



## Isolation and Characterization of Endophytic Bacteria from *Curcuma Aeruginosa* Roxb As Antibacterial Candidates Against Pneumonia

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Received: October 2025; Revised: November 2025; Accepted: December 2025; Published: December 2025

**Abstract:** This study aimed to isolate and characterize endophytic bacteria from black turmeric rhizomes (*Curcuma aeruginosa* Roxb) and test their potential as antibacterial agents against *Streptococcus pneumoniae*. This experimental research began with the isolation of endophytic bacteria from surface-sterilized black turmeric rhizomes. The obtained pure isolates were characterized macroscopically (colony shape, margin, elevation, pigment) and microscopically (cell shape, Gram staining). The growth curve of potential isolates was determined, and antibacterial activity was tested using the disc diffusion method against *S. pneumoniae*, with ciprofloxacin as a positive control. Six endophytic bacterial isolates (PS1, PS2, PS3, PS4, PS5, PS6) were successfully isolated, showing variations in colony morphology, and all were classified as Gram-negative bacilli. All isolates exhibited strong antibacterial activity against *Streptococcus pneumoniae*, with inhibition zone diameters ranging from 15.3 mm to 20.6 mm. Black turmeric rhizomes (*Curcuma aeruginosa* Roxb) harbor diverse endophytic bacteria with significant potential as antibacterial agents against the pneumonia pathogen, *Streptococcus pneumoniae*.

**Keywords:** Isolation; characterization; endophytic bacteria; pneumonia

**How to Cite:** Utami, A. W., Rezaldi, F., Ginaris, R. P., Nabilla, J., Bramantyo, Setiawati, P., ... Wahyuni, D. (2025). Isolation and Characterization of Endophytic Bacteria from *Curcuma Aeruginosa* Roxb As Antibacterial Candidates Against Pneumonia. *Bioscientist: Jurnal Ilmiah Biologi*, 13(4), 2648–2656. <https://doi.org/10.33394/bioscientist.v13i4.18148>



<https://doi.org/10.33394/bioscientist.v13i4.18148>

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### INTRODUCTION

Pneumonia is a major form of acute respiratory infection (ARI) and remains one of the leading causes of mortality among children worldwide, including in Indonesia. According to the World Health Organization (WHO), pneumonia is the single largest infectious cause of death in children under five years old, accounting for approximately 988,136 deaths globally. This places pneumonia as the primary cause of mortality among children aged 1–5 years (Sonartra et al., 2024). Beyond its acute clinical manifestations, pneumonia can lead to severe complications such as purulent pericarditis, thoracic empyema, pneumothorax, and extrapulmonary infections, including purulent meningitis. In the long term, pneumonia may impair growth and development in children and, in severe cases, result in death (Riyanto & Megasari, 2021).

Pneumonia is an acute infection of lung tissue caused by various pathogenic microorganisms, including bacteria, viruses, and fungi (E. Sari & Bahrina, 2024). Among bacterial pathogens, *Streptococcus pneumoniae* is recognized as the most predominant etiological agent of pneumonia globally (Novita et al., 2023). This bacterium possesses a polysaccharide capsule with more than 100 biochemically distinct serotypes, which contributes to its virulence and adaptability (Manna et al.,

2025). Antibiotic therapy has traditionally been the primary treatment strategy for pneumococcal infections. However, the increasing emergence of antibiotic resistance in *Streptococcus pneumoniae* has become a critical global health concern. Resistance to  $\beta$ -lactam antibiotics, including penicillin—which was once highly effective—has been increasingly reported, leading to reduced therapeutic efficacy and treatment failure (Hutchings et al., 2019; Amari et al., 2023).

In response to the growing challenge of antibiotic resistance, the exploration of alternative antibacterial agents derived from natural resources has gained significant attention. One promising source is the black turmeric rhizome (*Curcuma aeruginosa* Roxb.), which has long been utilized in Indonesian traditional medicine (Sukandaryah et al., 2023). Black turmeric rhizome is known to contain essential oils with antioxidant and antimicrobial properties (Sari & Supratman, 2022). Phytochemical analyses have revealed the presence of various bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and steroids, all of which contribute to its antimicrobial activity (Sari & Farmasi, 2024). Nevertheless, large-scale processing of black turmeric rhizomes poses economic and technical challenges, necessitating more efficient and sustainable strategies for bioactive compound production.

One such strategy involves the utilization of endophytic bacteria—microorganisms that inhabit internal plant tissues without causing harm to their host. Endophytic bacteria are known to establish mutualistic relationships with plants and are capable of producing secondary metabolites that are identical or similar to those synthesized by their host plants (Sains et al., 2024; Ali et al., 2024). In addition to enhancing plant immunity, these bacteria offer a sustainable and cost-effective alternative for sourcing bioactive compounds with antimicrobial potential.

Therefore, the novelty of this study lies in exploring the antibacterial potential of endophytic bacteria isolated from black turmeric rhizomes (*Curcuma aeruginosa* Roxb.) against pneumonia-causing pathogens, specifically *Streptococcus pneumoniae*. This research aims to isolate, characterize, and evaluate endophytic bacteria from black turmeric rhizomes as potential antibacterial agents against *Streptococcus pneumoniae*, contributing to the development of alternative therapeutic strategies for pneumonia management.

## METHOD

This research employs an experimental method, involving the stages of isolating endophytic bacteria from black turmeric rhizome (*Curcuma aeruginosa* Roxb.), characterizing the pure isolates, and testing the antibacterial activity of the endophytes against *Streptococcus pneumoniae*. This research was conducted from June to August 2025. The research took place at the Biology Pharmacy Laboratory of Stikes Tujuh Belas and the Microbiology Laboratory of the Faculty of Medicine, Universitas Sebelas Maret.

### Isolation and Purification of Endophytic Bacteria from *Curcuma aeruginosa* Roxb.

The black ginger rhizome sample, collected from Gondang Village, Sragen Regency, was washed under running water until clean and then cut into small pieces. Surface sterilization was performed by immersing the rhizome samples in 70% ethanol for 1 minute, followed by 5.25% sodium hypochlorite for 3 minutes, and then rinsing with sterile distilled water three times. The water from the final rinse was used as a control and was inoculated onto a Nutrient Agar (NA) medium in a separate Petri dish to confirm the success of the surface sterilization process. The black ginger rhizome

was then placed aseptically onto a Petri dish containing Nutrient Agar (NA) medium supplemented with 0.01% (w/v) nystatin. Incubation was carried out for 24 hours at 37°C. The bacterial colonies that grew were then made into pure culture stocks on NA slant media in test tubes (Ezeobiora et al., 2021).

### **Morphological Characterization of Endophytic Bacteria from Rhizome *Curcuma aeruginosa* Roxb**

Morphological characterization of the endophytic bacteria was performed both macroscopically and microscopically. Macroscopic morphological characterization was conducted by observing the bacterial colonies that grew, including their form, color, size, and margin. Microscopic characterization was carried out by performing Gram staining on the bacterial cells and observing them under a light microscope at 1000x magnification (Singh et al., 2017).

### **Inhibition Activity Test of Endophytic Bacterial Isolates Against *Streptococcus pneumoniae***

A 24-hour culture of the test bacteria (*Streptococcus pneumoniae* and *Klebsiella pneumoniae*) was sampled with one inoculating loop and mixed into 10 ml of 0.9% physiological NaCl solution until a concentration equivalent to McFarland 1.0 ( $3.0 \times 10^8$  CFU/mL) was achieved. Subsequently, 1 ml of the test bacterial suspension was poured into a Petri dish, and Nutrient Agar (NA) medium was added using the pour plate method. The bacterial test was conducted using the diffusion method with paper discs. Sterile paper discs were placed in a Petri dish and immersed in the endophytic bacterial supernatant for 2 hours. These paper discs were then transferred into the Petri dishes already inoculated with the test bacteria. The plates were incubated at 37°C for 24 hours. The negative control used a paper disc moistened with aquades, while the positive control used ciprofloxacin. The inhibition zones were measured using a caliper (Irvanita et al., 2024).

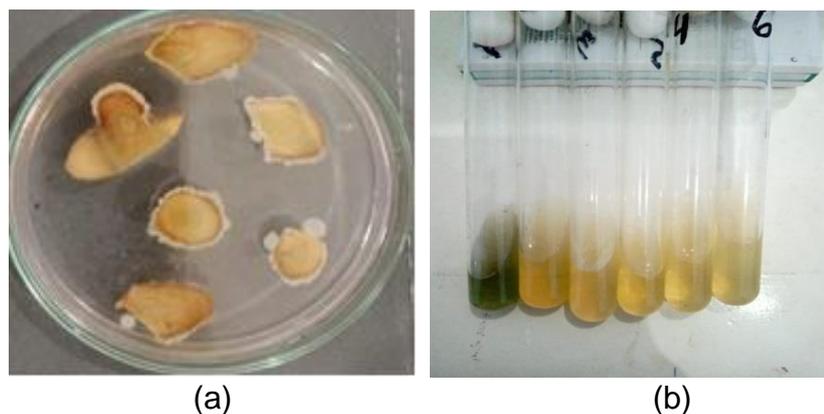
### **Data Analysis**

The obtained data were analyzed qualitatively by compiling the results from all observations of the isolates throughout the process and the antibacterial testing, including measuring the diameter of the formed inhibition zones. Data collection was carried out as follows: observing and identifying the endophytic bacterial isolates through cell morphology, colony morphology, analyzing the results from the endophytic bacterial growth curve, and measuring the diameter of the inhibition zones using a caliper from the bacterial testing process. The overall observational data results are presented descriptively, supplemented with figures and tables.

## **RESULTS AND DISCUSSION**

### **Isolation of Endophytic Bacteria from Black Ginger Rhizome (*Curcuma aeruginosa* Roxb.)**

The isolation of endophytic bacteria from the black ginger rhizome (*Curcuma aeruginosa* Roxb.) yielded 6 isolates. The results of the isolation and purification of the endophytic bacterial isolates from the black ginger rhizome (*Curcuma aeruginosa* Roxb.) are shown in Figure 1.



**Figure 1.** (a) Endophytic bacteria from black ginger rhizome (*Curcuma aeruginosa* Roxb.), (b) Pure isolates of endophytic bacteria from black ginger rhizome: PS1, PS2, PS3, PS4, PS5, PS6

Based on the results obtained, six isolates were observed on the Petri dishes cultured on Nutrient Agar (NA) medium, with the main characteristic being the growth of endophytic bacteria around the sample. If contamination occurred, irregular growth tending to appear farther from the sample would be observed. These bacteria produce various beneficial metabolites and are thus considered a source of biologically active compounds. Endophytic bacteria can exhibit similar antibacterial activity and produce metabolites akin to those of their host. The biologically active compounds of medicinal plants can influence the endophytic microbes living within the plant tissues and their physiological functions (Zulfarina et al., 2022). The exact number of bacteria within a plant cannot be determined precisely; however, these bacteria can be isolated using agar media (Rizqoh et al., 2021). Hundreds of endophytic bacterial species can be isolated from a single plant species, and environmental conditions, host plant type, and plant age can influence the population and profile of the endophytic microbes within (Zulfarina et al., 2022).

### ***Morphological Characterization of Endophytic Bacteria from Black Ginger Rhizome (Curcuma aeruginosa Roxb.)***

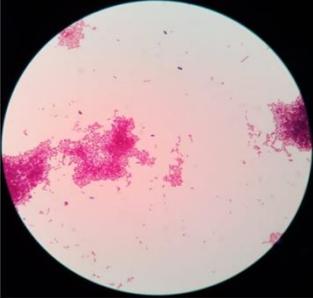
Macroscopic characterization included the form of individual colonies, the margin of individual colonies, colony elevation, and the pigment of individual endophytic bacterial colonies. The endophytic bacteria isolated from black ginger rhizome exhibited diversity in terms of morphology. The morphological characteristics of the endophytic bacterial colonies, including form, margin, elevation, and pigment, are presented in Table 1.

**Table 1.** Macroscopic characterization of endophytic bacterial isolates from black ginger rhizome

Isolate Code	Morphological Characterization			
	Form	Edge	Elevasi	Pigmen
PS1	Irreguler	Entire	Flat	White
PS2	Irreguller	Entire	Flat	White
PS3	Cirkuler	Undulate	Convex	Yellowish white
PS4	Irreguler	Lobate	Flat	White
PS5	Cirkuler	Entire	Flat	Yellowish white
PS6	Cirkuler	Undulate	Flat	Yellowish white

Microscopic characterization of endophytic bacteria in black turmeric rhizome plants can be seen in Table 2.

**Table 2.** Microscopic characterization of endophytic bacterial isolates from black ginger rhizome

Isolate code	Microscopis	Form	Gram stain
PS1		Basil	Negative
PS2		Basil	Negative
PS3		Basil	Negative
PS4		Basil	Negative
PS5		Basil	Negative

Isolate code	Microscopis	Form	Gram stain
PS6		Basil	Negative

Based on data from Table 1, the results of 6 endophytic bacterial isolates revealed differences in colony morphology, color, elevation, edges, and shape of the bacteria, namely white, yellowish-white, irregular and circular shapes, flat and convex elevations, flat, serrated, and grooved edges. This is because endophytic bacteria on a single host plant generally consist of several genera and species. This diversity occurs because the morphology of bacterial colonies on agar media depends on several factors such as culture media, temperature, incubation time and culture age (Rizqoh et al., 2021). Each bacterium has a different ability to adapt to survive in its environment, changes in the environment can affect the morphological and physiological properties of a bacterium (Handayani et al., 2023).

Table 2 presents the results of the microscopic observation of the bacteria. From the six isolates, it was found that the endophytic bacteria from the black ginger rhizome are classified as Gram- negative. The mechanism of Gram staining in these bacteria is based on the structure and composition of the bacterial cell wall. Gram-positive bacteria have a thick peptidoglycan layer in their cell wall, which is able to retain the crystal violet dye. In contrast, Gram- negative bacteria generally have a thin peptidoglycan layer. Furthermore, Gram-negative bacteria contain more lipids than Gram-positive bacteria. Therefore, during the staining process when alcohol is applied, if the bacteria are Gram-negative, a reaction occurs between the lipids and the alcohol. This reaction increases the permeability of the cell wall, allowing the crystal violet-iodine complex to be washed out (Apriyanthi, 2022).

**Inhibition Activity Test of Black Turmeric Endophytic Bacterial Isolates Against *Streptococcus pneumoniae***

The results of the inhibition zone test for endophytic bacteria isolated from black turmeric rhizomes using the well diffusion method can be seen in Table 3.

**Table 3.** Results of the inhibition activity test of black turmeric endophytic bacteria against *Streptococcus pneumoniae*

Isolate code	Inhibition Zone Diameter (mm)		Remarks
	Avarage (mm)	Inhibition Standard (mm)	
PS1	15,3	≤ 5 = weak	Strong
PS2	15,3	5-10 = moderate	Strong
PS3	16,3	10-20 = strong	Strong
PS4	20	≥ 21 = very strong	Strong
PS5	20,6		Strong
PS6	20		Strong
Control +	33		Very strong
Control -	0		

Information: K+: Ciprofloxacin (antibiotic), K-: Aquades

The results of the antibacterial activity test of endophytic bacteria against *Streptococcus pneumoniae*, as shown in Table 3, indicate that six bacterial isolates were able to inhibit the growth of *Streptococcus pneumoniae*. Isolate PS1 had an average inhibition zone of 15.3 mm, categorized as strong; isolate PS2 had an average inhibition zone of 15.3 mm, also categorized as strong. Isolate PS3 showed an average inhibition zone of 16.3 mm (strong), isolate PS4 showed an average of 20.0 mm (strong), isolate PS5 showed an average of 20.6 mm (strong), and isolate PS6 showed an average of 20.0 mm (strong). The positive control using ciprofloxacin showed a clear zone of 33.0 mm, classified as very strong. The inhibition zone categories consisted of four levels: weak (diameter  $\leq 5$  mm), moderate (diameter 5-10 mm), strong (diameter 10-20 mm), and very strong (diameter  $\geq 20$  mm) (Zulfarina et al., 2022). The larger the clear zone formed, the more potent the bioactive compounds are at inhibiting bacterial growth. The inhibitory activity, indicated by the formation of a clear zone, is caused by the interaction between the bioactive compounds produced by the endophytic bacteria and the cells of *Streptococcus pneumoniae*. This interaction likely affects the cell wall synthesis process and disrupts the permeability of the bacterial cell membrane. *Streptococcus pneumoniae* is a Gram-positive bacterium with a relatively simpler cell wall structure, composed of peptidoglycan approximately 40-80 nm thick, about 2% lipid, and teichoic acids. This characteristic makes it easier for the antibacterial compounds produced by the endophytic bacteria to penetrate the bacterial cells, rendering Gram-positive bacteria more susceptible to these antibacterial compounds (Yunita et al., 2022).

## CONCLUSIONS

Based on the research results, it can be concluded that the rhizome of black turmeric (*Curcuma aeruginosa* Roxb.) harbors diverse endophytic bacteria, all of which were identified as Gram-negative bacilli. These isolates demonstrated varying degrees of antibacterial activity against *Streptococcus pneumoniae*, with isolates PS5 and PS4 showing the highest potency, as indicated by their largest average inhibition zones of 20.6 mm and 20.0 mm, respectively. Therefore, endophytic bacteria from black turmeric represent a promising source of antibacterial agents against this significant pneumonia pathogen.

## RECOMMENDATION

Future research should be conducted in thorough phytochemical screening and bioassay-guided isolation of the specific bioactive compounds produced by the endophytic bacteria from *Curcuma aeruginosa* Roxb.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the research funding from Kemdiktisaintek and the laboratory support from STIKes Tujuh Belas and FK UNS.

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