BIODIVERSITAS
Volume 26, Number 2, February 2025
Pages: 981-991

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260247

Investigation of endophytic bacteria associated with *Paederia foetida*: Population, characterization, antibacterial, and pathogenicity analysis

MELDA YUNITA[1,♥], MORGAN OHIWAL[2], JUEN CARLA WARELLA[3], SITI NUR AZIZAH[4], ELPIRA ASMIN[5], PRESLI GLOVRIG SIAHAYA[6]

[1]Department of Microbiology, Faculty of Medicine, Universitas Pattimura. Jl. Ir. M. Putuhena, Ambon 97233, Maluku, Indonesia.
Tel./fax.: +62-82398709925, ♥email: melda.yunita@lecturer.unpatti.ac.id
[2]Department of Soil Science, Faculty of Fisheries and Forestry, Universitas Muhammadiyah Maluku. Wara, Ambon 97128, Maluku, Indonesia
[3]Department of Microbiology, Faculty of Medicine, Universitas Pattimura. Jl. Ir. M. Putuhena, Ambon 97233, Maluku, Indonesia
[4]Pharmacy Vocational Program, Politeknik Kesehatan Jember. Jl. Pangandaran No. 42, Jember 68121, East Java, Indonesia
[5]Department of Public Health, Faculty of Medicine, Universitas Pattimura. Jl. Ir. M. Putuhena, Ambon 97233, Maluku, Indonesia
[6]Department of Pharmacology, Faculty of Medicine, Universitas Pattimura. Jl. Ir. M. Putuhena, Ambon 97233, Maluku, Indonesia

Manuscript received: 23 October 2024. Revision accepted: 23 February 2025.

Abstract. *Yunita M, Ohiwal M, Warella JC, Azizah SN, Asmin E, Siahaya PG. 2025. Investigation of endophytic bacteria associated with* Paederia foetida*: Population, characterization, antibacterial, and pathogenicity analysis 26: 981-991.* The aim of this study was to obtain non-pathogenic endophytic bacterial isolates that can be developed as antibacterials for further applications. Endophyte isolation was carried out in 4 different media to obtain diverse endophytes and characterized macroscopically and microscopically. Endophyte screening was done through antibacterial tests against 10 different pathogenic bacteria. Pathogenicity analysis was performed on Blood Agar Plate (BAP) to differentiate pathogenic and non-pathogenic group of endophytic bacteria. The results showed that Starch Casein Agar was the best media for isolation in terms of total population and morphological characteristics compared to the other three media. A total of 27 isolates were collected according to the different morphology. Screening through antibacterial tests revealed that 19 endophytic isolates were significantly able to inhibit various species of pathogenic bacteria (p-value of 0.001). Pathogenicity analysis showed that 8 of 19 isolates (HR1, HR2, HR3, K2, NL3, S2, A2, and A9) were categorized as γ hemolysis, while the other 11 isolates were categorized as β hemolysis and α hemolysis. The study concluded that 8 endophytic bacterial isolates associated with *P. foetida* leaves had the strong potential to be developed and can be applied in advanced antibacterial applications.

Keywords: Antibacterial activity, *Paederia foetida,* α hemolysis, β hemolysis, γ hemolysis

INTRODUCTION

The rising global population experiencing health issues resulting in various infectious diseases, drug-resistant bacteria, parasitic protozoans, and fungal infections is a matter of concern (Aswani et al. 2020; Rustamova et al. 2020). It is crucial for researchers to focus on new antibiotic sources since powerful antimicrobial medications are being replaced by bacterial resistance (Wu et al. 2021). As a promising new source of potentially valuable pharmacological compounds, endophytes are currently the subject of a thorough inquiry to develop novel and more effective medications to address these disease challenges (Singh et al. 2017; Singh et al. 2022).

Endophytes are microorganisms, including bacteria, that inhabit the intercellular spaces of plants without inducing any symptom of disease in their host plant (Liu et al. 2016; Yunita et al. 2022a). In a symbiotic interaction, endophytes serve as the predominant microbial populations associated with plants. They exchange nutrients and protect the host from infections dan pathogen colonization by producing bioactive compounds (Singh et al. 2022). Endophytes primarily infiltrate plant tissue via the root zone; however, aerial parts of the plant, including cotyledons, leaves, stems, and flowers, may also serve as entrance points (Anjum and Chandra 2015). These bacteria invade tissues through germination radicles, secondary roots, stomata, or damaged areas. Endophytes within a plant may either remain localized at the entry point or inhabit the cells, intercellular spaces, or vascular system, and can also disseminate throughout the plant (Beiranvand et al. 2017).

Numerous studies have highlighted endophytes from medicinal plants and their use in various domains. The bioactive chemicals in medicinal plants warrant a comprehensive investigation of the endophytes linked to certain plant species, which may facilitate the identification of bioactive compounds from associated microbes for many scientific applications (Cardoso et al. 2020). Recently, several well-known endophytic bioactive metabolites and a few new ones have been found to possess biological activities, such as antibacterial properties (Singh et al. 2017; Yunita et al. 2022a; Yunita et al. 2022b), antioxidant (Sulistiyani et al. 2016; Anitha 2019; Triandriani et al. 2020), antiviral (Maela et al. 2022; Vinodkumar et al. 2018), anticancer and antitumor (Maela et al. 2022; Newman 2018), anti-inflammatory (dos Santos et al. 2018), and antifungal activity (Yunita et al. 2016, Yunita et al. 2023a).

Paederia foetida L. (Rubiaceae), often known as *sembukan* in Indonesia, is a perennial climbing shrub endemic to temperate and tropical Asia (Yunita et al. 2023b; Savitri et al. 2024). In folk medicine, the plant is thought to have antibacterial (Dutta et al. 2023), anti-inflammatory (Kumar et al. 2015), and anti-diarrheal properties (Chanda et al. 2013; Kumar et al. 2014). The plant's aerial portions, which contain iridoid glucosides, methyl mercaptan, alkaloids, and other compounds, are mostly used to treat gastrointestinal issues, such as dyspepsia, flatulence, gastritis, and enteritis (Pal et al. 2012; Dutta et al. 2023). A number of studies have been done to study the role of endophytic bacteria associated with *P. foetida* leaves as bioactive compound producers. Several studies have been conducted to investigate endophytic bacteria associated with *P. foetida* leaves yet are still limited in terms of diversity (Pal et al. 2012) as well as their potential as antimicrobial (Sushma et al. 2018) and antifungal (Widjajanti et al. 2021).

Numerous endophytes penetrate plant tissues and function as either endophytic or pathogenic. Many studies have revealed the benefits and roles of endophytic bacteria as bioactive compounds producer (Fatimah et al. 2024). However, the natural properties of endophytic bacteria are also important to evaluate. The possibility that endophytic bacteria do provide benefits but can also provide slight disadvantages due to their virulence needs to be considered. In future applications, this is undoubtedly an issue if the endophytic bacteria that are supposed to be dependable develop into infectious diseases. Additionally, when using endophytic bacteria or their secondary metabolites in the pharmaceutical industry, the cascade impact needs to be kept to a minimum (Dutta et al. 2023). To ensure that the endophytic isolates obtained for further application do not have the potential to become pathogens, it is essential to conduct pathogenicity analysis on isolated endophyte. The ability of endophytic bacteria to hemolyze blood in Blood Agar Plate (BAP) media serves as a criterion for classifying the obtained endophytic bacteria as pathogenic or non-pathogenic (Said et al. 2021). This is crucial during the screening phase to obtain prospective isolates that are completely non-pathogenic. The aim of this study was to obtain non-pathogenic endophytic bacterial isolates that can be developed as antibacterials for further applications.

MATERIALS AND METHODS

Study area

Paederia foetida leaves were taken from 5 areas of the Lease Islands, Maluku Province, Indonesia including Ambon (3°39'37.47"S, 128°11'28.38"E), Saparua (3°35'2.27"S, 128°37'16"E), Kairatu (3°20'23.66"S, 128°21'11.64"E), Haruku (2°32'41.92"S, 128°33'18.00"E) and Nusalaut (3°38'49.89"S, 128°45'46.58"E). Sampling site map is presented in Figure 1. Leaves were collected from each site, then kept in plastic zip bag, stored in a cool box with ice pack to prevent the leaves from wilting during the sampling. Leaf samples were taken to the Microbiology Laboratory of the Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia for bacterial isolation.

Procedures

Plant identification

Paederia foetida was identified in UPT. Herbal Laboratory of Materia Medica Batu with reference number: 000.9.3/2181/102.20/2024.

Preparation of leaf filtrate

Paederia foetida leaf filtrate was prepared according to (Yunita et al. 2022a). A total 10 g of fresh leaves were extracted using 100 mL sterile distilled water to obtain leaf filtrate and then filtered using 0.2 μ millipore which was used as a supplement for the growth medium of endophytic bacteria.

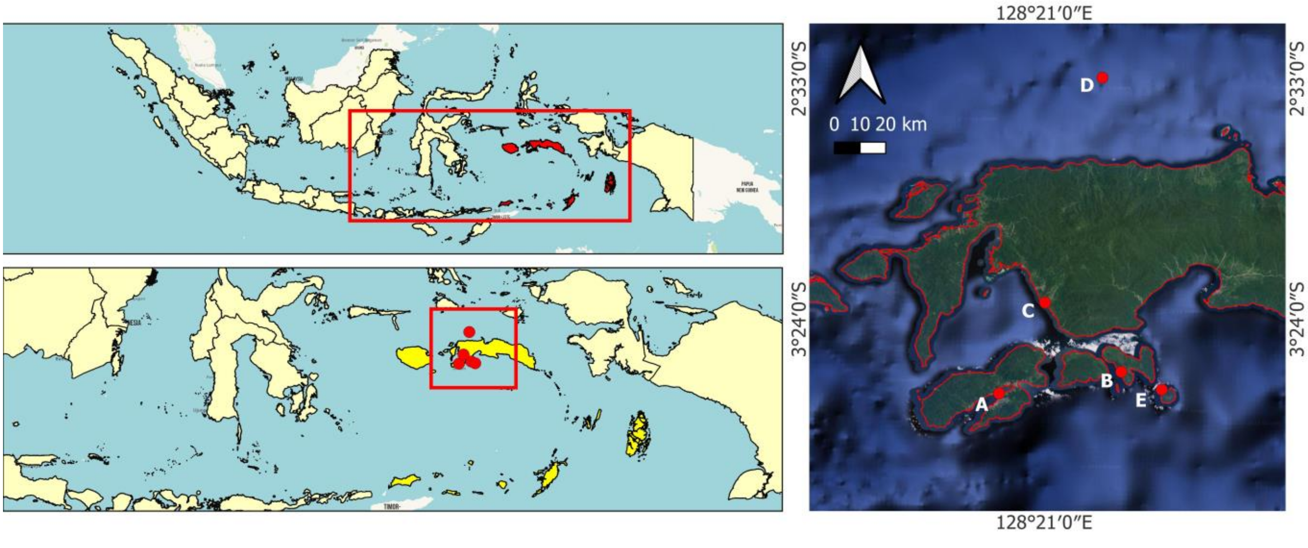


Figure 1. Sampling area (red spots) of *P. foetida* leaves at five sites in the Lease Islands, Maluku, Indonesia. A. Ambon; B. Saparua; C. Kairatu; D. Haruku; E. Nusalaut

Isolation of endophytic bacteria

Endophytic bacteria were isolated from *P. foetida* leaves of five areas in the Lease Islands, Maluku, Indonesia that are Ambon, Seram, Saparua, Nusalaut, and Haruku. Surface sterilization was done by soaking *P. foetida* leaves in 95% ethanol solution for 15 minutes, followed by 5% sodium hypochlorite for 10 minutes, and finally rinsed with sterile distilled water (Anjum and Chandra 2015). To obtain diverse endophytes with an emphasis on morphological variety and antibacterial capacity, as well as, if feasible, actinobacterial species, endophyte isolation was performed on four different media. Isolation of endophytic bacteria was initiated by preparing serial dilution. A total of 25 g of leaves were ground and dissolved in 225 mL of pH 7 phosphate buffer then homogenized for 30 minutes with a shaker. A total of 1 mL of the mixture was put into 9 ml of NaCl as the first dilution (10^{-1}) and continued up to 10^{-4}. A total of 100 µL of each dilution was planted into Starch Casein Agar (SCA, HiMedia); Water Yeast Extract Agar (WYA); Yeast Malt Agars ISP No.2 (HiMedia); and Humic Acid Vitamin Agar (HVA) media. Isolation was performed using spread plate method and incubated for 2-5 days at room temperature. The isolation media was added with nystatin (100 µg/mL) as an antifungal, nalidixic acid (100 µg/mL) as a gram-negative antibacterial, and *P. foetida* leaf filtrate as media supplements (El-Shatoury et al. 2006; Passari et al. 2018; Singh et al. 2018). The number of bacterial colonies that appear in the media was counted and analyzed for the total plate count using the formula:

$$\text{TPC (Colony Forming Unit/g)} = \text{Number of colonies} \times \frac{1}{\text{Dilution factor}}$$

Purification and preliminary characterization

Endophytic bacterial isolates were isolated and purified based on morphological differences. Pure cultures were prepared by streaking them on NA media containing nystatin and 10% plant filtrate and incubated for 1-3 days at room temperature. Macroscopic characterization was carried out by observing the shape, color, elevation, margin, and size of colony, while microscopic observations, included shape and color of bacterial cells (Gram staining) (Yunita et al. 2022b).

Antibacterial test

In order to screen the endophytic bacteria, antibacterial test was conducted using Kirby-Bauer agar diffusion method with few modification. Ten isolates of pathogenic bacteria were used, including *Escherichia coli*, *Morganella morgani*, *Klebsiella pneumoniae*, *Staphylococcus sciuri*, *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, *Staphylococcus hemolyticus*, *Rothia kristinae*, *Bacillus subtilis*, and *Salmonella gallinarum.* The pathogenic bacteria were swabbed on NA media containing 100 µg/mL nystatin and endophytic bacteria were spotted on the swabbed media. The media were incubated for 1-3 days at room temperature. The clear zone that appeared around the colony was observed and measured using a ruler. The clear zone formed represents the antibacterial index which was calculated using the following formula:

$$\text{Antibacterial index} = (DV - DC) + (DH - D)$$

Where:
DV: Diameter of vertical zone of inhibition
DH: Diameter of horizontal zone of inhibition
DC: Diameter of bacterial colony

While antibacterial category was examined as: weak (<5 mm), moderate (5-10 mm), strong (10-20 mm), very strong (>20 mm).

Pathogenicity analysis

Pathogenicity analysis was conducted to assess the pathogenic properties of endophytic bacteria. Selected endophytic bacterial isolates that inhibit pathogenic bacteria were further tested for pathogenicity analysis by streaking on 5% Blood Agar Plate (BAP) media and incubated for 1-3 days. Bacterial isolates capable of forming hemolysis zones on the agar media were examined and their hemolysis patterns were interpreted into 3 categories, namely complete hemolysis (β), partial hemolysis (α), and no hemolysis (γ) (Russell et al. 2006).

Ethical clearance

Ethical clearance was issued by the Ethics Committee of the Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia with reference number: 139/FK-KOM.ETIK/VIII/ 2024.

Data analysis

The data obtained were presented with tabulations and figures and analyzed descriptively qualitatively for preliminary characterization of endophytic bacteria and pathogenicity analysis. Bacterial population were analyzed using Ms Excel Program, while antibacterial test was analyzed with Kruskal-Wallis test using SPSS 16.

RESULTS AND DISCUSSION

Bacterial population

The total bacterial population was calculated using the Total Plate Count (TPC) method. Bacterial colonies grown in the media is presented in Figure 2. The bacterial colonies seen on SCA media looked more diverse and different macroscopically, while bacterial colonies on WYA media looked more uniform and did not show clear differences macroscopically. On the other hand, only a few bacterial colonies grew on HVA and ISP media, the colony was very small colony, so it was difficult to purify.

The five sampling sites under investigation showed different endophytic bacterial total plate count values. On an average, significantly more endophytic bacteria had grown in WYA and SCA than in HVA and ISP No. 2. However, compared to WYA media, more bacterial colonies grew on SCA media. The TPC analysis result is shown in Figure 3. This figure shows that bacterial population in SCA media ranged from approximately 3.30E+05 ± 1.4 to 6.90E+07 ± 3.5 CFU/g, 4.50E+05 ± 4.9 to 8.30E+06 ± 7.8 CFU/g for WYA media, 3.10E+02 ± 3.2 to 3.70E+03 ± 2.2 CFU/gr for HVA media, and 3.30E+02 ± 0.9 to 7.10E+03

± 2.1 for ISP media. According to this result, TPC analysis showed that in all media, endophytic bacteria isolated from Haruku area had the largest population compared to other locations such as Ambon, Kairatu, Saparua, and Nusalaut.

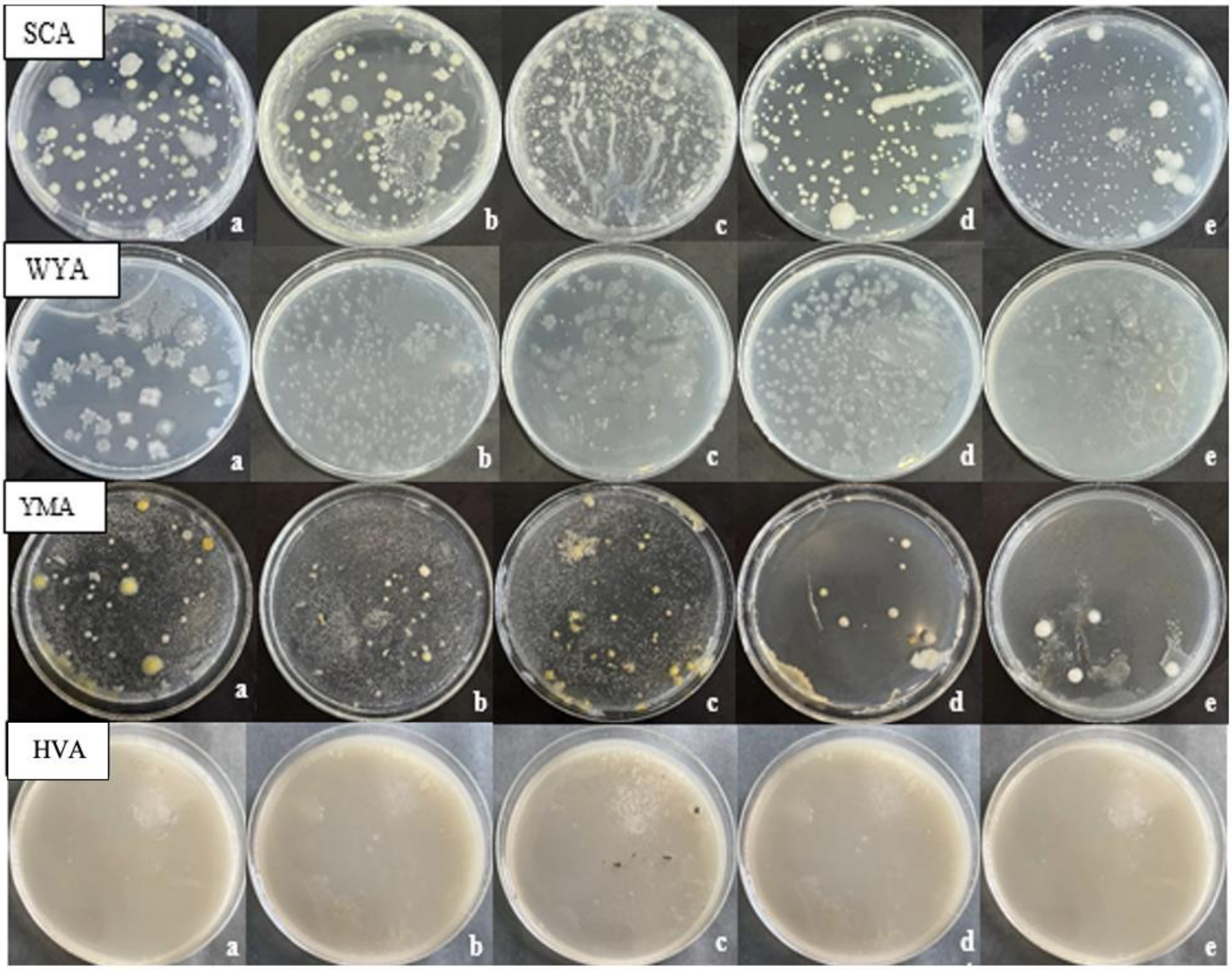
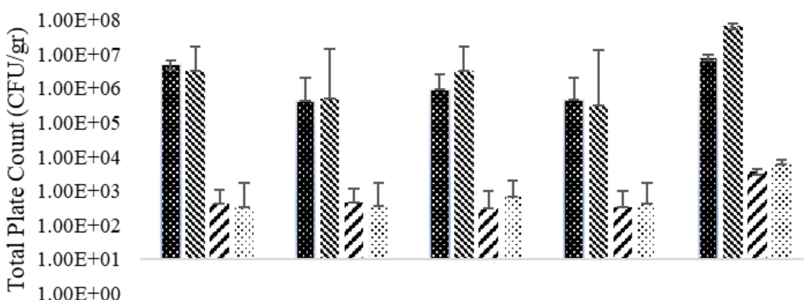


Figure 2. Endophytic bacterial isolated from five sampling sites using different media. a. Ambon; b. Saparua; c. Haruku; d. Nusalaut; e. Kairatu. SCA: Starch Casein Agar; WYA: Water Yeast Extract Agar; YMA Yeast Malt Agar ISP 2; HVA: Humic Acid Vitamin Agar



	Ambon	Kairatu	Saparua	Nusalaut	Haruku
WYA	5.10E+06	4.50E+05	9.40E+05	4.80E+05	8.30E+06
SCA	3.30E+06	4.90E+05	3.20E+06	3.30E+05	6.90E+07
HVA	4.10E+02	4.60E+02	3.10E+02	3.30E+02	3.70E+03
ISP	3.30E+02	3.70E+02	6.60E+02	4.20E+02	7.10E+03

Sampling Sites

Figure 3. Total plate count of endophytic bacteria isolated from five sampling sites

Preliminary characterization

The results of macroscopic and microscopic characterization are presented in Table 1. This table shows that 27 endophytic bacterial isolates were obtained with different macroscopic and microscopic characteristics. The colony size varied from small, medium, to large. Colony shape also showed a variety of results, ranging from circular, irregular, and filamentous, while colony color varied between white, milky white, pink, yellow, and orange. The colony elevation varied between flat, convex, and umbonate. On the other hand, microscopic characteristics, including cell shape and gram staining also show various results. The cell shape consists of bacilli, cocci, and diplobacilli. The gram staining results are dominated by Gram-positive bacteria of 16 isolates, followed by Gram-negative bacteria of 11 isolates.

Antibacterial test

Antibacterial test was performed against 10 pathogenic bacteria including *E. coli, M. morganii, K. pneumoniae, S. pseudintermedius, S. aureus, S. sciuri, S. haemolyticus, R. kristinae, B. subtilis*, and *S. gallinarum*. The clear zone that emerged around the colony exhibited the antibacterial activity produced by the endophytic bacteria (Figure 4).

Of the 27 endophytic bacteria tested, 19 isolates (HR1, HR2, A2, A3, A7, A12, A13, A14, K1, K2, NL2, NL3, A4, A8, A9, S2, SA6, HR3, HR4) were able to inhibit various pathogenic bacteria with varying inhibition zones categorized as weak to strong between 2-17.5 mm as shown in Table 2. While statistical analysis of antibacterial activity using Kruskal-Wallis showed a p-value of 0.001, which indicated that there was a significant difference between each isolate against 10 pathogenic bacteria.

Figure 5 shows that 19 endophytic bacterial isolates were able to inhibit various pathogenic bacteria. Three bacterial isolates had the best ability to inhibit pathogenic bacteria compared to other endophytes, which were isolates A3, HR1 and HR3. Isolate A3 was able to inhibit 7 pathogenic bacteria including *E. coli, S. pseudintermedius, S. aureus, S. sciuri, S. gallinarum, B. subtilis,* and *S. gallinarum*. Isolate HR1 was able to inhibit 4 pathogenic bacteria including *E. coli, K. pneumoniae, S. aureus*, and *S. sciuri*. While isolate HR3 was also able to inhibit 4 pathogenic bacteria including *E. coli, Morganella morganii, S. aureus*, and *S. Sciuri*. Meanwhile, the other isolates had relatively lower antibacterial activity compared to 3 isolates mentioned.

Table 1. Macroscopic and microscopic characterization of endophytic bacteria

Isolates code	Macroscopic and microscopic characterization						
	Size	Shape	Color	Margin	Elevation	Cell shape	Gram staining
A1	Large	Irregular	White	Undulate	Flat	Bacili	Positive
A2	Small	Circular	Yellow	Entire	Convex	Bacili	Positive
A3	Small	Irregular	Yellow	Undulate	Convex	Cocci	Negative
A4	Small	Circular	Yellow	Entire	Convex	Bacili	Positive
A5	Small	Irregular	White	Curled	Flat	Bacili	Positive
A6	Small	Irregular	Yellow	Entire	Convex	Diplo bacil	Negative
A7	Moderate	Irregular	White	Rhizoid	Flat	Bacili	Negative
A8	Moderate	Circular	Milky White	Undulate	Flat	Bacili	Negative
A9	Large	Circular	Milky White	Entire	Flat	Bacili	Negative
A10	Moderate	Irregular	Milky White	Entire	Umbonate	Bacili	Positive
A11	Moderate	Irregular	Yellow	Undulate	Flat	Bacili	Positive
A12	Small	Irregular	White	Undulate	Flat	Bacili	Positive
A13	Moderate	Irregular	White	Undulate	Umbonate	Bacili	Negative
A14	Large	Irregular	White	Undulate	Flat	Bacili	Positive
S2	Small	Irregular	Milky White	Undulate	Umbonate	Bacili	Positive
S3	Moderate	Irregular	Yellow	Undulate	Raised	Cocii	Negative
S4	Moderate	Irregular	Yellow	Undulate	Raised	Cocii	Negative
S6	Moderate	Circular	White	Entire	Convex	Bacili	Positive
S8	Small	Irregular	Pink	Undulate	Convex	Bacili	Positive
HR1	Small	Irregular	White	Entire	Flat	Bacili	Positive
HR2	Large	Fillamentous	White	Filamentous	Convex	Diplo bacil	Negative
HR3	Large	Circular	Milky white	Entire	Umbonate	Cocci	Positive
HR4	Small	Irregular	Orange	Undulate	Umbonate	Bacili	Positive
K1	Moderate	Irregular	White	Undulate	Flat	Bacili	Positive
K2	Moderate	Irregular	White	Lobate	Flat	Bacili	Positive
NL2	Moderate	Circular	Orange	Entire	Flat	Bacili	Negative
NL3	Large	Irregular	Pink	Undulate	Flat	Bacili	Positive

Table 2. Measurement results of antibacterial tests of endophytic bacteria against various pathogenic bacteria (mean ± SD)

Isolates code	Antibacterial zone (mm ± SD)									
	Ec	Mm	Kp	Sp	Sa	Sc	Sh	Rk	Bs	Sg
A1	0.0 ± 00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A3	5.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.4	5.0 ± 0.0	2.5 ± 0.4	0.0 ± 0.0	3.5 ± 0.7	5.5 ± 0.0	4.5 ± 0.1
A5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A11	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
S3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
S4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HR1	6.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	6.0 ± 1.4	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HR2	3.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A2	0.0 ± 0.0	5.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.5 ± 0.0	0.0 ± 0.0
A7	0.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A12	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0
A13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.0 ± 0.0	0.0 ± 0.0
A14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
K1	0.0 ± 0.0	4.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
K2	0.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NL2	4.0 ± 0.7	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NL3	0.0 ± 0.0	1.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 0.0	13.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A8	4.5 ± 0.0	3.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A9	0.0 ± 0.0	7.8 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 4.6	3.0 ± 4.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
S2	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
S6	15 ± 1.4	4.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
S8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HR3	17.5 ± 0.7	8.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	8.5 ± 0.7	7.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HR4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.0 ± 0.0	0.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Notes: Ec: *Escherichia coli;* Mm: *Morganella morganii;* Kp: *Klebsiella pneumoniae;* Sp: *Staphylococcus pseudintermedius;* Sa: *Staphylococcus aureus*; Sc: *Staphylococcus sciuri;* Sh: *Staphylococcus haemolyticus;* Rk: *Rothia kristinae;* Bs: *Bacillus subtilis;* Sg: *Salmonella gallinarum*

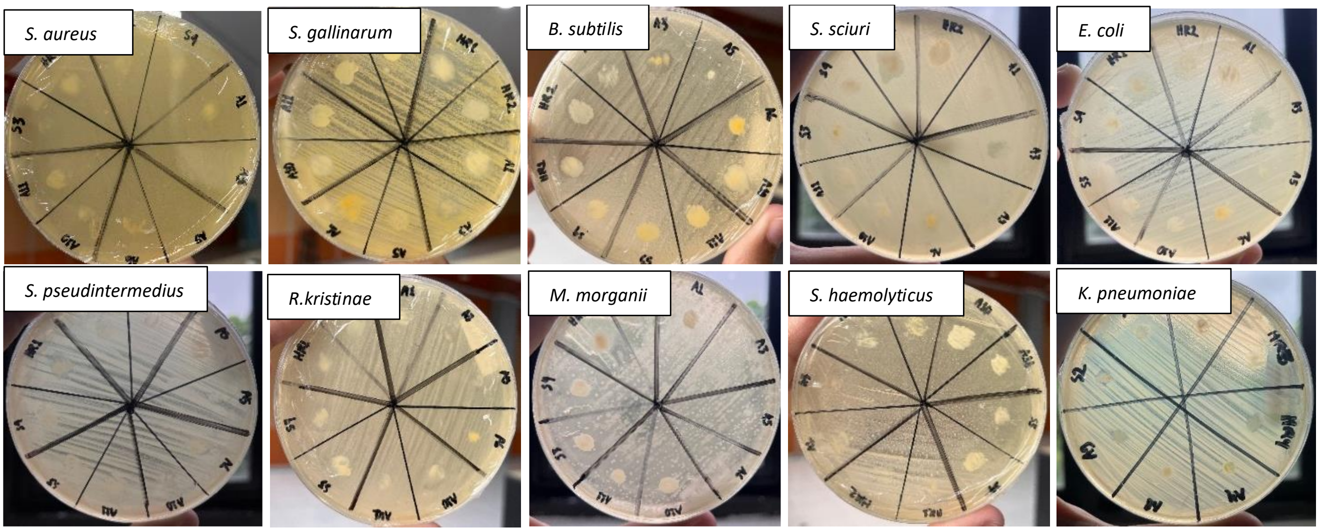


Figure 4. Antibacterial test of endophytic bacteria against various pathogenic bacteria

Pathogenicity analysis

To ensure the pathogenicity of endophytic bacteria, pathogenicity test was further carried out. Nineteen endophytic bacteria that inhibit pathogenic bacteria were further tested for pathogenicity analysis on 5% BAP media and incubated for ± 24 hours. The results of pathogenicity analysis showed that 8 (isolates HR1, HR2, HR3, K2, NL3, S2, A2, and A9) of the 19 isolates tested did not show any hemolytic activity against blood in BAP media or called as γ-hemolysis. Meanwhile, 11 isolates had hemolytic activity that was classified into the categories of α-Hemolysis (isolates NL2, A13, S6, A8, HR4, A4) and β-Hemolysis (isolates K1, A3, A7, A14, A12) (Table 3, Figure 6).

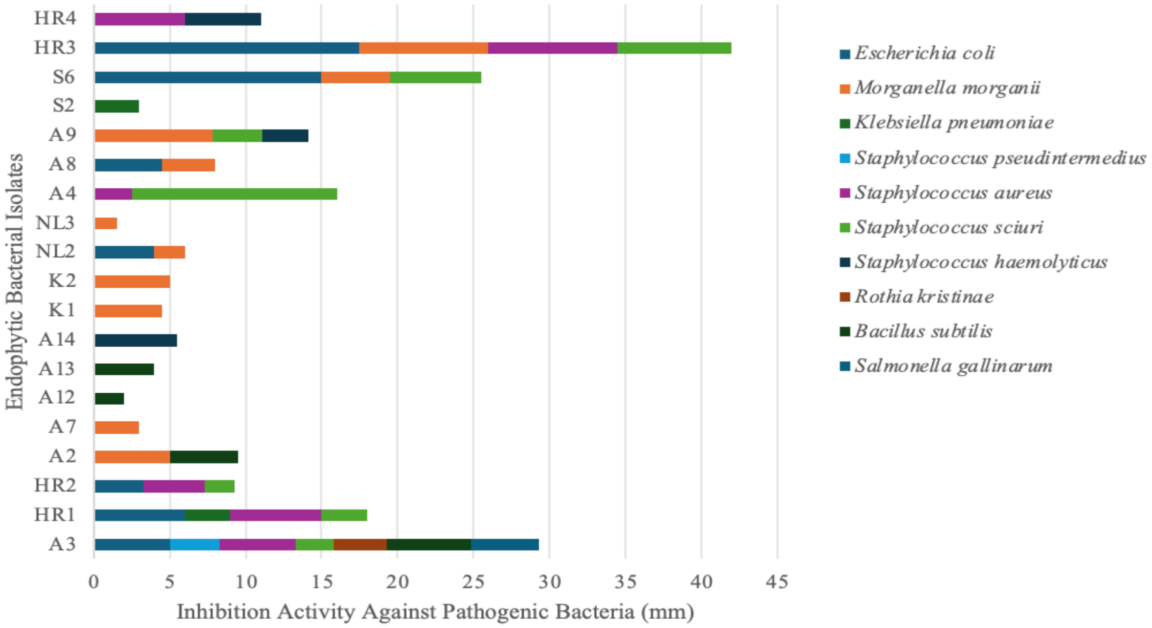


Figure 5. Inhibition activity of 19 endophytic isolates against 10 pathogenic bacteria

Table 3. Results of pathogenicity analysis of endophytic bacteria in blood agar media containing 5% sheep blood

Isolates code	Hemolysis category	Description
A2	γ-Hemolysis	No hemolytic zone
A3	β-Hemolysis	Very clear hemolytic zone
A4	α-Hemolysis	No hemolytic zone
A7	β-Hemolysis	Fairly clear hemolytic zone
A8	α-Hemolysis	Color change in the media
A9	γ-Hemolysis	No hemolytic zone
A12	β-Hemolysis	Very clear hemolytic zone
A13	α-Hemolysis	No hemolytic zone
A14	β-Hemolysis	Very clear hemolytic zone
HR1	γ-Hemolysis	No hemolytic zone
HR2	γ-Hemolysis	Very clear hemolytic zone
HR3	γ Hemolysis	No hemolytic zone
HR4	α-Hemolysis	No hemolytic zone
S2	Γ-Hemolysis	Scattered green color colony
S6	α-Hemolysis	No hemolytic zone
K1	β-Hemolysis	Discoloration of the media around the colony
K2	γ-Hemolysis	Very clear hemolytic zone
NL2	α-Hemolysis	No hemolytic zone
NL3	γ-Hemolysis	Discoloration of the media around the colony

Note: β: complete hemolysis, α: partial hemolysis, γ: no hemolysis

Discussion

Endophytic bacteria are one of microorganisms that naturally reside in healthy plant tissues and do not infect or damage the host plant. The majority of endophytic bacteria reside in vascular tissue, leaves, roots, and intracellular and intercellular components (Afzal et al. 2019). Through nutrient absorption, endophytic bacteria typically coexist in mutualistic symbiosis with plants, regulating plant development and affecting phytohormones in stressed plant (Khare et al. 2018). Indirectly, endophytic bacteria have an important function in increasing plant defense by targeting pests and pathogens by producing antibiotic compounds, hydrolytic enzymes, and nutrient limitations (Wu et al. 2021; Kandasamy and Kathirvel 2023). The strong association of endophytic bacteria and host plants has important benefits in the production of secondary metabolite compounds that are expected to have lower toxicity effects on normal cells, because they are not harmful to eukaryotic host cells (dos Santos et al. 2018). Endophytic bacteria produce secondary metabolite compounds that are similar to their hosts because they have the same cell signaling pathways through gene mutations or exchange of genetic information (Khare et al. 2018).

Plant-endophytic bacteria can be isolated using rich and complex media that are high in nutrients. On the other hand, there are several groups of bacteria that can be grown on nutrient-poor media (Eevers et al. 2015). According to the present study, the isolation of endophytic bacteria originating from *P. foetida* leaves using four growth media resulted in different macroscopic characteristics and numbers of colonies. Differences in nutrients in the media affect the number and diversity of endophytic bacteria that can be isolated from certain plant tissues (Yunita et al. 2022a). The results indicate that SCA media was the best media for isolating endophytic in terms of number of colonies. This is in line with previous studies which revealed that the isolation of endophytic bacteria is effective on SCA media because it has a low nutrient content so that it can limit the growth of fungi and other microorganisms (El-Shatoury et al. 2006; Passari et al. 2018). In the isolation process, some endophytic bacterial colonies were able to grow but could not grow in the re-culturing process. This is influenced by the presence of specific compounds in plants that are still present during the isolation process but are lost during the re-culturing process (Singh et al. 2022). In addition, plant tissue contains specific compounds that are not present in synthetic growth media so that the addition of leaf extracts is needed to enhance the growth of endophytic bacteria (Singh et al. 2022) as has been done in our study.

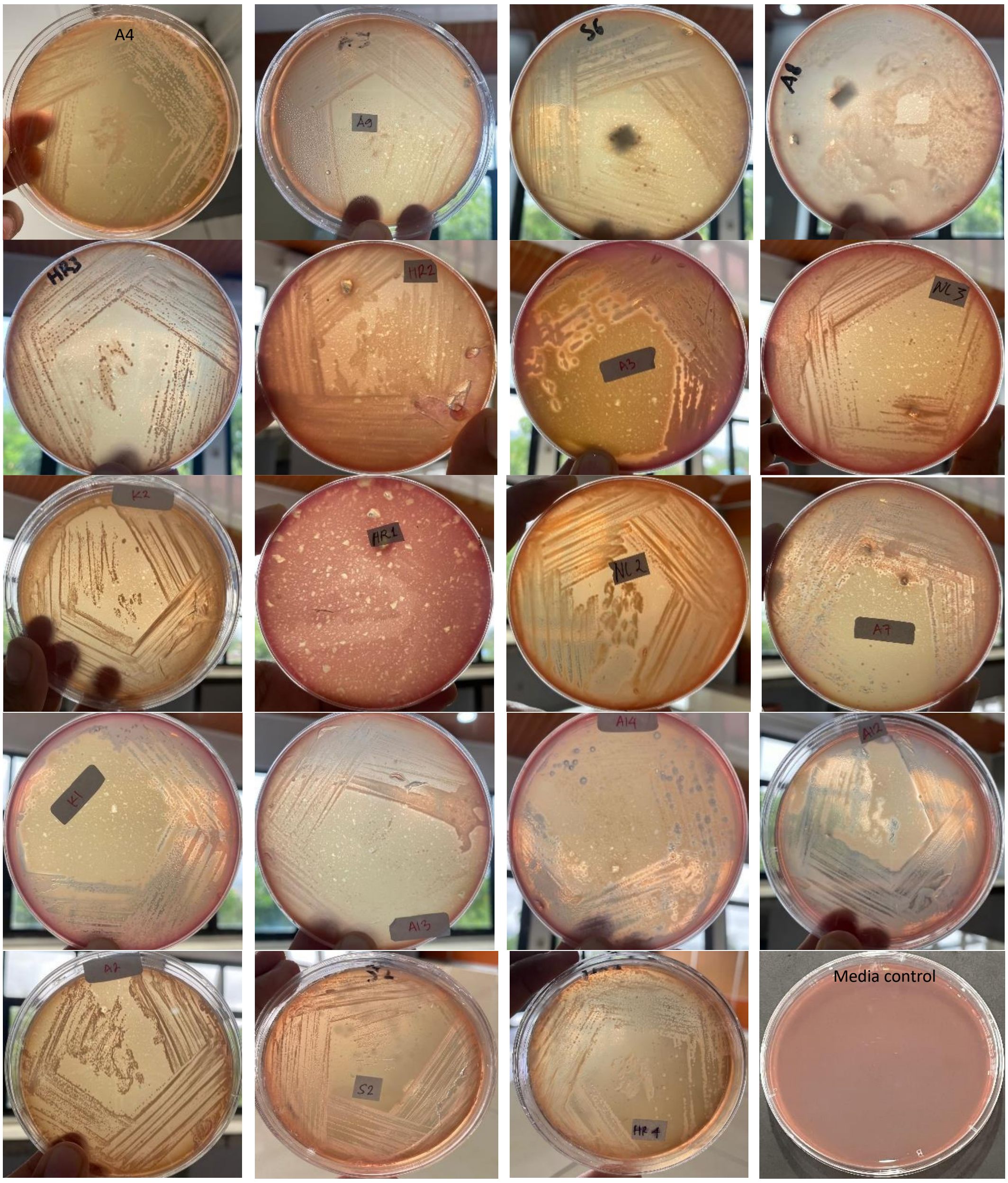


Figure 6. Visualization of endophytic bacteria in BAP media containing 5% sheep blood

Macroscopic characterization showed that endophytic bacteria that grew had differences in morphological characteristics, including size, shape, color, margin, and elevation, while microscopic characterization showed that 21 isolates were in the form of bacilli (A1, A2, A4, A5, A7, A8, A9, A10, A11, A12, A13, A14, S2, S6, S8, HR4, K1, K2, NL2, NL3), 2 isolates were diplobacilli (A6; HR2), 4 isolates were cocii (A3, S3, S4, HR3). Microscopic characterization according to gram staining showed that 17 isolates were gram-positive bacteria (A1, A2, A4, A5, A10, A11, A12, A14, S2, S6, S8, HR1, HR3, HR4, K1, K2, NL3), while 10 isolates were negative bacteria (A3, A6, A7, A8, A9, A13, S3, S4, HR2, NL2). These results are supported by previous studies showing that endophytic bacteria have varied morphologies and can be grouped into Gram-negative and Gram-positive bacteria (Duhan et al. 2020; Mugiastuti et al. 2023).

The antibacterial tests performed used the agar diffusion method against 10 pathogenic bacteria. The results showed that 19 endophytic bacteria had antibacterial activity as evidenced by the presence of a clear zone around the endophytic colony. The ability of endophytic bacteria to inhibit the growth of pathogenic bacteria is due to the content of secondary metabolites (Fatimah et al. 2024). The mechanism of action of endophytic bacteria is through the production of antimicrobial compounds such as surfactin, fengycin, iturin, subtilosin A, bacillaene, fusaricidin, viscosinamide which are known to have antimicrobial activity (Morales-Cedeño et al. 2021). Hydrolytic enzymes produced by endophytic bacteria function in the degradation of the cell walls of pathogenic bacteria (Woźniak et al. 2023).

Endophytic bacteria can also interfere with quorum sensing in pathogenic bacteria which allows resistance to antibiotics by forming biofilms. This is reinforced by (Rajesh and Ravishankar 2014) by finding that the production of *Pseudomonas aeruginosa* bacterial biofilms can be inhibited when tested with endophytic bacteria. In addition, endophytic bacteria are also competitive for nutrients and substrates (Ali et al. 2024). In this study, it was found that *P. foetida* has a high content of secondary metabolites such as iridoid glycosides, miscellaneous glycosides, anthraquinones, and terpenoids which have the main function as antibacterials (Dutta et al. 2023), so that endophytic bacteria associated with *P. foetida* leaves are also thought to have the similar bioactive compound content.

In contrast to rhizosphere bacteria, which are often highly competitive because of the nutrient-rich environment, endophytic bacteria associated with leaves are typically obligatory. Although obligatory endophytes usually have a lesser arsenal of metabolites due to limited competition, they may nevertheless produce additional specialized metabolites that facilitate their interaction with the host. Endophytic bacteria must first effectively compete in the rhizosphere before they may enter the plant because many of them are facultative plant colonists (Brader et al. 2014).

Pantoea agglomerans DAPP-PG 734, an endophytic bacterium, was previously isolated from olive knots infected by *Pseudomonas savastanoi* pv. *savastanoi* DAPP-PG 722. Whole-genome analysis of the *P. agglomerans* strain revealed the presence of a hypersensitive response and pathogenicity (Moretti et al. 2021). This suggests that endophytic bacteria's hypersensitive response can be influenced by pathogenic bacteria in the community within the plant tisue.

Pathogenicity analysis was performed using Blood Agar Plate (BAP) media to detect the hemolytic ability of bacteria. The BAP media is a versatile medium that is frequently used to differentiate bacteria according to their hemolytic characteristics. Complete lysis of red blood cells and hemoglobin is categorized as β-hemolysis; partial lysis of red blood cells and hemoglobin is called α-hemolysis; and no hemolysis, or no color change in the media, is categorized as γ-hemolysis (Hikmawati et al. 2019).

The results revealed that among 19 endophytic bacteria tested, eight of them had (isolates A2, A9, HR1, HR2, HR3, S2, K2, and NL3) the potential to be antibacterials without the capacity to cause disease as they did not hemolyze the blood contained in the media. While the other 12 isolates were able to hemolyze blood cells with complete and partial hemolysis categories indicated by the presence of clear zone or discoloration in the media. In particular, isolates K1, A3, A7, A14, and A12 were classified as β-hemolysis, whereas isolates NL2, A13, S6, A8, HR4, A4 were classified as β-hemolysis. The presence of a highly clear zone on BAP media is caused by the activity of β-hemolysin toxin, however the dark zone in α-hemolysis category is caused by the presence of α-hemolysin toxin (Parul et al. 2014). The ability of endophytic bacteria to hemolyze blood is related to the pathogenicity of bacteria. Generally, bacteria that are unable to destroy red blood cells or those included in the γ-hemolysis type are non-pathogenic bacteria. Many studies have reported that several pathogenic bacteria that are able to hemolyze blood and form clear zones in blood agar media are *E. coli* (Parul et al. 2014), *S. aureus* (Said et al. 2021), *Enterococcus* sp. (Ira et al. 2013), and *Vibrio* spp. (Hikmawati et al. 2019).

In conclusion, the present study successfully obtained many endophytic bacterial isolates from the leaves of *P. foetida* that had antibacterial potential. Isolates HR1 and HR3 were promising endophytic bacteria which required further research. Isolate HR1 demonstrated the capacity to suppress the growth of *E. coli, K. pneumoniae*, *S. aureus,* and *S. sciuri*, with moderate to strong categories. Meanwhile, isolate HR3 demonstrated a strong category of inhibition against *E. coli, M. morganii, S. aureus,* and *S. sciuri*. Both isolates, HR1 and HR3, were non-pathogenic in blood hemolysis tests. Further research on molecular identification is required to determine the species of the two bacteria. The profile of bioactive compounds must be known to define the direction of possible uses, as well as to verify the similarity of active molecules between endophytic bacteria and host plant.

ACKNOWLEDGEMENTS

Authors thank the Ministry of Education, Culture, Research, and Technology in association with the Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research and Technology (DRTPM) for funding our research by contract number: 077/E5/PG.02.00.PL/2024; 376/UN13.3/PT/2024 through fundamental scheme. We also thank the Faculty of Medicine of Universitas Pattimura, Ambon, Indonesia, for providing the laboratory to conduct this research.

REFERENCES

Afzal I, Shinwari ZK, Sikandar, S, Shahzad, S. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiol Res 221: 36-49. DOI: 10.1016/j.micres.2019.02.001.

Ali MA, Ahmed T, Ibrahim E, Rizwan M, Chong KP, Yong JWH. 2024. A review on mechanisms and prospects of endophytic bacteria in biocontrol of plant pathogenic fungi and their plant growth-promoting activities. Heliyon 10: e31573. DOI: 10.1016/j.heliyon.2024.e31573.

Anitha KG. 2019. Antioxidant producing endophytic bacterial consortium as biological tool for enhancing the antioxidant activity of bhendi under salt stress. J Pharm Phytochem 8 (5): 934-937.

Anjum N, Chandra R. 2015. Endophytic bacteria: Optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J Pharm Clin Res 8 (4): 233-238.

Aswani R, Jishma P, Radhakrishnan EK. 2020. Endophytic bacteria from the medicinal plants and their potential applications. In: Ajay Kumar, Vipin Kumar Singh (eds). Food Science, Technology and Nutrition. Woodhead Publishing, Cambridge. DOI: 10.1016/B978-0-12-818734-0.00002-4.

Beiranvand M, Amin M, Hashemi-Shahraki A, Romani B, Yaghoubi S, Sadeghi P. 2017. Antimicrobial activity of endophytic bacterial populations isolated from medical plants of Iran. Iran J Microbiol 9 (1): 11-18.

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A. 2014. Metabolic potential of endophytic bacteria. Curr Opinion Biotechnol 27 (1): 30-37. DOI: 10.1016/j.copbio.2013.09.012.

Cardoso VM, Campos FF, Santos ARO, Ottoni MHF, Rosa CA, Almeida VG, Grael CFF. 2020. Biotechnological applications of the medicinal plant *Pseudobrickellia brasiliensis* and its isolated endophytic bacteria. J Appl Microbiol 129: 926-934. DOI: 10.1111/jam.14666.

Chanda S, Sarethy IP, De B, Singh K. 2013. *Paederia foetida* - a promising ethno-medicinal tribal plant of northeastern India. J For Res 24 (4): 801-808. DOI: 10.1007/s11676-013-0369-2.

dos Santos ML, Berlitz DL, Wiest SLF, Schünemann R, Knaak N, Fiuza, LM. 2018. Benefits associated with the interaction of endophytic bacteria and plants. Braz Arch Biol Technol 61 (1): e18160431. DOI: 10.1590/1678-4324-2018160431.

Duhan P, Bansal P, Rani S. 2020. Isolation, identification and characterization of endophytic bacteria from medicinal plant *Tinospora cordifolia*. S Afr J Bot 134: 43-49. DOI: 10.1016/j.sajb.2020.01.047.

Dutta PP, Marbaniang K, Sen S, Dey BK, Talukdar NC. 2023. A review on phytochemistry of *Paederia foetida* Linn. Phytomed Plus 3 (1): 100411. DOI: 10.1016/j.phyplu.2023.100411.

Eevers N, Gielen M, Sánchez-López A, Jaspers S, White JC, Vangronsveld J, Weyens N. 2015. Optimization of isolation and cultivation of bacterial endophytes through addition of plant extract to nutrient media. Microb Biotechnol 8 (4): 707-715. DOI: 10.1111/1751-7915.12291.

El-Shatoury S, Abdulla H, El-Karaaly O, El-Kazzaz W, Dewedar A. 2006. Bioactivities of endophytic actinomycetes from selected medicinal plants in the world heritage site of Saint Katherine, Egypt. Intl J Bot 2 (3): 307-312. DOI: 10.3923/ijb.2006.307.312.

Fatimah, Asritafriha L, Salsabila S, Dewi EK, Geraldi A, Ramadhan R, Suwito H, Rahman A, Riyadi L. 2024. Potential of antimicrobial-producing endophytic bacteria from Balikpapan endemic ginger (*Etlingera balikpapanensis* A.D. Poulsen). Biodiversitas 25 (9): 3005-3013. DOI: 10.13057/biodiv/d250922.

Hikmawati F, Susilowati A, Setyaningsih R. 2019. Deteksi jumlah dan uji patogenitas *Vibrio* spp. pada kerang hijau (*Perna viridis*) di kawasan wisata Pantai Yogyakarta. J Pros Sem Nas Masy Biodiv Indo 5 (2): 334-339. [Indonesian]

Ira P, Sujatha S, Chandra PS. 2013. Virulence factors in clinical and commensal isolates of Enterococcus species. Indones J Pathol Microbiol 56 (1): 24-30. DOI: 0.4103/0377-4929.116144.

Kandasamy GD, Kathirvel P. 2023. Insights into bacterial endophytic diversity and isolation with a focus on their potential applications-A review. Microbiol Res 266: 127256. DOI: 10.1016/j.micres.2022.127256.

Khare E, Mishra J, Arora NK. 2018. Multifaceted interactions between endophytes and plant: Developments and prospects. Front Microbiol 9: 2732. DOI: 10.3389/fmicb.2018.02732.

Kumar V, Al-Abbasi FA, Ahmed D, Verma A, Mujeeb M, Anwar F. 2015. *Paederia foetida* Linn. inhibits adjuvant induced arthritis by suppression of PGE2 and COX-2 expression via nuclear factor-κB. Food Funct 6 (5): 1652-1666. DOI: 10.1039/c5fo00178a.

Kumar V, Anwar F, Ahmed D, Verma A, Ahmed A, Damanhouri ZA, Mishra V, Ramteke PW, Bhatt PC, Mujeeb M. 2014. *Paederia foetida* Linn. leaf extract: An antihyperlipidemic, antihyperglycaemic and antioxidant activity. BMC Compl Alter Med 14: 76. DOI: 10.1186/1472-6882-14-76.

Liu YH, Guo JW, Salam N, Li L, Zhang YG, Han J, Mohamad OA, Li WJ. 2016. Culturable endophytic bacteria associated with medicinal plant *Ferula songorica*: Molecular phylogeny, distribution and screening for industrially important traits. 3 Biotech 6 (2): 209. DOI: 10.1007/s13205-016-0522-7.

Maela MP, van der Walt H, Serepa-Dlamini MH. 2022. The antibacterial, antitumor activities, and bioactive constituents' identification of *Alectra sessiliflora* bacterial endophytes. Front Microbiol 1 (1): 870821. DOI: 10.3389/fmicb.2022.870821.

Morales-Cedeño LR, Orozco-Mosqueda M del C, Loeza-Lara PD, Parra-Cota F, de los Santos-Villalobos S, Santoyo G. 2021. Plant growth-promoting bacterial endophytes as biocontrol agents of pre- and post-harvest diseases: Fundamentals, methods of application and future perspectives. Microbiol Res 242: 126612. DOI: 10.1016/j.micres.2020.126612.

Moretti C, Rezzonico F, Orfei B, Cortese C, Moreno-Pérez A. van den, Burg HA, Buonaurio R. 2021. Synergistic interaction between the type III secretion system of the endophytic bacterium *Pantoea agglomerans* DAPP-PG 734 and the virulence of the causal agent of olive knot *Pseudomonas savastanoi* pv. *savastanoi* DAPP-PG 722. Mol Plant Pathol 22 (10): 1209-1225. DOI: 10.1111/mpp.13105.

Mugiastuti E, Manan A, Soesanto L. 2023. Biological control of maize downy mildew with the antagonistic bacterial consortium. Biodiversitas 24 (9): 4644-4650. DOI: 10.13057/biodiv/d240904.

Newman DJ. 2018. Are microbial endophytes the actual producers of bioactive antitumor agents?. Trends Cancer 4 (10): 662-670. DOI: 10.1016/j.trecan.2018.08.002.

Pal A, Chattopadhyay A, Paul AK. 2012. Diversity and antimicrobial spectrum of endophytic bacteria isolates from *Peaderia foetida* L. Intl J Curr Pharm Res 4 (3): 32.

Parul B, Sharma B, Jain U. 2014. Virulence associated factors and antibiotic sensitivity pattern of *Escherichia coli* isolated from cattle and soil. Vet World 7: 369-372. DOI: 10.14202/vetworld.2014.369-372.

Passari AK, Leo VV, Chandra P, Kumar B, Nayak C, Hashem A, Abd Allah EF, Alqarawi AA, Singh BP. 2018. Bioprospection of actinobacteria derived from freshwater sediments for their potential to produce antimicrobial compounds. Microb Cell Fact 17 (1): 68. DOI: 10.1186/s12934-018-0912-0.

Rajesh PS, Ravishankar RV. 2014. Quorum quenching activity in cell-free lysate of endophytic bacteria isolated from *Pterocarpus santalinus* Linn., and its effect on quorum sensing regulated biofilm in *Pseudomonas aeruginosa* PAO1. Microbiol Res 169 (7-8): 561-569. DOI: 10.1016/j.micres.2013.10.005.

Russell FM, Biribo SSN, Selvaraj G, Oppedisano F, Warren S, Seduadua A, Mulholland EK, Carapetis JR. 2006. As a bacterial culture medium, citrated sheep blood agar is a practical alternative to citrated human blood agar in laboratories of developing countries. J Clin Microbiol 44 (9): 3346-3351. DOI: 10.1128/JCM.02631-05.

Rustamova N, Wubulikasimu A, Mukhamedov N, Gao Y, Egamberdieva D, Yili A. 2020. Endophytic bacteria associated with medicinal plant *Vernonia anthelmintica*: Diversity and characterization. Curr Microbiol 77 (8): 1457-1465. DOI: 10.1007/s00284-020-01924-5.

Said A, El-Gamal MS, Abu-Elghait M, Salem SS. 2021. Isolation, Identification and antibiotic susceptibility pattern of urinary tract infection bacterial isolates. Lett Appl NanoBioSci 10 (4): 2820-2830. DOI: 10.33263/LIANBS104.28202830.

Savitri L, Tiyas EW, Krissanjaya R. 2024. Testing antibacterial activity of daun kentut (*Paederia foetida* L.) leaf extract against *Escherichia coli* in vitro. Biol Med Nat Prod Chem 13 (1): 277-283. DOI: 10.14421/biomedich.2024.131.277-283.

Singh AK, Sharma RK, Sharma V, Singh T, Kumar R, Kumari D. 2017. Isolation, morphological identification and in vitro antibacterial activity of endophytic bacteria isolated from *Azadirachta indica* (neem) leaves. Vet World 10: 510-516. DOI: 10.14202/vetworld.2017.510-516.

Singh R, Bharose R, Singh VK. 2018. Effect of Modern agricultural practices on biological properties of rhizospheric and non rhizospheric soils of chaka block of Allahabad District, U.P. Intl J Curr Microbiol Appl Sci 7 (12): 1496-1504. DOI: 10.20546/ijcmas.2018.712.176.

Singh R, Pandey KD, Singh M, Singh SK, Hashem A, Al-arjani ABF, Abd_allah EF, Singh PK, Kumar A. 2022. Isolation and characterization of endophytes bacterial strains of *Momordica charantia* L. and their possible approach in stress management. Microorganisms 10 (2): 290. DOI: 10.3390/microorganisms10020290.

Sulistiyani S, Ardyati T, Winarsih S. 2016. Antimicrobial and antioxidant activity of endophyte bacteria associated with *Curcuma longa* rhizome. J Exp Life Sci 6: 45-51. DOI: 10.21776/ub.jels.2016.006.01.11.

Sushma M, Jayasangkar M, Vinu AK. 2018. Antibacterial activity of endophytic bacteria isolated from few medicinal plants of BR hills, Karnataka. J Pharm Phytochem 7 (5): 2338-2342.

Triandriani W, Sogandi, Saputri DD, Suhendar U. 2020. Antioxidant activity of endophytic bacterial extract isolated from clove leaf (*Syzygium aromaticum* L.). J Agri Appl Biol 1 (1): 9-17. DOI: 10.11594/jaab.01.01.02.

Vinodkumar S, Nakkeeran S, Renukadevi P, Mohankumar S. 2018. Diversity and antiviral potential of rhizospheric and endophytic *Bacillus* species and phyto-antiviral principles against tobacco streak virus in cotton. Agric Ecosyst Environ 267: 42-51. DOI: 0.1016/j.agee.2018.08.008.

Widjajanti H, Handayani CV, Nurnawati E. 2021. Antibacterial activity of endophytic fungi from Sembukan (*Paederia foetida* L.) leaves. Sci Technol Indonesia 6: 189-195. DOI: 10.26554/sti.2021.6.3.189-195.

Woźniak M, Tyśkiewicz R, Siebielec S, Gałązka A, Jaroszuk-Ściseł J. 2023. Metabolic profiling of endophytic bacteria in relation to their

potential application as components of multi-task biopreparations. Microbiol Ecol 86: 2527-2540. DOI: 10.1007/s00248-023-02260-4.
Wu W, Chen W, Liu S, Wu J, Zhu Y, Qin L, Zhu B. 2021. Beneficial relationships between endophytic bacteria and medicinal plants. Front Plant Sci 12 (1): 646146. DOI: 10.3389/fpls.2021.646146.
Yunita M, Mubarik NR, Solihin DD. 2016. Isolation and identification of chitinolytic bacteria as biocontrol agent of pathogenic fungi on gold silkworm cocoon *Cricula trifenestrata*. Malay J Microbiol 12(1): 69-75. DOI: 10.21161/mjm.74415.
Yunita M, Ohiwal M, Dirks CS, Angkejaya OW, Sukmawati S, Ilsan NA. 2022a. Endophytic bacteria associated with *Myristica fragrans*: Improved media, bacterial population, preliminary characterization, and potential as antibacterials. Biodiversitas 23 (8): 4047-4054. DOI: 10.13057/biodiv/d230824.
Yunita M, Manse Y, Wulandari MC, Sukmawati S, Ohiwal M. 2022b. Isolation of endophytic bacteria from nutmeg plant as antibacterial agents against pathogenic bacteria. Jurnal Pendidikan Biologi 7 (1): 115-122. DOI: 10.31932/jpbio.v7i1.1522.
Yunita M, Ohiwal M, Elfitrasya MZ, Rahawarin H. 2023a. Antibacterial activity of *Paederia foetida* leaves using two different extraction procedures against pathogenic bacteria. Biodiversitas 24 (11): 5920-5927. DOI: 10.13057/biodiv/d241110.
Yunita M, Lumbantobing RM, Tahitu R. 2023b. Antifungal activity of endophytic bacteria isolated from miana plants (*Coleus scutellarioides* (L.) Benth.) against *Candida albicans*. Folia Med Indonesia 59 (3): 274-281. DOI: 10.20473/fmi.v59i3.43989.