positive control the mean colony count was 46.5 × 1013 CFU / mL. In sorghum agar (Sorghum bicolor, 2.50 grams of sorghum flour with an average number of colonies 70.75 × 1013 CFU / ml. Sorghum flour 6.25 grams with an average number of colonies 42 × 1013 CFU) / ml, 5.00 gram sorghum flour had a colony count average of nearly the same as the gold standard.

Post Hoc Multiple Comparison Test. The results of the Post Hoc Multiple Comparison test showed that the variations in the mass variation of sorghum flour 2.50 grams and 3.75 grams had a significant value <α = 0.05, namely 0.00 and 0.004. significant with positive control, while the variation in the mass of sorghum flour was 5.00 grams, and 6.25 grams had significant values> α = 0.05, there was no significant difference with the positive control.

Table 2. The results of the number of Escherichia coli colonies calculation using the method total plate count on the substitute media for sorghum agar (*Sorghum bicolor*) and media (nutrient agar) anufacturers as gold standard

Media replication	Positive control	Negative control	Variation in the mass of sorghum flour on sorghum substitute media *(Sorghum bicolor)*			
			2.50 g	3.75 g	5.00 g	6.25 g
I	50	0	75	67	54	47
II	44	0	73	54	45	40
III	52	0	65	60	50	42
IV	40	0	70	62	48	39
Total	186	0	283	242	197	168
Colony average ($\times 10^{13}$CFU/mL)	46.5	0	70.75	60,75	49,25	42

DISCUSSION

Sorghum flour in 100 grams contains 10.6 grams of protein, 72.0 grams of carbohydrates, 28 milligrams of calcium, 4.4 milligrams of iron, 287 milligrams of phosphorus, and 0.38 milligrams of vitamin B1, while the NA medium (nutrient agar) contains 0, 5 grams of peptone in 100 mL This research expects that 10.6 grams of sorghum flour protein can replace peptone in Nutrien Agar media. The results of the study used variations in the mass of 5.00 gram sorghum flour 100 mL media had the same number of colonies as the gold standard because 5 grams of sorghum flour was equivalent to 0.5 grams of peptone on gold standard NA (nutrient agar) media, so that the nitrogen requirement for cell division fulfilled. Sorghum flour also contains elements C, O, H, N, P, K, Ca, vitamins which are suitable for the metabolic processes and growth of Escherichia coli. Living microorganisms are influenced by physical factors (temperature, pH, osmotic pressure, and light), and chemical factors (nutrients/culture media) [10]. Sorghum can be used as an alternative medium for the growth of Candida albicans. [5]

The limitation of this research is that it does not use extracts from sorghum which can produce pure protein, so it still contains other ingredients that can affect the growth of Escherichia coli, as evidenced by the highest mass variation of 6.25 grams giving the smallest growth results

CONCLUSION

All variations of the mass of sorghum flour on sorghum agar *(Sorghum bicolor)* can grow Escherichia coli bacteria. The average number of bacterial colonies in the mass variation of the sorghum flour was 2.50 grams, namely 70.75 × 1013 CFU / mL, the variation of the mass of sorghum flour was 3.75 grams, namely 60.75 × 1013 CFU / mL, the variation of the massofsorghumflourwas 5.00 gram, namely 49.25 × 1013 CFU / mL and the variation of the mass of sorghum flour 6.25 grams, namely 42 × 1013 CFU / mL. However, the effective mass variation used as a substitute medium for NA (nutrient agar) is 5.00 grams because the average number of colonies is almost the same as NA (nutrient agar) media as the Gold Standard. For futur eresearchers, it is hoped that sorghum flour will beused as a substitute medium for EMB (Eosin Methylene Blue) as a selective medium

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