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RESEARCH **Open Access**

The potential of Cilembu sweet potato (Ipomoea batatas L.) as a growth medium for Staphylococcus aureus and Escherichia coli

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Abstract
The media aims to store, reproduce, and identify bacteria but has disadvantages such as being expensive, containing chemicals, and being easily damaged due to contamination. Therefore we need an alternative media that can overcome these limitations. Cilembu sweet potato (Ipomoea batatas, L.) is a natural ingredient that is easy to obtain and contains sufficient nutrients so it has the potential to be used as a basic ingredient for growth media. The purpose of this study was to determine the potential of Cilembu sweet potato flour as an alternative medium for the growth of Staphylococcus aureus and Escherichia coli bacteria with a quasi-experimental design. Cilembu sweet potato flour is obtained by cleaning, chopping, drying in the oven, crushing with a blender, and sifting the tubers to obtain fairly fine flour. The flour was dissolved in agar and then inoculated with S. aureus and E. coli, each with 16 replications. The results showed that the average number of S. aureus colonies was 119.12 CFU (169.2 CFU in control) while E. coli was 160.56 CFU (221.2 CFU in control). The Mann-Whitney test showed that there was a difference in the number of S. aureus colonies on alternative media and NA ($p = 0.006 \leq 0.05$), but there was no difference between the number of E. coli colonies on alternative media and NA ($p = 0.057 > 0.05$). Finally, there was a difference in the number of S. aureus and E. coli colonies on alternative media ($p = 0.04 \leq 0.05$). The nutritional composition shows that Cilembu sweet potato flour has more potential to replace NA as a growth medium for E. coli than for S. aureus.

Keywords: Cilembu Sweet Potato, *Staphylococcus aureus*, *Escherichia coli*, Alternative Media.

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1. INTRODUCTION

To study the properties or identify bacteria, a medium is needed so that the bacteria can grow. Growth media must meet the nutritional requirements needed by microorganisms. The nutrients needed by microorganisms to fulfill their growth include sources of carbon, nitrogen, non-metal elements such as sulfur and phosphorus, metal elements such as calcium, zinc, natrium, potassium, copper, manganese, magnesium, and iron, vitamins, water, and energy (Juariah, 2021; Tille, 2022).

The commonly used media to grow microorganisms is nutrient agar (NA) which can be obtained in the market in a ready-to-use form. NA is a basic culture medium used to subculture organisms for maintenance purposes or to check the purity of subcultures from isolation plates prior to biochemical or serological tests. This media is in solid form made from a mixture of 5.0 gr peptone, 5.0 gr sodium chloride, 1.0 gr lab-lemco powder, 2.0 gr yeast extract, and 15.0 gr agar as a solidifier (Bridson, 2006).

Unfortunately, commercial media are generally synthetic materials that have drawbacks such as being expensive, cannot be self-mixed, contains chemicals, decreases in quality due to microbial contamination so requires additional costs, and is not always easy to obtain on the market. Natural materials such as Cilembu sweet potato can be used as an alternative for growth media because they have several advantages compared to synthetic commercial media such as being cheap, not containing chemicals (such as preservatives, dyes, or indicators), being easy to obtain, and containing nutrients that can support bacterial growth.

Cilembu sweet potato (*Ipomea batatas*, L.) originates from Central America but can be grown and bred worldwide. The delicious taste supports its benefits as an anti-cancer, anti-inflammatory and anti-diabetic. This advantage can be achieved because Cilembu sweet potatoes contain macronutrients such as carbohydrates, protein and fat, as well as micronutrients such as vitamins (beta-carotene, lutein, zeaxanthin, thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, folate, vitamin C, vitamin E) and minerals (calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc). (Mohanraj & Sivasankar, 2014). These components, apart from being complete nutrition for humans, are also very necessary for the growth of bacteria. The high content of carbohydrates (up to 20.1%) and protein (1.6%) in Cilembu sweet potato is expected to replace the functions of peptone and yeast extract in NA. Thus, the nutritional needs for bacteria can be fulfilled.

Based on the structure of the cell wall, bacteria can be classified into Gram-positive and Gram-negative, which was classified by Christian Gram in 1884 (Silhavy et al., 2010). Gram-positive bacteria have a thicker peptidoglycan layer than Gram-negative (Sizar & Unakal, 2022). The main pathogens in humans can come from these two groups. For example, among the most commonly found in sepsis isolates are *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) (Ramachandran, 2013).

S. aureus causes a wide range of clinical symptoms. Both community-acquired infections and hospital-acquired infections are frequent, and therapeutic management is still difficult due to the emergence of multi-drug resistance strains like Methicillin-Resistant *Staphylococcus aureus* (MRSA). *S. aureus* can be found on healthy people's skin and mucous membranes, most frequently in the nasal region, as well as in the environment and in normal human flora. On healthy skin, *S. aureus* usually does not cause infection; however, if the bloodstream or internal tissues are opened up to the germs, a number of potentially dangerous diseases may result (Park & Seo, 2022).

The majority of *E. coli* strains are safe in the intestines and infrequently damage healthy people. Yet, both healthy and immunocompromised people might develop diarrhea or extraintestinal disorders as a result of a number of pathogenic strains. In addition to being a serious public health issue, diarrheal diseases are a leading cause of morbidity and mortality in newborns and young children, particularly in poor nations (Gomes et al., 2016).

Apart from being important pathogens, these two species are the objects most often used in research to represent the Gram-positive and Gram-negative groups respectively (Silhavy et al., 2010). Therefore, it is necessary to have sufficient stock media to support the life of these bacteria as a first step prior to further research. Cilembu sweet potato has been studied as a natural ingredient that contains quality nutrients and bioactive compounds (Eduardo Cartabiano Leite et al., 2020), which act as antidiabetic, cytotoxic, antioxidant, and antibacterial (Das et al., 2019). The use of Cilembu sweet potato as an alternative medium has also been studied but is limited to *E. coli* only (Patricia et al., 2022). The purpose of this research is to explore the potential of Cilembu sweet potato flour as a basic composition for alternative media, as well as take an approach to find out whether this alternative media is more effective against Gram-positive or Gram-negative bacteria.

2. RESEARCH METHOD

This research was carried out from July to September 2022 at the Bacteriology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak. The research design is quasi-experimental where the temperature and incubation time, the composition of Cilembu sweet potato flour, and the number of bacteria inoculated can be controlled while the maturity of Cilembu sweet potato and its nutritional composition cannot be controlled. As research samples, we chose Cilembu sweet potato tubers that were not rotten, oval in shape, and had yellowish-white tuber skin to standardize the maturity of the tubers and ensure that the nutrients in them were not lost or decomposed. The way to obtain sweet potato tuber flour is as follows: five kilograms of tubers are cleaned of the skin and finely chopped with a clean knife, then dried in the oven at 60^{o}C for 12 hours. This dry material is ground with a blender and then sifted through a 60 mesh sieve with a diameter of 20 cm and a height of 5 cm to obtain fairly fine flour. The flour obtained was sufficient to make 16 alternative media for each bacteria so the total treatment was 32 treatments.

Table 1. Prepare alternative media from Cilembu sweet potato tuber flour

Alternative media composition in 1000 mL	
Cilembu sweet potato tuber flour	8 g
Agar	15 g
Aquadest	1000 mL

To make alternative media, the ingredients were prepared as shown in Table 1 and then dissolved in Erlenmeyer and autoclaved at 121^{o}C and 15 psi for 30 minutes. 20 mL of sterile media was poured into a sterile petri dish and then allowed to solidify at room temperature. After that, the media is stored in a cooler at 4^{o}C until it is used. As a control, NA was prepared by weighing 28 gr of NA powder into an Erlenmeyer and then dissolving it with 1000 mL of distilled water. The next procedure is the same as for preparing alternative media (Bridson, 2006). The 8 grams of Cilembu sweet potato flour used is considered to be equivalent to the content of 5.0 grams of peptone, 1.0 grams of lab-lemco powder, 2.0 grams of yeast extract as in NA; while 15 agar is also equivalent to the number of agar in NA.

A suspension of *S. aureus* (ATCC 29737, Culti-Loops, Thermo Scientific) and *E. coli* (ATCC 25922, Culti-Loops, Thermo Scientific) was prepared by mixing 5 mL of 0.9% NaCl in a test tube with bacterial colonies and equalizing the turbidity with the McFarland standard 0.5 so that the concentration was 1.5 x 10^{8} CFU/mL (Eduardo et al., 2018). After that, 1 loop of each bacterial suspension was taken and streak-inoculated into alternative media and NA, then incubated at 37^{o}C for 24 hours. Growing colonies were counted manually under the colony counter and expressed in colony-forming units (CFU). These two strains are standards that are often used in various studies, including antimicrobial susceptibility testing (Minogue et al., 2014; Yehia et al., 2020).

Finally, the number of *S. aureus* and *E. coli* colonies on alternative and control media was tested statistically with Mann-Whitney U to determine whether there was a difference between the groups at a significance level of 0.05.

3. RESULTS AND DISCUSSION

This research was carried out in September 2022, involving laboratory tests for 7 consecutive days at the Bacteriology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak.

On average, the number of *S. aureus* colonies in the alternative media was lower than the number of *E. coli* colonies (119.12 CFU and 160.56 CFU respectively), and so did the control media (169,2 CFU and 221,2 CFU respectively). pH measurements with a pH meter showed that all media had a pH value of 7 which supports the optimal life of both bacteria because *S. aureus* live in the pH range of 4.5 to 9.3 (optimal pH is 7.0 to 7.5) (Bennett et al., 2013) and *E. coli* in the pH range of 4.5 to 9.5 (optimal pH is 7.0) (Basavaraju, & Gunashree, 2022). Observations of the characteristics of the colonies on all media also showed similar characteristics, namely the colonies were round, white, small in size, and smooth, indicating that both media are universal and do not show properties as differential media or inhibit the emergence of certain characteristics of bacteria. Both alternative media and NA do not contain pigments or indicators so that the colonies that grow appear uniform. On non-cultured media, whether alternative media or NA, there were no growing colonies, this indicated that all colonies growing in the treatment group were indeed bacteria from suspension made the day before, and this ensures that no contaminating bacteria grow during the inoculation and incubation stages. All of this data is shown in table 2.

What can be seen from the results of this study indicates that the nutrients contained in the Cilembu sweet potato alternative media play an important role in influencing the growth of both bacteria in terms of the number of colonies. These nutrients can be used by both bacteria to generate energy and grow, and *E. coli* seems to use them more optimally.

For example, sugar (in the form of glucose) is used by *E. coli* and other Gram-negative bacteria to produce energy. Glucose enters the cell by passive diffusion through the porins protein and then penetrates the deeper membrane with the help of the permease enzyme. In the cytoplasm, glucose is successively converted into glucose-6-phosphate, pyruvate, acetyl coenzyme A and then enters the citric acid cycle and produces adenosine triphosphate (ATP) (Glover et al., 2022). Referring to the research conducted by El-Hadedy & Abu El-Nour, (2012), it was shown that *E. coli* was at least able to ferment glucose, lactose, mannitol, and sucrose.

Table 2. Laboratory culture results and descriptive statistics

Groups	Replications	Number of colonies (CFU)	pH of medium and colony characteristics	Descriptive and univariate statistics
S. aureus on alternative media	1	103	pH 7, colonies round, white, small size, smooth	Min. 95 CFU Max. 208 CFU Mean 119.12 CFU Test of normality (Shapiro-Wilk) significance value
	2	110		
	3	105		
	4	99		
	5	100		
	6	114		

	7	111		0.000
	8	98		
	9	95		
	10	119		
	11	140		
	12	121		
	13	160		
	14	208		
	15	102		
	16	121		
E. coli on alternative media	1	105	pH 7, colonies round, white, small size, smooth	Min. 100 CFU Max. 423 CFU Mean 160,56 CFU Test of normality (Shapiro-Wilk) significance value 0,000
	2	125		
	3	100		
	4	189		
	5	180		
	6	167		
	7	160		
	8	219		
	9	101		
	10	135		
	11	178		
	12	423		
	13	151		
	14	102		
	15	132		
	16	102		
S. aureus on NA	1	205	pH 7, colonies round, white, small size, smooth	Min. 129 CFU Max. 205 CFU Mean 169,2 CFU
	2	162		
	3	129		
	4	163		
	5	187		
E. coli on NA	1	363	pH 7, colonies round, white, small size, smooth	Min. 115 CFU Max. 363 CFU Mean 221,2 CFU
	2	208		
	3	115		
	4	197		
	5	223		
Uncultured alternative medium	1	0		
Uncultured NA medium	1	0		

In managing protein, *E. coli* has a variety of protease enzymes that break down their substrates into small peptide fragments consisting of amino acids (Bittner et al., 2017). Amino acids such as serine, aspartate, cysteine, glycine, glutamate, and alanine can be used by *E. coli* as sole carbon and nitrogen sources under aerobic conditions (Liu et al., 2020).

The content of various vitamins in Cilembu sweet potato also has a good effect on the growth of *E. coli*, in line with Monk et al., (2016) which state that the addition of vitamins in the media can increase the average growth of *E. coli*, and Tramonti et al., (2021) who describes the metabolism of vitamin B6 group by *E. coli*. Putnam & Goodman (2020) explained that gut commensal bacteria, including *E. coli*, can acquire various types of B vitamins for use in various metabolic pathways, which is also supported by Peterson et al. (2020). On the other hand, vitamin B6 is reported to have antibacterial effects against *S. aureus* and *S. epidermidis*

(Kayumov et al., 2015; Mikkelsen & Apostolopoulos, 2019), which may be the reason why the number of *S. aureus* colonies on alternative media is lower than *E. coli*.

Table 3. Statistical test

Mann-Whitney U statistics	**Asymp. Sig. (2-tailed)**
S. aureus on alternative media vs NA	0.006
E. coli on alternative media vs NA	0.057
S. aureus vs *E. coli* on alternative media	0.040

Furthermore, *S. aureus* is also known to be able to utilize carbohydrates and amino acids. *S. aureus* uses the tricarboxylic acid cycle (TCA, also known as the citric acid cycle), pentose phosphate (PPP), and the Embden-Meyerhof-Parnas (EMP) pathway to catabolize carbohydrates (Ferreira et al., 2013). Through biochemical tests, it is proven that *S. aureus* is at least able to ferment glucose, lactose, sucrose, fructose, and mannitol (El-Hadedy & Abu El-Nour, 2012); and consumed the amino acids alanine, serine, and aspartate during growth under aerobic conditions in vitro (Zühlke et al., 2016).

In this study, we also found that the number of colonies that grew on alternative media was less than in NA, both by *S. aureus* and *E. coli*. This shows that peptone and yeast extract in NA are truly in the ideal and simpler composition. Conversely, the composition of nutrients in alternative media, although many types are in a larger and more complex form so that the growth of microorganisms requires more time to decompose these components into simpler forms that can be absorbed by cells and used for cell synthesis and energy (Juariah, 2021). The process that requires time to break down complex nutrients to be simpler is clearly seen by *S. aureus* where the number of colonies in alternative media is significantly different from the number of colonies in NA; whereas *E. coli* can be said to be more efficient because the difference is not significant. In addition, it should be noted that vitamins also have the effect of inhibiting bacterial growth, for example vitamin C (Hassuna et al., 2023; Razzaq & Askar, 2023) and vitamin E (Vergalito et al., 2020). This could also be the reason why the number of bacterial colonies on alternative media is less than on NA.

To prove whether the number of E. coli and S. aureus colonies had a significant difference, the Mann-Whitney U statistical test was used. The Mann-Whitney U test between the number of *S. aureus* colonies on alternative media and NA media obtained a significance value of 0.006 ($p \leq \alpha$ 0.05), which means that there was a difference in the number of colonies in the two groups. Meanwhile, in the test between the number of *E. coli* colonies on alternative media and NA, a significance value of 0.057 ($p > \alpha$ 0.05) was obtained, which meant that there was no difference in the two groups. Next, between the number of *S. aureus* and *E. coli* colonies on the alternative media, a significance value of 0.040 ($p \leq \alpha$ 0.05), was obtained, which means that there was a difference in the number of colonies in the two groups (Table 3).

4. CONCLUSION

This study shows that Cilembu sweet potatoes contain the nutrients needed by S. aureus and E. coli, as evidenced by the presence of colonies that grow with characteristics similar to NA. However, because the nutrients in it must be decomposed into a simpler form, we remind that the use of alternative media is more intended for simple Gram-negative bacteria such as E. coli, rather than Gram-positive like S. aureus. Further research is needed by combining Cilembu sweet potato flour with other nutritional sources that can better support the growth of S. aureus.

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