# The Utilization of Taro (Colocasia esculenta) as a Bacterial Growth Medium to Support Microbiology Laboratory Independence: A Study of Local Papuan Food Resources

p-ISSN: 2442-9481

e-ISSN: 2685-7332

# Sarima Sarima(\*), Makmum Ashari, Rimba Lukas Kiding, Estervina Rebekah Ance Yoku

Biology Education Study Program, Faculty of Teacher Training and Education, Universitas Cenderawasih Jln. Raya Sentani, Abepura, Papua 99351, Indonesia

\*Corresponding Author: sarima@fkip.uncen.ac.id Submitted June 20 Th 2025 and Accepted August 30 Th 2025

#### **Abstract**

Background: In universities, the availability of bacterial growth media, which is typically synthetic media such as Nutrient Agar (NA), is extremely important for microbiology practicum activities. Nevertheless, in eastern Indonesia, such as Papua, the high cost of commercial media, the reliance on distribution from outside the region, and the limited budget and laboratory facilities frequently present obstacles to access. Currently, there is a significant lack of research on the use of Papuan local food resources as an alternative medium for bacterial growth. Consequently, further research is required to address this gap. The objective of this investigation is to investigate the potential of taro (Colocasia esculenta), a local source of Papuan carbohydrates, as the foundation for the development of an alternative medium for the growth of Escherichia coli bacteria. This is due to the amylose content of 20–25% and amylopectin of 75– 80%, which have the potential to serve as an energy source for bacteria. Methodology: The media was made from taro extract formulated with the addition of agar, glucose, and MSG, then sterilized and inoculated with E. Coli bacteria. The data were analyzed using the parameters of the number of colonies (CFU/mL) with the ALT calculation standard, as well as a descriptive comparison between the control medium (NA) and the taro media. Findings: The Taro alternative media is capable of supporting bacterial growth at a rate of  $8.3 \times 10^6$  CFU/mL, which is approximately 75.5% of the NA media's capacity of  $11 \times 106$  CFU/mL. Although the color of the colonies differs slightly, the morphological characteristics of colonies growing in alternative media are similar to those of colonies in NA media in terms of shape, elevation, size, and edges. **Contribution:** These findings indicate that taro has significant potential as a substitute for microbiological media that is based on local resources. Not only does the utilization of this medium offer practical and cost-effective solutions for laboratory activities, but it also promotes educational independence and innovation that are rooted in local knowledge in restricted regions. The implications of this research provide opportunities for laboratory independence in the 3T area and promote the utilization of local wisdom for sustainable microbiology research and education.

**Keywords:** Alternative Media; <u>E</u>. <u>coli</u> Bacteria; Local Foodstuff; Papua; Taro



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<u> https://doi.org/10.36987/jpbn.v11i3.7972</u>

### **INTRODUCTION**

The primary means of supporting practicum activities in higher education, particularly in the context of science and biotechnology education, is the existence of laboratories. This relationship is inextricably linked to microbiology learning. The bacterial growth medium is a crucial component of the microbiology practicum, as it functions as a substrate for the growth and development of microorganisms. In the field of microbiology, the utilization of media is essential for the identification of bacteria by growing, isolating, quantifying, and testing their physical properties (Rafika et al., 2024). Nutrient Agar (NA) and Potato Dextrose Agar (PDA) are synthetic media that have become the standard in various microbiological activities. This advantage is due to their balanced nutrient composition and their capacity to promote the growth of a diverse array of heterotrophic bacteria (Cappuccino et al., 2013; Benson, 2021). However, particularly in eastern Indonesia, including Papua, the availability and accessibility of these media remain restricted.

p-ISSN: 2442-9481

e-ISSN: 2685-7332

The limitations that result from these factors include the dependence on distribution from outside the region, relatively expensive media prices, and non-optimal budgets and laboratory facilities in universities in the 3T (frontier, outermost, and disadvantaged) areas (Fitri, 2019). The effectiveness of microbiology learning is diminished as a result of the limited frequency of practicum implementation. Practicum activities are critical in the development of student competencies, including the ability to isolate microorganisms, identify colonies, and comprehend the dynamics of bacterial growth (Madigan et al., 2018). Consequently, inventive and sustainable solutions are required to replace the commercial synthetic media that has been employed thus far by maximizing the potential of local resources.

One potential approach is the use of local foods that are rich in carbohydrates as the basic ingredients for making alternative media (Sundari et al., 2021). Several previous studies have evaluated the effectiveness of local food as an alternative medium for bacterial growth. Khaerunnisa et al., (2019) reported that yellow tuber boiled water was able to support the growth of E. coli up to  $284.83 \times 10^5$  CFU/mL (>100% effectiveness compared to Nutrient Agar/NA), while purple tubers showed growth equivalent to NA in E. coli (173.16 × 10<sup>5</sup> CFU/mL) but lower for S. aureus  $(45.33 \times 10^5 \text{ CFU/mL})$  vs  $207.5 \times 10^5 \text{ CFU/mL}$ ). Wulandari et al., (2019) found that vegetable flour-based media (a combination of carrots, tomatoes, and cabbage) supported the growth of E. coli by  $53 \times 10^{5}$  CFU/mL (about 21% of NA) and S. aureus by 36 × 10<sup>5</sup> CFU/mL (about 18% of NA), showing lower effectiveness than NA. Meanwhile, Deivanayaki & Iruthayaraj (2012) demonstrated that the formulation of fresh vegetables (carrots, tomatoes, cabbage, and pumpkin) on solid media is able to promote the growth of several bacteria: E. coli 250 CFU/0.1 mL (125% of NA), Staphylococcus sp. 230 CFU/0.1 mL (191% of NA), and Klebsiella sp. 150 CFU/0.1 mL (150% of NA). Another result was shown by Rofiyanti et al., (2024), who used soybean flour with dosage variations. The medium with 3 g of soybean flour produced an E. coli growth of  $41.4 \times 10^{13}$  CFU/mL, which is about 51% effectiveness compared to NA (81.8  $\times$  10<sup>13</sup> CFU/mL), while the 4–6 g variation showed low effectiveness (<35% of NA). Research by Anisah & Rahayu (2015) reported that gembili tubers can support the growth of *E. coli* and *S. aureus* with higher effectiveness than NA, indicated by larger colonies, larger numbers, and easy-to-observe morphology.

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e-ISSN: 2685-7332

In general, prior research has demonstrated that local foodstuffs possess genuine potential as alternative media, albeit the degree of efficacy is contingent upon the ingredients and test bacteria employed. Nevertheless, there is a significant lack of research that specifically investigates the microbiological properties of Papuan local food.

Taro (*Colocasia esculenta*) is a traditional food ingredient that has been widely used in Papua due to its abundant biological wealth and local food resources (Wenda & Nangio, 2020; Wulanningtyas et al., 2019). Taro is recognized for its complex carbohydrates, which contain an amylose content of approximately 20-25% and amylopectin of 75-80%, as well as other critical nutrient components, including proteins, minerals, and vitamins (Karmakar et al., 2014; Soekarto, 1990). This material has the potential to serve as an energy source for the proliferation of microorganisms due to its substantial carbohydrate content.

The availability of carbon sources is a critical factor in the success of microorganism growth in the field of microbiology (Prescott et al., 2017). Consequently, the utilization of taro as a fundamental component of bacterial growth media is a pertinent solution to the issue of laboratory independence, particularly in regions with restricted access to commercial media. The objective of this investigation is to evaluate the efficacy of taro (*Colocasia esculenta*) as an alternative medium for bacterial growth in comparison to Nutrient Agar (NA). This research stands out due to its utilization of Papuan taro, a plant that has yet to receive extensive study. Consequently, it is anticipated that it will promote laboratory independence in the 3T sector by fostering innovations that are informed by local knowledge. It is anticipated that this method will result in the development of solutions that are not only economically viable and applicable but also contribute to the contextual and sustainable growth of microbiology education capacity in the Papua region.

## **METHOD**

Laboratory experiments were implemented in this investigation. The research was conducted on May 2025 at the Microbiology Laboratory, Faculty of Medicine, Cenderawasih University.

# **Tools and Materials**

Hot plates, analytical scales, magnetic stirrers, incubators, autoclaves, ovens, petri dishes, test tubes, Erlenmeyers, pipettes, ose, and microscopes were among the employed instruments. Additionally, aluminum foil and laminar air flow as the sterilization support equipments were employed. The following ingredients were used: Nutrient Agar media (NA, Himedia brand), pure *Escherichia coli* isolate (collection of the Microbiology Laboratory of the Faculty of Medicine of Cenderawasih University), 100 grams of taro (*Colocasia esculenta*) obtained from Youtefa Market, Jayapura, 4 grams of powdered gelatin (Swallow), 0.9% physiological NaCl, 7 grams of glucose (Merck), 1 gram of monosodium glutamate (Ajinomoto), and sterile aquades.

# **Sterilization Tools**

All appliances in direct contact with the media and samples were sterilized in a 170 °C oven for one hour. Before the sterilization process, aluminum foil was used to wrap glass tools, including petri dishes, Erlenmeyer flasks, and test tubes. The objective of this procedure was to prevent cross-contamination during inoculation and incubation, based on Cappuccino et al., (2013).

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## Creation of Agar Nutrient Media (NA)

A total of 14 grams of NA media were weighed and dissolved in 500 mL of aquades. The mixture was heated until it was homogeneous and transparent, and it was subsequently sterilized in an autoclave at 121°C for 15 minutes. This medium was employed as a comparison due to its standardization for the cultivation of heterotrophic microorganisms (Juariah & Sari, 2018).

# Taro Media Manufacturing

Small pieces of 100 g taro were chopped, boiled in 500 mL of aquades, and subsequently filtered to produce an extract. This extract was combined with 6 g of agar, 7.5 g of glucose, and 1 g of MSG. The volume was increased to 500 mL, and the mixture is then brought to a boil. The medium's pH was maintained within the range of 6.8–7.2. The autoclave was used to sterilize the media at 121°C for 15 minutes (Ariyanti & Rahayu, 2016).

### Manufacture of Suspension and Bacterial Inoculation

*E. coli* suspensions were made based on the McFarland standard of 0.5, then multi-stage dilution ( $10^{-1}$  to  $10^{-6}$ ) using NaCl 0.9%. Each dilution ( $10^{-4}$  to  $10^{-5}$ ) was inoculated into the media (NA and taro) using the spread plate method of 100 μL per petri dish aseptically three times. The incubation was carried out at 37 °C for 48 hours (Deivanayaki & Iruthayaraj, 2012). A control filled with sterile NaCl 0.9 % was also spread on the petri dish media of each medium.

### Colony Calculation and Morphological Observation

After incubation, the number of colonies was calculated at dilution resulting in 30–300 colonies (CFU/plate) and analyzed descriptively. The colony calculation is carried out with the formula 1. 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 by Atlas (2010); Benson (2021).

$$CFU/mL = \frac{Number of Colonies}{Dilution Factor X Inoculum Volume (mL)}$$
 (1)

## **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.

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# **RESULT AND DISCUSSION**

# Results

This study aims to test the effectiveness of taro-based alternative growth media (*Colocasia esculenta*) against the growth of *Escherichia coli* bacteria, compared to NA standard media. The sterilized media was tested by E. *coli* suspension inoculation through the spread plate method and incubated for 48 hours at 37 °C. The observation results were carried out macroscopically and microscopically, and the number of growing colonies (CFU/mL) was calculated in each medium. Table 1 and Figure 2 display the study's results.

**Table 1.** Calculation of Total Plate Number (ALT) Colonies in NA Media and Taro Alternative Media

Media	Dilution	Deuteronomy			A	AIT
Type		1	2	3	Average	ALT
Media NA	103	TNTC*	TNTC	TNTC	TNTC	11X10 <sup>6</sup> CFU/mL
	104	212	231	298	247	
	105	115	81	46	87	
	Control	0	0	0	0	0
Taro Alternative Media	103	TNTC	TNTC	TNTC	TNTC	8.3X10 <sup>6</sup>
	104	290	209	228	242	CFU/mL
	105	71	57	49	59	
	Control	0	0	0	0	0

Caption: \*TNTC = Too numerous to count

The calculation of the number of colonies shows that there is a variation in growth between NA media and taro media (Table 1 and Figure 2). At  $10^{-4}$  dilution, the NA media yielded an average of 247 colonies (equivalent to  $11 \times 106$  CFU/mL). Alternative media from taro yielded an average of 242 colonies (8.3 ×  $10^6$  CFU/mL). The number of colonies at  $10^{-3}$  dilution was entirely in the TNTC (*Too Numerous To Count*) category, which exceeded 300 colonies per cup (Prescott et al., 2017).

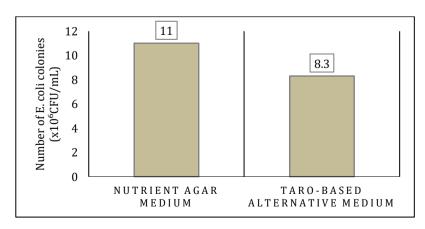
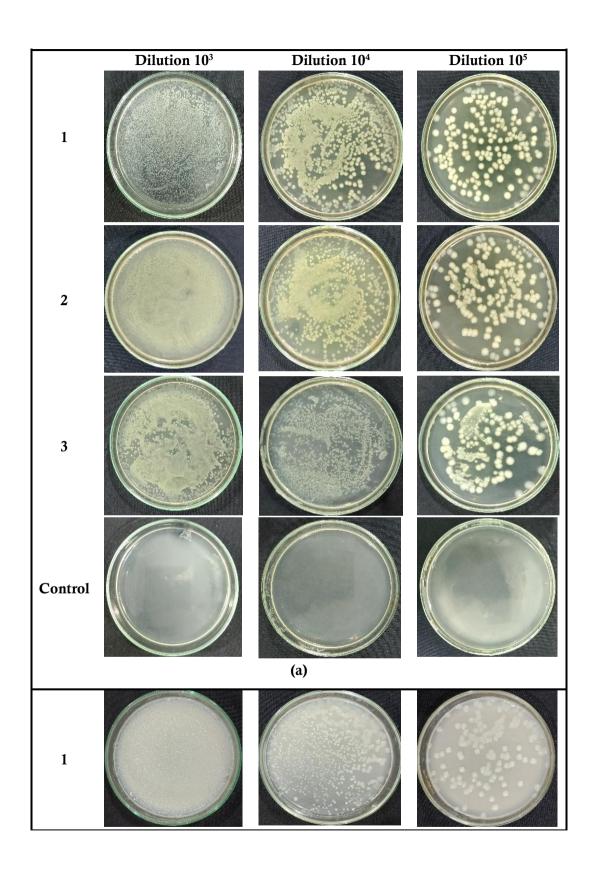
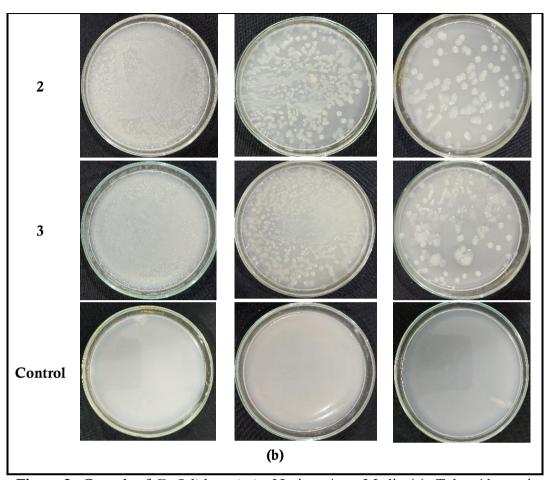


Figure 1. Comparison of the Average Number of E. Coli Bacterial Colonies



p-ISSN: 2442-9481

e-ISSN: 2685-7332



p-ISSN: 2442-9481

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**Figure 2.** Growth of *E. Coli bacteria in*: Nutient Agar Media (a); Talas Alternative Media (b); with three repetitions of treatment and control each.

These findings suggest that *E. coli* is able to grow well on both types of media, but the highest growth effectiveness is still demonstrated by NA media. The medium from taro showed an effectiveness of about 75.5% of the NA (Table 1); this result suggests that the alternative medium from taro was able to support the growth of bacteria in quantifiable quantities and replicated consistently on three repetitions. Observation data on the characteristics of *E. coli* bacterial colonies after 48 hours of incubation is presented in Table 2.

**Table 2.** Growth of *E. coli* bacteria in NA Media and Taro Alternative Media

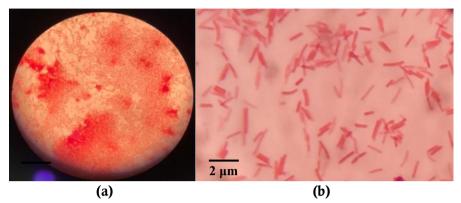
Characteristics of E. coli	Media NA	Taro Alternative Media
Shape	Round	Round
Size	Big	Big
Color	Yellow	Milky White
Margin	Flat	Flat
Elevation	Convex	Convex

Visual observation of *E. coli* colonies shows that colonies growing on both types of media have the characteristics of a circular shape, flat edges (*entire*), large colony size, and convex elevation. However, there are differences in terms of color. The

colonies in NA media are yellow, while they are milky white in taro media. Microscopic observation through Gram staining shows that the bacterial cells that grew on all media were Gram-negative bacteria in the form of short rods (*bacillus*) (Figure 3).

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**Figure 3.** (a) Gram staining of *E. coli bacteria* observed under a microscope with 100x magnification; (b) Microscopic morphology *of E. Coli* bacteria

## Discussion

The potential of local Papuan foodstuffs, specifically taro (*Colocasia esculenta*), to serve as an alternative medium for the growth of *Escherichia coli* bacteria is the focus of this investigation. The results suggested that *E. coli* was capable of establishing colonies and thriving in both the NA standard media and the taro alternative media. The calculations suggest that NA media generates an average of 1.1 × 10<sup>7</sup> CFU/mL, whereas taro media generates 8.3 × 10<sup>6</sup> CFU/mL, which is approximately 75.5 % more effective than NA. These results are consistent with previous research: In comparison to NA, yellow tubers demonstrate a greater than 100% efficacy (Khaerunnisa et al., 2019), soybean flour 51% (Rofiyanti et al., 2024), and vegetable flour only 21% (Wulandari et al., 2019). As a result, taro's efficacy is classified as medium-high in comparison to that of other alternative ingredients. This research contributes to the development of microbiological media that are derived from local natural resources. This study advocates for the autonomy of educational laboratories by employing Papuan taro, an alternative culture media that is cost-effective, accessible, and contextual in regions with logistical constraints.

In general, the media's capacity to support bacterial growth is significantly influenced by the available nutrient content, particularly the availability of carbon, nitrogen, and other essential compound sources (Maghfiroh et al., 2020). NA media is specifically engineered to meet the nutritional requirements of bacteria in the most efficient manner possible. This medium contains peptone and beef extract, which are sources of amino acids, polypeptides, proteoses, and glycogen that are readily absorbed by bacterial cells (Cappuccino et al., 2013). The compound can be directly utilized for protein synthesis and cellular energy, which enables bacteria to grow and develop rapidly due to its nutrient content. This conclusion is in accordance with the report by Rahmawati & Anliza (2024), which posits that the growth of *E. coli* is more optimal in

NA media due to the fact that this medium contains simple components that are well-suited to the metabolic requirements of bacteria.

p-ISSN: 2442-9481

e-ISSN: 2685-7332

In contrast, alternative media that are derived from local ingredients, such as taro, are composed of more complex compounds, specifically starch, which must be hydrolyzed into a simpler form before being available to microorganisms. To utilize taro as an energy source, bacteria must exhibit additional enzymatic activity due to the presence of complex carbohydrates, including amylose and amylopectin (Anisah & Rahayu, 2015; Ramadhan et al., 2021; Arum & Wahyudi, 2022; Djala et al., 2023; Martsiningsih et al., 2023). This is one of the primary reasons why bacterial growth in alternative media is typically slower than in synthetic media.

However, the taro-derived media demonstrates a moderate ability to promote the growth of *E. coli*. The nutritional composition of taro, comprising carbohydrates, proteins, and minerals such as phosphorus, potassium, and magnesium, substantially enhances bacterial metabolism (Onwueme & Charles, 1994; Wang et al., 2011). Furthermore, the physical characteristics of taro media, which are similar to the consistency of NA, contribute to the provision of an optimal growth surface for bacterial colonization. This condition enables optimal cell division and the adhesion of cells to the media surface.

The practice of calculating the number of colonies also documents the phenomenon of colony mergers and the formation of colony chains. This procedure is a prevalent occurrence in microbiological tests, particularly on media with concentrated inoculum. To prevent quantification bias, the joined colonies were counted as a single unit in this study (Cheesbrough, 2006). This phenomenon implies that the media's capacity to sustain the initial density of bacteria remains competitive, even when it is composed of local ingredients.

Apart from the quantity of colony growth, this study also pays attention to the morphological characteristics of colonies that grow in each medium. In general, the colonies formed in both media have a similar shape, which is round, with flat edges and convex elevation. This characteristic corresponds to the general characteristics of E. coli colonies that are typically found in solid nutrient media (Benson, 2021; Pelczar et al., 2001). This similarity suggests that alternative media from taro remains able to support the growth of bacterial cells with a distinctive morphological structure and does not undergo deformations or abnormalities that could indicate extreme nutritional stress. Nevertheless, the difference in colony color remains visible between NA and taro media. E. coli colonies in NA media show a yellowish color, while in alternative media they are milky white. The color of the colony is strongly influenced by the interaction between the substrate of the media and the metabolites of bacteria as well as local pH changes due to metabolic activity. According to Pelczar et al. (2001), colony discoloration can be caused by pigment compounds produced during growth, as well as by byproducts such as organic acids or bases resulting from carbohydrate metabolism. Colonies in the alternative media that are white indicate that the substrate and metabolites produced lack the yellow pigment, unlike in the NA media.

From the technical side, this study also shows that taro media has satisfactory physical stability after the sterilization process and during the incubation period. The medium does not liquefy, shows no contamination, and still supports bacterial growth

consistently on three repetitions. This data shows that the media formulation has met the basic stability criteria required in microbiology practice (Cheesbrough, 2006). The absence of microorganism growth in the control medium also indicates that the sterilization procedure is effective.

p-ISSN: 2442-9481

e-ISSN: 2685-7332

In addition to macroscopic observations, verification of cell morphology is carried out through Gram staining. The results showed that the growing bacteria were Gram-negative in the shape of a short rod, consistent with the morphological characteristics of *E. coli* commonly found in various microbiological literature (Tortora et al., 2019; Khaerunnisa et al., 2019). The pink staining on the Gram results indicated that the bacterial cell wall was unable to retain the violet crystals and absorb the saffron reverse dye, typical of the Gram-negative cell wall structure.

The novelty of this study is that it is the first study to use Papuan taro (*Colocasia esculenta*) specifically as an alternative medium for bacterial growth. There has been no previous study that specifically explored Papuan taro in the context of microbiology, so this study fills an important literature gap. This study is in line with the findings of Deivanayaki & Iruthayaraj (2012) that the formulation of fresh vegetables (carrots, tomatoes, cabbage, and pumpkin) can achieve 125 % effectiveness compared to NA, and Anisah & Rahayu (2015), which show that gembili tubers are able to support the growth of E. coli and S. aureus higher than NA. Compared to the study, the effectiveness of taro (75.5 %) is quite competitive and promising, although not as high as yellow bulbs or gembili. The study of Zhang et al., (2013) showed that alternative media based on local carbohydrate ingredients can produce competitive microbial growth if the formula is tailored to the needs of the target microorganisms.

Thus, the use of taro as a basic material for microbiological media opens up great opportunities in reducing dependence on commercial synthetic media, especially in areas with limited logistics and budgets. This strategy is in line with efforts to build educational laboratory independence and strengthen sustainable local potential-based contextual education practices. The novelty of this research makes an important contribution to the efforts of laboratory independence in the 3T region by providing local media options that are cheap, easy to obtain, and contextual with the needs of microbiology education.

#### **CONCLUSION**

This study indicated that taro media (*Colocasia esculenta*) was able to support the growth of E. coli by  $8.3 \times 10^6$  CFU/mL, which is 75.5% of the effectiveness of NA ( $1.1 \times 10^7$  CFU/mL). Thus, taro has more potential than other local ingredients such as vegetable flour ( $\leq 21\%$ ) or soybean flour (51%), although it is still lower than yellow bulbs (>100%) or fresh vegetable formulations (125%). The implication of this research is the opening of opportunities for the use of local Papuan food ingredients to support the independence of microbiology laboratories, reduce dependence on expensive synthetic media, and encourage the development of innovations based on local wisdom. The limitation of this study is that it only uses one type of test bacteria (E. coli, Gram-negative). Further research needs to test the effectiveness of taro on Gram-positive bacteria (Staphylococcus aureus) and other microorganisms, as well as

explore taro formulations in the form of flour or in combination with additional nitrogen sources to improve its effectiveness.

p-ISSN: 2442-9481

e-ISSN: 2685-7332

#### ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support from the Faculty of Teacher Training and Education (FKIP) through the DIPA PNBP BLU Universitas Cenderawasih, Fiscal Year 2025. This support was essential in facilitating the research activities and the completion of this work. The authors also wish to thank Universitas Cenderawasih for its continuous encouragement and institutional assistance throughout the research process.

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# How To Cite This Article, with APA style:

Sarima, S., Ashari, M., Kiding, R. L., & Yoku, E. R. A. (2025). Utilization of Taro (*Colocasia esculenta*) as a Bacterial Growth Medium Supporting Microbiology Laboratory Independence Based on Local Papuan Food Resources. *Jurnal Pembelajaran dan Biologi Nukleus*, 11(3), 1174-1186. https://doi.org/10.36987/jpbn.v11i3.7972

Conflict of interest: The authors declare that they have no known competing

financial interests or personal relationships that could have

p-ISSN: 2442-9481

e-ISSN: 2685-7332

appeared to influence the work reported in this paper.

Author contributions: All authors contributed to the study's conception and design.

Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [Sarima]. All authors contributed on previous version and revisions process of the manuscript. All authors

read and approved the final manuscript.