

## Potential of *Metroxylon sago* Flour as an Alternative Medium for *Escherichia coli* Growth: A Papuan Carbohydrate Source for Microbiology Laboratory Independence

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### Abstract

**Background:** Commercial microbiological media such as Nutrient Agar (NA) are widely used for cultivating non-fastidious bacteria, but their cost and procurement time may limit routine microbiology practicums in eastern Indonesia. This study evaluated Papuan sago (*Metroxylon sago* Rottb.) flour as a local alternative medium for *Escherichia coli* growth.


**Methodology:** A comparative laboratory experiment was conducted using NA as the control and a sago flour-based medium formulated from 10 g sago flour, 4 g agar, 7 g glucose, and 1 g monosodium glutamate in 500 mL distilled water. *E. coli* suspensions standardized to 0.5 McFarland were serially diluted, spread-plated in triplicate, and incubated at 37 °C for 48 h.

Colony counts were calculated from the 10<sup>-4</sup> dilution because all replicate plates were within the countable range. **Findings:** The sago flour medium supported *E. coli* growth up to 4.5 × 10<sup>6</sup> CFU/mL, lower than the 11 × 10<sup>6</sup> CFU/mL obtained on NA, but still showing consistent growth viability. The relative effectiveness of the sago flour medium reached 40.9% of the standard medium. Colonies on sago medium remained circular, entire, and convex but were smaller and milky white. The reduced growth suggests that sago flour mainly supplies carbohydrates, while nitrogen and mineral availability may remain limiting. **Contribution:** Papuan sago flour is feasible as a low-cost educational medium for demonstrating bacterial growth, although further enrichment with nitrogen and mineral sources is required before it can approach the performance of NA.

**Keywords:** Alternative Medium; *E. coli*; Laboratory Independence; Papua; Sago Flour



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## INTRODUCTION

Growth media are essential components in microbiology because they provide water, carbon and nitrogen sources, minerals, and other growth factors required by microorganisms to grow, form colonies, and express their phenotypic characteristics (Atlas, 2010; Basu et al., 2015; Bonnet et al., 2020). In microbiology practicum and research, Nutrient Agar (NA) remains a commonly used medium for cultivating non-fastidious heterotrophic bacteria because its nutrient composition is stable and easily reproducible (Cappuccino & Sherman, 2013; Benson, 2021; Elbing & Brent, 2019). However, dependence on commercial dehydrated media is problematic for institutions located far from national supply centers. In Indonesia, 500 g of commercial NA is commonly sold in the range of approximately IDR 1.65–2.19 million, depending on brand and supplier (LabMart, 2025; Blibli, 2026). For laboratories in Papua, this cost is compounded by procurement lead time and distribution constraints. Broader logistics analyses have shown that eastern Indonesia is affected by limited connectivity, high logistics costs, and price disparities relative to western Indonesia (World Bank, 2015; Zen, 2022). These constraints may reduce practicum frequency and encourage the search for locally available media ingredients.

The search for more affordable alternative media has encouraged many studies to use local food materials and biological wastes as nutrient sources for microbial growth. Previous studies have shown that boiled yellow and purple tubers, lesser yam, sweet potato, soybean flour, vegetables, legumes, and materials derived from mangrove fruits and sago seeds can support the growth of *Escherichia coli* and other bacteria, although with varying levels of effectiveness (Deivanayaki & Iruthayaraj, 2012; Anisah & Rahayu, 2015; Khaerunnisa et al., 2019; Arum & Wahyudi, 2022; Ramadhan et al., 2021; Rofiyanti et al., 2024; Rahmah et al., 2025; Gamit et al., 2023). This variation suggests that the success of an alternative medium is strongly influenced by the availability of carbon, nitrogen, and minerals, the physical properties of the medium, and its compatibility with the physiological requirements of the target microorganism (Madigan et al., 2018; Prescott et al., 2017).

Papua possesses abundant biological resources, one of which is sago (*Metroxylon sagu* Rottb.), a food commodity that has major social, cultural, and economic importance in many parts of Papua. Sago is rich in starch, particularly amylose and amylopectin, and therefore has potential as a carbon source to support microbial metabolism (Flach & Rumawas, 1996; Karim et al., 2008; Rahmawati et al., 2019; Moeljono et al., 2025). In addition to its role as a staple food, sago also has development potential in industry, biomaterials, and biotechnology, including as a substrate for fermentation processes and microbial growth media (Zhang et al., 2013; Anastassiadis, 2016). Nevertheless, the potential of sago flour as a microbiological medium has received far less attention than its food and physicochemical properties (Karim et al., 2008; Moeljono et al., 2025).

Previous studies indicate that local Papuan food resources have potential to be developed as alternative media for bacterial growth. Sarima et al., (2025) reported that a taro-based medium supported *Escherichia coli* growth up to  $8.3 \times 10^6$  CFU/mL, or approximately 75.5% of the effectiveness of Nutrient Agar. This finding strengthens

the view that local carbohydrate sources can be utilized as more contextual and affordable microbiological media. Sago is also a highly promising local carbohydrate source, yet its use as a bacterial growth medium remains insufficiently discussed in scientific articles. This gap is important because sago has distinctive characteristics, particularly its starch dominance, low protein content, and different mineral composition, all of which may affect the ability of the medium to support bacterial growth (Flach & Rumawas, 1996; Purwandari et al., 2014; Rahmawati et al., 2019).

*Escherichia coli* was selected as the test bacterium because it is a Gram-negative model organism widely used in basic microbiology practica and in assessments of growth-medium effectiveness. Its nutritional requirements are well understood, and its growth response on different media is frequently used to evaluate the suitability of alternative media (Tortora et al., 2019; Elbing & Brent, 2019). In addition, its colony morphology and microscopic characteristics are relatively easy to recognize, making it suitable for laboratory teaching contexts (Benson, 2021; Pelczar et al., 2001).

Based on this background, this study aimed to evaluate the potential of Papuan sago flour as an alternative medium for *E. coli* growth using NA as the standard control. Specifically, the study compared total viable counts expressed as CFU/mL, described macroscopic and microscopic colony characteristics, calculated the relative effectiveness of the sago medium against NA, and interpreted the findings in relation to the nutritional profile of sago. The novelty of this study lies in providing a preliminary quantitative benchmark for sago flour as a local Papuan medium ingredient to support contextual and more independent microbiology practicums.

## **METHOD**

### **Experimental Design**

This study used a comparative true experimental laboratory design with two media treatments: Nutrient Agar (NA) as the standard control and sago flour-based medium as the alternative medium. Each medium was inoculated at three serial dilution levels ( $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$ ) with three replications per dilution. Sterile controls were prepared for each medium. Thus, the study used 18 inoculated plates (2 media  $\times$  3 dilutions  $\times$  3 replications) and 6 sterile-control plates, for a total of 24 Petri dishes. The final quantitative comparison was based on the  $10^{-4}$  dilution because all replicate plates in both media were within the countable range of 30–300 colonies.

### **Materials and Medium Preparation**

Sago flour was obtained from Youtefa Market, Abepura, Papua. The control medium was prepared by dissolving 14 g of NA powder in 500 mL distilled water, heating until fully dissolved, and sterilizing at 121°C for 15 min. The sago flour medium was prepared by suspending 10 g sago flour, 4 g agar, 7 g glucose, and 1 g monosodium glutamate in distilled water to a final volume of 500 mL. The mixture was homogenized, heated gradually until gelatinization occurred, adjusted to pH 6.8–7.2, and sterilized at 121°C for 15 min. Approximately 15–20 mL of each sterile medium was poured into 90 mm Petri dishes under aseptic conditions and allowed to solidify. No proximate analysis of the sago flour was performed in this

study; therefore, interpretation of its starch, protein, ash, and mineral characteristics was based on published literature on sago starch composition.

### **Bacterial Culture and Inoculation**

The test bacterium was *Escherichia coli* ATCC 25922. The bacterial suspension was adjusted to the turbidity of 0.5 McFarland standard and serially diluted in sterile 0.9% NaCl up to  $10^{-5}$ . A 100  $\mu$ L aliquot from each dilution was spread onto NA and sago flour medium using the spread-plate method. All inoculations were carried out aseptically in a laminar air flow cabinet.

### **Incubation Conditions**

All inoculated plates and sterile controls were incubated aerobically at 37 °C for 48 h. Sterile controls were inspected to confirm the absence of contamination during medium preparation, pouring, and incubation.

### **Data Collection and Calculation**

After incubation, the number of colonies was calculated at dilution resulting in 30 – 300 colonies (CFU/plate) and analyzed descriptively. The colonies were also observed macroscopically (shape, size, color, margin, and elevation) and microscopically through Gram staining to identify the morphological characteristics of bacterial cells (Atlas, 2010; Benson, 2021). The colony calculation is carried out with the formula:

$$\text{CFU/mL} = \frac{\text{Number of Colonies}}{\text{Dilution Factor} \times \text{Inoculum Volume (mL)}}$$

### **Data Analysis**

The data were presented in the form of tables and graphs, describing the average number of colonies per type of media and qualitative observations of colony morphology. Interpretation was carried out descriptively and compared with the NA control media.

## **RESULT AND DISCUSSION**

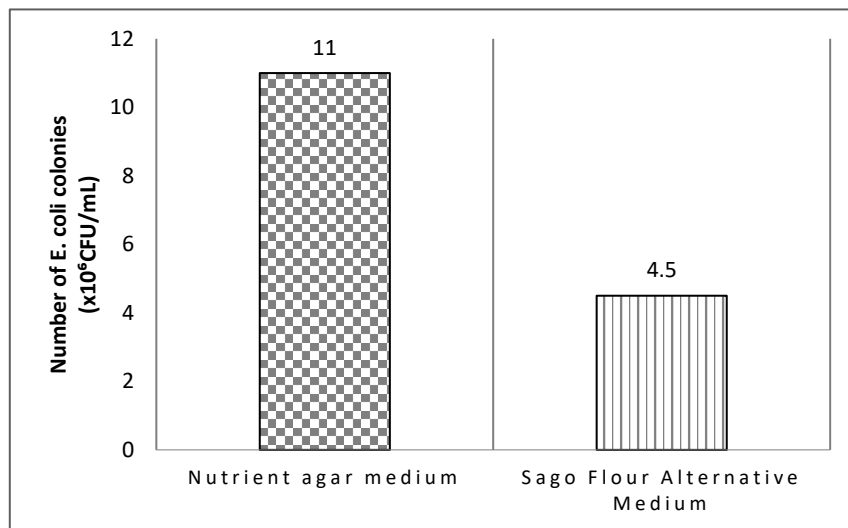
### **Result**

Before inoculation, the NA medium appeared transparent yellow, whereas the sago flour medium was turbid white. Neither medium showed contaminant microbial growth during the initial incubation period, indicating that the sterilization process was successful and that both media were sufficiently stable to be used as bacterial growth substrates. This physical stability is important because a homogeneous, sterile, and non-synergetic solid medium better supports the formation of colonies that can be observed clearly (Cheesbrough, 2006; Bonnet et al., 2020). Observations were conducted macroscopically and microscopically, and the number of colonies growing on each medium was calculated (CFU/mL) (Table 1 & Figure 2).

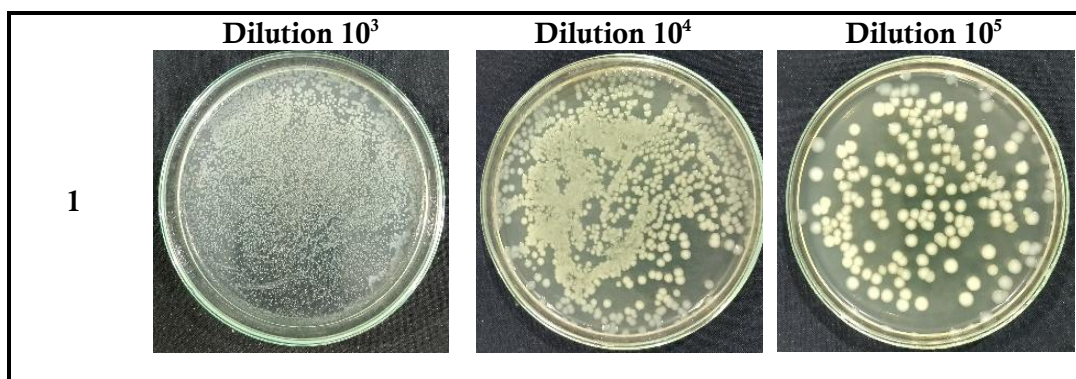
**Table 1.** Total Plate Count of *E. coli* Colonies on Nutrient Agar and Sago Flour Alternative Medium

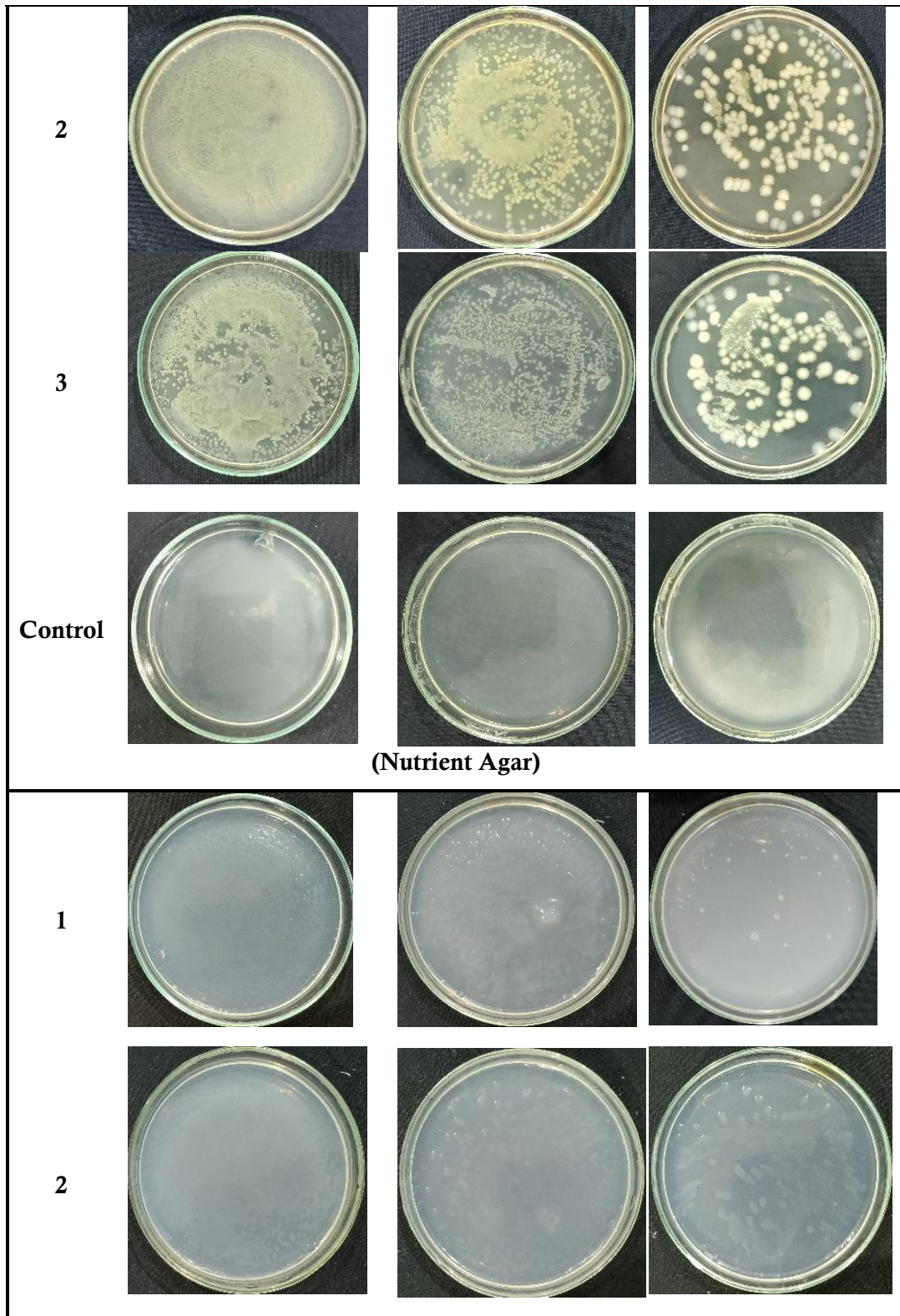
Medium	Dilution	Replicate			Average	TPC (CFU/mL)
		1	2	3		
Nutrient Agar	10 <sup>3</sup>	TNTC*	TNTC	TNTC	TNTC	11X10 <sup>6</sup>
	10 <sup>4</sup>	212	231	298	247	
	10 <sup>5</sup>	115	81	46	87	
	Control	0	0	0	0	
Sago flour medium	10 <sup>3</sup>	TNTC	TNTC	TNTC	TNTC	4,5X10 <sup>6</sup>
	10 <sup>4</sup>	62	51	79	64	
	10 <sup>5</sup>	85	31	21	39	
	Control	0	0	0	0	

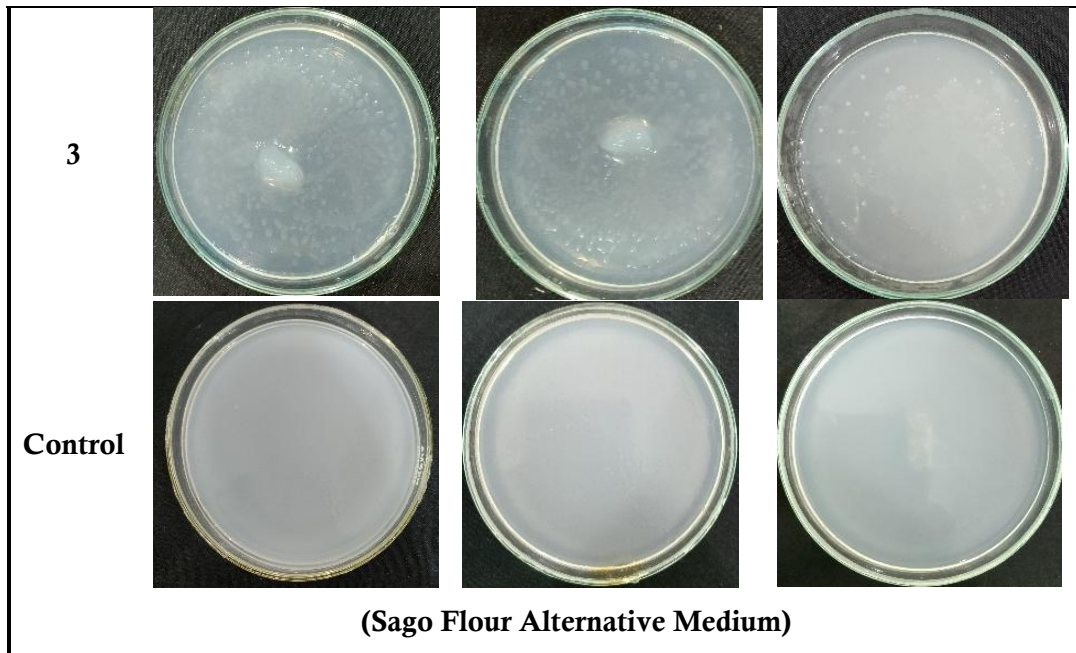
\*TNTC = Too numerous to count



**Figure 1.** Comparison of the Average Number of *E. coli* Colonies

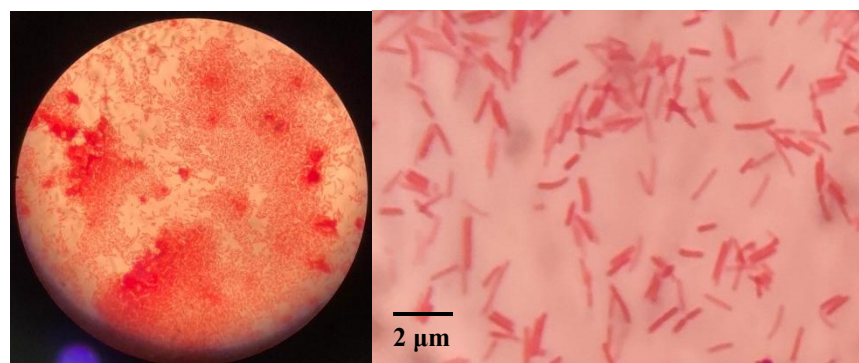






**Figure 2.** Growth of *E. coli* on Nutrient Agar and Sago Flour Alternative Medium (each treatment with three replications and sterile control across serial dilutions)

Colony counts showed that *E. coli* was able to grow on the sago flour medium, although the number of colonies was lower than on the NA control medium. On NA, total growth reached  $11 \times 10^6$  CFU/mL, whereas on the sago flour medium it reached  $4.5 \times 10^6$  CFU/mL. Thus, the relative effectiveness of the sago flour medium was 40.9 % compared with NA. At the  $10^{-4}$  dilution, the sago flour medium yielded an average of 64 colonies, whereas NA yielded an average of 247 colonies. At the  $10^{-3}$  dilution, all plates were categorized as TNTC (too numerous to count), whereas the sterile controls showed no colony growth.



**Figure 3.** Gram-stained *Escherichia coli* cells grown on sago flour medium, observed using oil immersion with the 100× objective lens (1000×total magnification)

Macroscopically, *E. coli* colonies on the sago flour medium were circular, entire, and convex, similar to colonies on NA. The main differences were observed in colony size and color. Colonies on the sago flour medium tended to be smaller and milky white, whereas colonies on NA were larger and yellowish. Microscopic observation

through Gram staining showed short rod-shaped Gram-negative bacterial cells, consistent with the typical characteristics of *E. coli*.

**Table 2.** Colony Characteristics of *E. coli* on Nutrient Agar and Sago Flour Alternative Medium

Characteristic	Nutrient Agar	Sago flour alternative medium
Shape	Circular	Circular
Size	Large	Small
Color	Yellowish	Milky white
Margin	Entire	Entire
Elevation	Convex	Convex

### Discussion

The results of this study confirm that sago flour has a real capacity to support *E. coli* growth, even though its performance remains below that of Nutrient Agar (NA). Biologically, this finding is reasonable because sago is rich in starch that can function as a carbon source, whereas its protein, mineral, and other growth-factor contents are far lower than those of synthetic media such as NA (Flach & Rumawas, 1996; Karim et al., 2008; Madigan et al., 2018). NA supplies peptone and beef extract rich in amino acids, peptides, vitamins, and other nitrogen-containing compounds that are directly available for bacterial biosynthesis and energy production (Cappuccino & Sherman, 2013; Benson, 2021). In contrast, sago flour-based media are dominated by complex carbohydrates, so *E. coli* must first access metabolizable fractions before optimal growth can occur.

The performance of the sago medium, which reached 40.9 % of NA, can still be considered meaningful in the context of educational laboratories. In alternative-medium exploration, the primary purpose is not necessarily to replace every function of synthetic media completely, but rather to assess whether local materials can support bacterial growth consistently and produce colonies that can still be observed, counted, and identified (Basu et al., 2015; Gamit et al., 2023). From this perspective, the sago flour medium met the initial feasibility criteria because *E. coli* still grew, colonies formed clearly, and the observed cell morphology corresponded to the target bacterium.

The high starch content of sago is the main strength of this medium. Sago is known to contain proportions of amylose and amylopectin that make it a potentially useful carbon substrate for food and biotechnological applications (Karim et al., 2008; Rahmawati et al., 2019; Moeljono et al., 2025). The heating process up to gelatinization during medium formulation may have increased substrate availability for the bacterium because starch granules became more hydrated and more open to hydrolysis (Karmakar et al., 2014). However, the dominance of carbohydrates without adequate nitrogen and micronutrients can limit cell division rate and colony size, as reflected by the smaller *E. coli* colonies on the sago medium (Prescott et al., 2017; Tortora et al., 2019).

These findings are consistent with studies on alternative media derived from local resources, which generally report lower performance than synthetic media but

still indicate suitability as partial substitutes or teaching media. Boiled yellow and purple tubers, lesser yam, soybean flour, sweet potato, and various vegetables have all been reported to support the growth of *E. coli* and *Staphylococcus aureus* with varying degrees of effectiveness depending on material composition, bacterial species, and medium formulation methods (Deivanayaki & Iruthayaraj, 2012; Khaerunnisa et al., 2019; Ramadhan et al., 2021; Rofiyanti et al., 2024; Arum & Wahyudi, 2022; Djala et al., 2025). In this comparative context, the performance of the sago medium can be categorized as moderate: it has not reached the effectiveness of alternative materials enriched with additional nutrients, but it remains promising given that sago contributes mainly carbon and only small amounts of protein.

Compared with previous research on taro-based medium, the sago flour medium in the present study still showed lower effectiveness. Sarima et al., (2025) was reported Taro medium to support *E. coli* growth of  $8.3 \times 10^6$  CFU/mL, or around 75.5 % of the performance of Nutrient Agar, whereas the sago flour medium in this study reached only  $4.5 \times 10^6$  CFU/mL, or around 40.9% of Nutrient Agar. This difference is understandable because taro is not only rich in starch but also contains relatively more diverse protein, fiber, vitamins, and minerals than sago flour (Soekarto, 1990; Wang et al., 2011). In contrast, sago flour is dominated by carbohydrates and contains more limited protein and micronutrients, so it functions more as a primary carbon source than as a complete nutrient matrix for bacterial growth. Even so, Sago flour still has important prospects as an alternative medium because of its abundant availability, relatively low cost, and close connection to everyday life in Papuan communities.

The colony characteristics on the sago medium—remaining circular, entire, and convex—indicate that this medium did not cause extreme distortion of colony morphology. Colony forms that remain consistent with the general characteristics of *E. coli* on solid media suggest that, although nutritionally limited, the basic physical properties of the medium were still able to support normal morphological expression (Pelczar et al., 2001; Benson, 2021). The shift in colony color from yellow on NA to milky white on the sago medium may be related to substrate composition, biomass accumulation, and interactions between cellular metabolites and the medium matrix (Pelczar et al., 2001). Similar findings have been reported in other alternative-media studies, where non-synthetic media tend to generate variation in colony color and size without altering the fundamental identity of the growing microorganism.

Beyond its scientific value, this study also has important practical implications for microbiology education. Teaching laboratories in frontier, outermost, and disadvantaged areas frequently face constraints related to cost, supply, and the sustainability of commercial media procurement. In such situations, alternative media based on local materials can function as pedagogical bridges for basic practicums, bacterial culture demonstrations, and colony-counting exercises (Bonnet et al., 2020; Basu et al., 2015). The use of sago flour also has a strong contextual dimension because it connects microbiology learning with Papuan local biological resources. This approach can strengthen science literacy, local relevance, and educational innovation based on regional potential.

However, the sago flour medium cannot yet be positioned as a full substitute for standard media. The lower level of growth observed indicates that formula

optimization is still necessary. Realistic strategies include adding organic or inorganic nitrogen sources, yeast extract, peptone, or specific minerals; adjusting sago concentration; carrying out starch pre-hydrolysis to make the substrate more accessible; and evaluating post-sterilization pH stability (Zhang et al., 2013; Anastassiadis, 2016; Rahmah et al., 2025). Optimization should also be accompanied by testing on more than one type of microorganism, including Gram-positive bacteria and possibly yeasts or fungi, so that the scope of application becomes broader (Kambuno et al., 2021; Maghfiroh et al., 2020).

## CONCLUSION

This study demonstrates that Papuan sago flour can function as a local basal ingredient for cultivating *Escherichia coli*, but its growth-supporting capacity remains significantly lower than that of Nutrient Agar. The sago flour medium produced visible, countable, and morphologically recognizable colonies, making it feasible for basic teaching activities such as growth demonstration, colony observation, and Gram staining. However, its lower CFU yield and smaller colonies indicate that starch-dominated sago flour does not provide a sufficiently balanced supply of nitrogen, minerals, and growth factors. The main contribution of this study is the provision of a preliminary quantitative benchmark for sago flour as a Papuan local-resource medium, showing both its educational potential and its nutritional limitations. Further formulation optimization, particularly nitrogen and mineral enrichment and possible starch pre-hydrolysis, is required before sago flour medium can be developed as a more robust substitute for standard synthetic media.

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