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Utilizing Maize Cob Media as a Replacement for Nutrient Agar Media in the Growth of Escherichia coli and Staphylococcus aureus Bacteria

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Abstract :*Bacteria are microorganisms that require adequate media and nutrients to grow and develop. The media commonly used to grow bacteria is Nutrient agar media, but the price of Nutrient agar media is quite expensive. Corn cobs are lignocellulosic wastes that contain nutrients that can meet the nutritional requirements of bacterial growth media. This study aims to investigate the viability of corn cobs as a cost-effective alternative to Nutrient agar media. Specifically, we explore the variance in bacterial colony growth when utilizing corn cobs as a growth medium for Escherichia coli and Staphylococcus aureus bacteria. Employing a true experimental design with a posttest-only control structure, we analyze the treatment's effects using the One Way ANOVA test and Independent Sample T-test. These statistical methods enable us to discern differences between groups utilizing corncob media with and without added sugar.Our findings reveal noteworthy insights into bacterial colony growth. For Escherichia coli, the average number of colonies on corncob media with sugar was 222 CFU/ml, on corncob media without sugar, it was 164 CFU/ml, and on Nutrient agar media, it reached 361 CFU/ml. In the case of Staphylococcus aureus, colonies on corncob media with sugar averaged 871 CFU/ml, on corncob media without sugar, they averaged 657 CFU/ml, and on Nutrient agar media, they reached 942 CFU/ml.Statistical analysis using the One Way ANOVA test for both Escherichia coli and Staphylococcus aureus demonstrated p-values <0.05, signifying significant differences in their growth on corncob media with sugar, corncob media without sugar, and Nutrient agar media. Furthermore, the Independent Sample T-test results for both bacterial strains yielded p-values <0.05, indicating dissimilar growth patterns on corncob media with sugar compared to corncob media without sugar.In conclusion, our study underscores significant disparities in the growth of Escherichia coli and Staphylococcus aureus bacteria among Nutrient agar media, corncob media with sugar, and corncob media without sugar. Notably, we observed a marked increase in bacterial colony counts on corncob media with sugar and corncob media without sugar.*

Keywords: *Corncob media, Escherichia coli bacteria, and Staphylococcus aureus bacteria*

INTRODUCTION

Microorganisms are creatures that have very small cell sizes, including bacteria. In order to grow and develop, bacteria require a suitable environment and sufficient nutrients (8). The nutrients needed by bacteria come in the form of simple compounds that are either readily available or derived from complex compounds that are subsequently broken down by microorganisms into simple compounds through enzymatic processes (9,11).

The development of bacterial culture media as a growth medium for microorganisms plays a crucial role in the field of microbiology. By isolating a bacterium and cultivating it in a medium, we can identify and study the characteristics of a bacterium (2,20). The growth medium must fulfill the nutritional requirements of microorganisms. The nutrients needed by microorganisms for their growth include carbon (C), nitrogen (N), non-metal elements such as sulfur (S) and phosphorus (P), metal elements such as calcium (Ca), zinc (Zn), sodium

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(Na), potassium (K), copper (Cu), manganese (Mn), magnesium (Mg), and iron (Fe), as well as vitamins, water, and energy (1).

The media commonly used for bacterial cultivation in laboratories is Nutrient agar (2,17). Nutrient agar is a solid medium made from a mixture of meat extract and peptone, with agar used as a solidifying agent. One carbohydrate source is corn (Zea mays). In Indonesia, corn is the second most important commodity after rice (6). Timor Island is one region in the East Nusa Tenggara Province (NTT) that is rich in local food sources such as corn. Corn has an advantage over other food commodities due to its higher nutritional content compared to rice. In addition to being a staple food, corn can also be used as animal feed, industrial material, and an export commodity (7,18,19).

Corn are known to contain a significant amount of dietary fiber. This dietary fiber consists of complex compounds such as lignin, hemicellulose, cellulose, and lignocellulose. Each of these compounds has the potential to be converted into other compounds through biological processes. Cellulose, in particular, serves as a carbon source that can be utilized by microorganisms as a substrate in the fermentation process (3,5).

There is currently no research on the utilization of corn cobs in the production of bacterial growth media. The objective of this study is to examine the utilization of corn cobs as a substitute for Nutrient agar media by comparing the growth of Escherichia coli and Staphylococcus aureus bacteria colonies on corn cob media.

MATERIAL & METHODS

This study is a true experiment with a posttest-only control design. The research was conducted at the Bacteriology Laboratory of the Medical Laboratory Technology Study Program at Poltekkes Kupang for the production of corn cob media, while the biochemical analysis of corn cob content was carried out at the Integrated Laboratory of Nusa Cendana University.

The materials and equipment used in this study included dried corn cobs, swallow agar, granulated sugar, Nutrient agar media, Escherichia coli ATCC 25922 bacterial strain, Staphylococcus aureus ATCC 25923 bacterial strain, distilled water, 70% alcohol, spiritus, 0.85% NaCl, iodine reagent, 10% NaOH, red litmus paper, picric acid, AgNO3, cotton, label paper, matches, brown paper, isolation, Pyrex beaker glass, Pyrex Erlenmeyer flask, Pyrex test tubes, Pyrex measuring glass, hot plate, spatula, Bunsen burner, incubator, Laminar Air Flow (LAF), oses, autoclave, petri dishes, digital weighing scale, glass stirring rod,

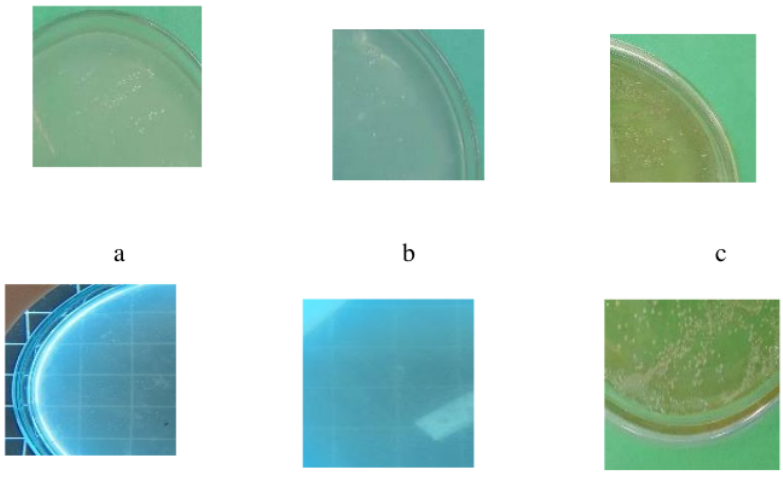
stationery, sieve, camera, test tube rack, microscope, glass slides, and personal protective equipment (PPE).

The research implementation began with the sterilization of the materials and equipment. Next, a boiling water extract of 100 g of corn cobs was prepared in 500 ml of distilled water, and an identification test of the corn cob content was conducted to determine the carbohydrate, nitrogen, Na+, and Cl- content. A total of 250 ml of corn cob media was prepared by adding 5 g of sugar and 5 g of agar for the sugar-added corn cob media, and 5 g of agar for the sugar-free corn cob media. Suspensions of *Escherichia coli* and *Staphylococcus aureus* bacteria were prepared according to *the McFarland* 0.5 standard. The bacterial suspensions were inoculated using the streak plate method on each corn cob medium and Nutrient agar medium, with three repetitions for each medium. The plates were then incubated at 37°C for 24-48 hours, and bacterial growth was observed by identifying macroscopic characteristics and counting the number of bacterial colonies using a colony counter.

The data collection technique in this study involved primary data obtained from the calculation of bacterial colony counts on corn cob media and Nutrient agar. The data analysis was performed by counting the number of bacterial colonies using a colony counter and then tested using the Independent Sample T-test and One-Way ANOVA.

RESULT & DISCUSSION

On the corn cob media with sugar and sugar-free corn cob media, there is bacterial growth of *Escherichia co*li and *Staphylococcus aureus*, as shown in the image below.



a b c

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Figure 1. Bacterial colonies of Escherichia coli and Staphylococcus aureus on media. Caption: a= Escherichia coli bacteria on corn cob media with sugar, b= Escherichia coli bacteria on sugar-free corn cob media, c= Escherichia coli bacteria on Nutrient agar control media, d= Staphylococcus aureus bacteria on corn cob media with sugar, e= Staphylococcus aureus bacteria on sugar , f= Staphylococcus aureus bacteria on Nutrient agar control media.

The growth of *Escherichia coli* and *Staphylococcus aureus* based on the colony counts on corn cob media and Nutrient agar control media can be seen in the table below:

Table 1. The number of bacterial colonies of Escherichia coli and Staphylococcus aureus on corn cob media with sugar, corn cob media without sugar, and Nutrient agar media.

Bacteria	The colony count CFU/ml on the media.		
	Corn cob with sugar.	Corn cob without sugar.	*Nutrient agar* (CFU)
Escherichia coli	222	164	361
Staphylococcus aureus	871	657	942

Based on the data in Table 1, it is evident that the average growth of *Escherichia coli* bacteria on corn cob media with sugar is greater compared to the growth on sugar-free corn cob media. Both the corn cob media with sugar and sugar-free corn cob media exhibit smaller colony sizes of Escherichia coli compared to the Nutrient agar control media. Similarly, the average growth of *Staphylococcus aureus* bacteria on corn cob media with sugar is higher than the growth on sugar-free corn cob media. The colony sizes of *Staphylococcus aureus* bacteria on both the corn cob media with sugar and sugar-free corn cob media are smaller than those on the Nutrient agar control media.

Discussion

Based on the research findings, the bacterial growth on the media infected with *Escherichia coli* and *Staphylococcus aureus* was analyzed using the One-Way ANOVA statistical test due to meeting the assumptions of normality and homogeneity. The One-Way ANOVA test for *Escherichia coli* bacterial growth yielded a significance value of 0.004

($p<0.05$), indicating significant differences in the growth capability of *Escherichia coli* on corn cob media with sugar, sugar-free corn cob media, and Nutrient agar control media. The One-Way ANOVA test for *Staphylococcus aureus* bacterial growth yielded a significance value of 0.002 ($p<0.05$), indicating significant differences in the growth capability of *Staphylococcus aureus* on corn cob media with sugar, sugar-free corn cob media, and Nutrient agar control media.

Furthermore, the Independent Sample T-test was conducted to compare the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria on corn cob media with sugar and sugar-free corn cob media. The test results for *Escherichia coli* bacterial growth on corn cob media with sugar and sugar-free corn cob media yielded a significance value of 0.032 ($p<0.05$), indicating significant differences in the growth capability of the bacteria.

Escherichia coli on corn cob media with sugar and sugar-free corn cob media. The test results for the growth of *Staphylococcus aureus* bacteria on corn cob media with sugar and sugar-free corn cob media yielded a significance value of 0.001 ($p<0.05$), indicating significant differences in the growth capability of *Staphylococcus aureus* bacteria on corn cob media with sugar and sugar-free corn cob media.

In order to grow well, a bacterial growth medium must meet certain requirements, including containing all the nutrients that microorganisms can easily utilize, having suitable osmotic pressure, surface tension, and pH, being free from inhibitory substances, and being sterile (5).

In this study, Nutrient agar media was used, which is commonly employed as a medium for bacterial growth, particularly bacteria found in water, waste, feces, and other materials. The composition of Nutrient agar typically consists of peptone, beef extract, and agar, providing nutrients for bacterial growth, including *Escherichia coli* and *Staphylococcus aureus* bacteria. Beef extract contains water-soluble substances such as carbohydrates, vitamins, organic nitrogen, and mineral salts, while peptone serves as a nitrogen source necessary for microorganisms, mainly in the form of amino acids, and agar acts as a solidifying agent (2,20,18).

Based on the results of the bacterial colony counts of *Escherichia coli* and *Staphylococcus aureus* on corn cob media and Nutrient agar control media, there are differences in colony size and count in bacterial growth that can be attributed to the different compositions of the test media compared to Nutrient agar as the control medium. Nutrient agar media has a composition that provides more adequate nutrients for bacterial growth, such as beef extract, while the test media prepared from corn cob did not contain beef extract.

The variations in the colony counts of both Escherichia coli and *Staphylococcus aureus* on corn cob media with sugar, sugar-free corn cob media, and Nutrient agar media can be influenced by the differing compositions of each type of media used, resulting in suboptimal bacterial growth on the test media compared to the Nutrient agar control media. The differences in colony growth of both bacteria can also be attributed to the pH of the media. During the preparation of the test media, the pH was not controlled. Neutral pH is one of the requirements for preparing growth media.

Additionally, the factor of media sterilization also affects bacterial growth, as non-sterile media can lead to the formation of contaminants such as fungi (14,15,16).In this study, a qualitative biochemical analysis of corn cob content was conducted, yielding negative results for carbohydrate content (-), negative results for protein content (-), positive results for Na+ content (+), and positive results for Cl- content (+). According to Ariyanto and Slamet (2014), the nutritional content of corn cob includes water content, crude fiber, crude protein, crude fat, and ash. The majority of the content in corn cob is crude fiber. Cellulose serves as a carbon source that microorganisms can use as a substrate in the fermentation process. The presence of cellulose in corn cob may contribute to the growth of bacterial colonies on corn cob media because cellulose is a type of carbohydrate that contains carbon (3,13)

Corn cob contains high levels of dietary fiber but low levels of crude protein (10). The low protein content in corn cob media may allow bacterial growth on the test media to result in smaller colony sizes compared to colonies grown on Nutrient agar control media. Nutrient agar media contains peptone, which serves as a high-protein source for microbial growth. Bacteria will hydrolyze proteins as an energy source required for colony size growth (4,20).

The qualitative test results for Na+ and Cl- content in corn cob indicate the presence of Na and Cl metal ions that are needed by bacteria. The presence of these metal ions can also be a factor contributing to bacterial growth on corn cob test media.

CONCLUSION

Based on the research findings, the average colony counts of Escherichia coli bacterial growth were 222 CFU/ml on corn cob media with sugar and 164 CFU/ml on sugar-free corn cob media. The average colony counts of Staphylococcus aureus bacterial growth were 871 CFU/ml on corn cob media with sugar and 657 CFU/ml on sugar-free corn cob media. There is a significant difference in the growth of Escherichia coli and Staphylococcus aureus bacteria among Nutrient agar media, corn cob media with sugar, and sugar-free corn

cob media, but there is bacterial colony growth on both corn cob media with sugar and sugar-free corn cob media.

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