BIODIVERSITAS
Volume 25, Number 9, September 2024
Pages: 3005-3013

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250922

Potential of antimicrobial-producing endophytic bacteria from Balikpapan endemic ginger (*Etlingera balikpapanensis* A.D. Poulsen)

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Manuscript received: 22 July 2024. Revision accepted: 13 September 2024.

Abstract. *Fatimah, Asritafriha L, Salsabila S, Dewi EK, Ni'matuzahroh, 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: 3005-3013. Etlingera balikpapanensis* A.D. Poulsen is a new species of the Zingiberaceae family, which was discovered as an endemic species in the Balikpapan, Kalimantan. This study aimed to isolate endophytic bacteria from endemic Balikpapan ginger (*E. balikpapanensis*) and evaluate its antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* and to identify the most promising antimicrobial isolates. Plant samples include roots, stems, and leaves. The process of isolating bacteria from various plant parts, such as roots, stems, and leaves, involved repeated subculturing until pure isolates were obtained. Seven pure isolates were successfully obtained, coded as 6D, 5R, 2R, 3D1, 10D, 4R2, and 2D. Macroscopic, microscopic, and biochemical characterization of the seven isolates was carried out. The seven endophytic bacteria had varied macroscopic characteristics and were Gram-positive. The disc diffusion method was used to determine the ability of isolates to produce antimicrobial agents. The isolates showed antibacterial activity against *E. coli* and *S. aureus*. Isolates 3D1 and 5R showed the highest antibacterial activity against *E. coli* (8.20±0.47 mm and 8.57±0.08 mm), and 5R also showed the highest antibacterial activity against *S. aureus* (8.14±0.49 mm). These results demonstrate the potential of these isolates as effective antimicrobial agents. The 16S rRNA gene analysis revealed that isolate 3D1 was identified as *Bacillus cereus* strain PJA4.2 and isolate 5R as *Micrococcus luteus* strain SGAir0127 with similarity of 99.55% and 100%, respectively.

Keywords: Antimicrobial agent, endemic ginger, endophytic bacteria, *Etlingera balikpapanensis*, 16S rRNA gene

INTRODUCTION

Plants, with their secondary metabolite content, are a crucial source of natural substances for drug development (Begum et al. 2022). These secondary metabolite compounds, produced during plants' metabolic processes, possess bioactive properties. They serve as mechanisms for chemical adaptation to environmental stress and self-defense, enabling plants to repel insects, herbivores, and microorganisms (Divekar et al. 2022). However, the extraction of these natural compounds from plants requires a significant biomass, leading to the potential risk of overexploitation and endangering their sustainability. This underscores the urgent need to find alternative sources of bioactive compounds that are more accessible and efficient.

Endophytic microorganisms, a significant source of bioactive compounds, reside within their hosts without causing symptoms at various stages of their life cycle. They play a vital role in the growth, development, fitness, and diversification of plants (Rana et al. 2020). Endophytic microbes can produce bioactive compounds with characteristics similar to their host due to genetic transfer (Chaudhry et al. 2017). These microorganisms live in plant tissues and represent the natural resource abundance to be used as a source for new drug discovery. The growth rate of endophytes exceeds that of the host, making the exploration of endophytes as a source of new drug discovery not just profitable but also promising for the future of drug development (Frank et al. 2017).

Etlingera (Zingiberaceae) is one of a group of high-value medicinal plants that have long been known to the people of Indonesia for their benefits and uses (Saudah et al. 2022). Many species from the Zingiberaceae family have the potential to treat various diseases, including degenerative disorders, digestive health issues, cardiovascular disorders, and cancer (Masshhadi et al. 2013). Furthermore, *E. balikpapanensis* also has the potential as an antimicrobial agent capable of addressing infectious diseases (Amer and Ibrahim 2019). Endophytic bacteria have been studied for producing bioactive compounds categorized into various groups, including alkaloids, terpenoids, steroids, lactones, phenolic compounds, quinones, and lignans, among others. These compounds are also present in host plants to enhance defense mechanisms, including combating pathogens (Mei and Flinn 2010; Anjum and Chandra 2015; Manurung et al. 2019).

Etlingera balikpapanensis is a new species of the Zingiberaceae family discovered by Danish botanist Axel Dalberg in 2006 (Poulsen 2006). This plant is found as an endemic species in the Balikpapan region, specifically in the protected forest area of Sungai Wain Balikpapan, East Kalimantan, Indonesia (Manurung et al. 2019). Rhamadhani et al. (2016) revealed that leaf extracts of *E. balikpapanensis* exhibit antimicrobial activity against bacteria such as *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus* due to the presence of secondary metabolites such as phenolics and tannins known for their antibacterial properties. Leaf extracts of *E. balikpapanensis* were found to contain alkaloids, phenolics, flavonoids, and steroids and exhibit high antioxidant activity. Therefore, it is hoped that these compounds will also be produced by endophytic bacteria of *E. balikpapanensis* as antimicrobial agents (Manurung et al. 2019). It is known that the conservation status of *E. balikpapanensis* is threatened due to deforestation and forest fires in East Kalimantan (Poulsen 2006). There has been significant exploitation of plant parts for medicinal purposes, leading to a decline in natural resources. Therefore, the isolation of endophytic microbes capable of producing bioactive compounds to reduce exploitation can be considered a viable solution (Raimi and Adeleke 2021).

Further research into the content of bioactive compounds produced by bacteria associated with this species is of great interest, given its classification as a new species. Hence, the study aimed to isolate endophytic bacteria from the roots, stems, and leaves of *E. balikpapanensis*, assess the antimicrobial activity of the isolated endophytic bacteria, and identify their species names. Antimicrobial activity testing was conducted against three types of test pathogens: *E. coli*, *S. aureus*, and *Candida albicans*. Based on the background above, it is anticipated that antimicrobial compounds produced by endophytic bacteria from *E. balikpapanensis* can be developed into active antimicrobial agents to combat infectious diseases.

MATERIALS AND METHODS

Plant materials

The roots, stems, and leaves of *Etlingera balikpapanensis* A.D. Poulsen were collected from Balikpapan Botanical Garden, Balikpapan City, East Kalimantan, Indonesia, in October 2019. The Coordinates range of sampling sites was 01°07.823'S to 01°07.960'S and 116°51.321'E to 116°51.515'E (Figure 1).

Isolation of endophytic bacteria

The endophytic bacteria were isolated from the stems, roots, and leaves of *E. balikpapanensis*. Samples were sterilized with 70% ethanol (1 minute), 5.25% natrium hypochlorite (5 minutes), and 70% ethanol three times. Then, it was washed with sterile aquadest (1 minute) twice. Next, 2-3 mL extract of these surface sterilized samples were inoculated with the pour plate method to nutrient agar, which contained Nistatyn 30µg/mL. Incubation is carried out at 28±2°C for 48 hours (Purwanto et al. 2014; Anjum and Chandra 2015).

Characterization of endophytic bacterial isolates

The character of endophytic bacteria is observed macroscopically and microscopically. Macroscopic characters of colonies include size, shape, color, margin, elevation, and consistency. Microscopic characteristics of cells include size, shape, and response of cells to Gram dyes (color) (Desriani et al. 2013).

Biochemical test of endophytic bacteria

Biochemical tests were performed using the Oxoid™ Microbact™ GNB Reagents kit. Physiological test results were read after an incubation period of 24-48 hours and analyzed using the Oxoid™ Microbact™ software program.

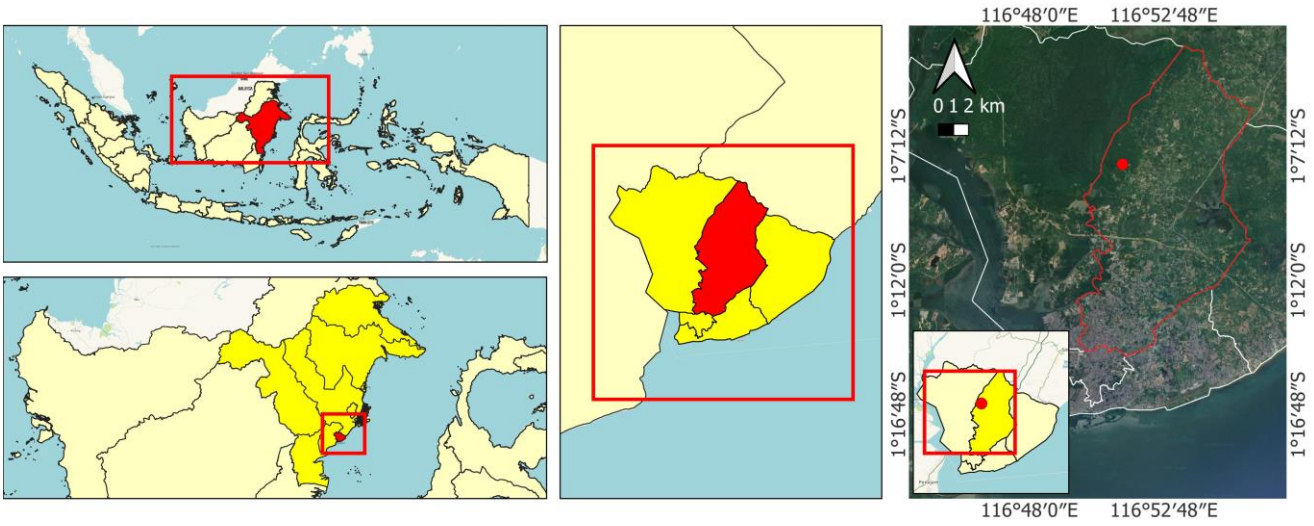

Figure 1. Location of plant material, *Etlingera balikpapanensis* collection in North Balikpapan, Balikpapan, East Kalimantan, Indonesia

Screening of antimicrobial activity of endophytic bacteria

All endophytic bacteria isolates were grown in a nutrient broth medium for 2 days at room temperature. Each endophytic bacterial culture was centrifuged for 20 minutes at 3000 rpm, and the supernatant was used for an antimicrobial activity test (Kumala et al. 2006). Next, the antimicrobial activity was determined by the disc diffusion method against three pathogenic bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *C. albicans* ATCC 10231). Overnight grown cultures of the test microorganisms were spread by sterile cotton swabs onto the surface of Mueller Hinton Agar (MHA) medium. Filter paper discs (6 mm) containing 20 µL of the endophytic bacteria supernatant are placed on MHA. Ciprofloxacin and nystatin (100.000 IU) were used as positive control instead of sterile aquadest as negative control and incubated at 35ºC for 24-48 hours (Radji et al. 2011). Antimicrobial activity is expressed by the diameter of the inhibition zone (mm) formed around the paper disc containing the endophytic bacterial supernatant. Then, a caliper is used to measure the diameter of the inhibition zone.

Identification of endophytic bacteria isolates

Endophytic bacteria with antimicrobial potential were identified using 16S rRNA. DNA extraction of each isolate was carried out using Wizard Genomic DNA Purification Kit (Promega 2023). The universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') were used to amplify the 16S rRNA gene. (Kusharyati et al. 2020). Amplification was carried out with mixed reactions that contained Gotaq Green PCR master mixture, primers, and DNA in a total volume of 50 µL. This reaction was performed for 30 cycles using the following conditions: Preheat at 94ºC for 2 minutes, DNA template denaturation at 94ºC for 30 seconds, annealing at 55ºC for 30 seconds, extension at 72ºC for 30 seconds, and final extension at 72ºC for 7 minutes. The PCR products were separated by agarose gel electrophoresis and visualized under UV light, then sequenced and purified by 1st Base in Singapore. Nucleotide sequences were edited using Bioedit. The obtained consensus sequences were BLAST with genomic database GenBank in NCBI (Li et al. 2017). The microorganism strains with the highest level of identity were chosen to construct a phylogeny tree. The data were further analyzed by aligning the obtained sequences with strain from NCBI using the MEGA X Software. Phylogenetic trees were then constructed using Neighbor-Joining with a bootstrap of 1.000 times.

RESULTS AND DISCUSSION

Morphology characteristics of endophytic bacteria

Figures 2 and 3 show the colony and cell morphology of the seven endophytic bacterial isolates from Balikpapan ginger (*E. balikpapanensis*). The isolates were coded as 6D, 5R, 2R, 3D1, 10D, 4R2, and 2D. Table 1 presents the Colony characteristics of endophytic isolates.

Table 1. Characteristics of colony and cell of endophytic bacteria *Etlingera balikpapanensis*

Isolate code	Color	Shape	Margin	Elevation	Cell shape	Gram
6D	White	Irregular	Undulate	Flat	Bacill	+
5R	Red	Circular	Entire	Convex	Coccus	+
2R	White	Filamentous	Filiform	Flat	Bacill	+
3D1	Bone White	Irregular	Undulate	Flat	Bacill	+
10D	Cream	Circular	Undulate	Raised	Bacill	+
4R2	White	Irregular	Undulate	Flat	Bacill	+
2D	White	Irregular	Filiform	Flat	Bacill	+

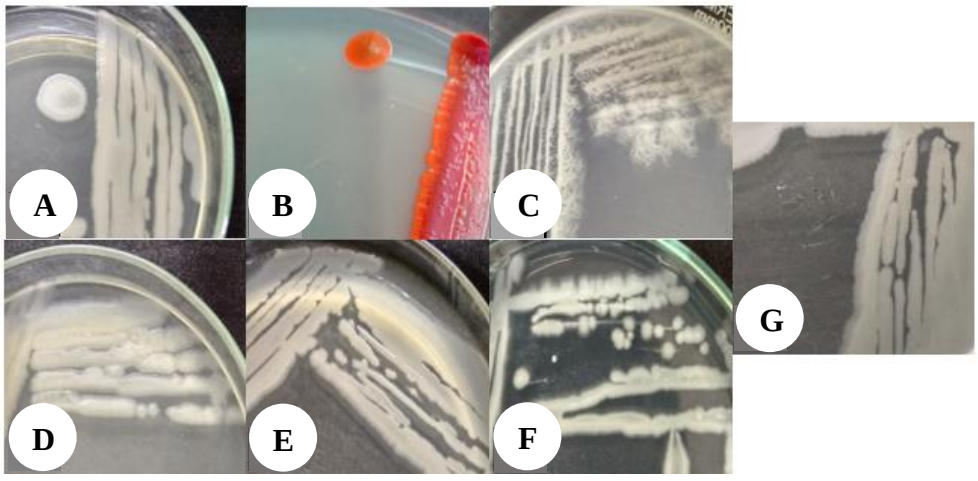

Figure 2. Colony morphology of endophytic bacteria isolated from *Etlingera balikpapanensis*. A. 6D; B. 5R; C. 2R; D. 3D1; E. 10D; F. 4R2; G. 2D

Biochemical test

Based on Table 2, all seven endophytic bacterial isolates showed positive reactions in oxidase, nitrate, and Voges-Proskauer (V-P) tests and negative reactions in ornithine, H_2S, indole, urease (except 2D), malonate, rhamnose, lactose, raffinose, and arginine tests. All isolates except 5R were motile and positive for TDA, inositol, adonitol, and salicin tests. Isolates from *E. balikpapanensis* were positive for catalase (except 6D and 2R) and xylose (except 6D and 10D), and negative for arabinose (except 4R2 and 2D), sucrose and sorbitol (except 6D and 5R), ONPG and citrate (except 5R and 10D), lysine (except 5R, 2R, and 10D), glucose and mannitol (except 5R and 2D), and gelatin (except 5R, 10D, and 4R2).

Antimicrobial activity test

Table 3 show the diameters of the inhibition zones resulting from the antimicrobial activity of endophytic bacteria against *E. coli*, *S. aureus*, and *C. albicans*, respectively.

All endophytic bacterial isolates have a greater inhibition zone diameter after 48 hours than after 24 hours of incubation against *S. aureus* (Table 3). Isolate 5R has the largest inhibition zone diameter of 8.14±0.49 mm. Isolate 2D has the smallest inhibition zone of 6.78±0.44 mm. For comparison, the positive control, ciprofloxacin, has a mean inhibition zone diameter of 24.35±0.30 mm.

The antifungal activity of Balikpapan ginger (*E. balikpapanensis*) isolates against *C. albicans* showed that no isolates formed clear zones during either the 24-hour or 48-hour incubation. Therefore, it can be interpreted that no isolates are active as antifungal agents against *C. albicans*. In the antifungal test, sterile distilled water was used as a negative control, and nystatin was the positive control.

Table 2. Biochemical tests of endophytic bacteria *Etlingera balikpapanensis*

Biochemical test	Endophytic bacteria						
	6D	5R	2R	3D1	10D	4R2	2D
Catalase	-	+	-	+	+	+	+
Oxidase	+	+	+	+	+	+	+
Motility	+	-	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+
Lysine	-	+	+	-	+	-	-
Ornithine	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-
Glucose	-	+	-	-	-	-	+
Mannitol	-	+	-	-	-	-	+
Xylose	-	+	+	+	-	+	+
ONPG	-	+	-	-	+	-	-
Indole	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	+
V-P	+	+	+	+	+	+	+
Citrate	-	+	-	-	+	-	-
TDA	-	+	-	-	-	-	-
Gelatine	-	+	-	+	-	+	-
Malonate	-	-	-	-	-	-	-
Inositol	-	+	-	-	-	-	-
Sorbitol	+	+	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-
Sucrose	+	+	-	-	-	-	-
Lactose	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	+	+
Adonitol	-	+	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-
Salicin	-	+	-	-	-	-	-
Arginine	-	-	-	-	-	-	-

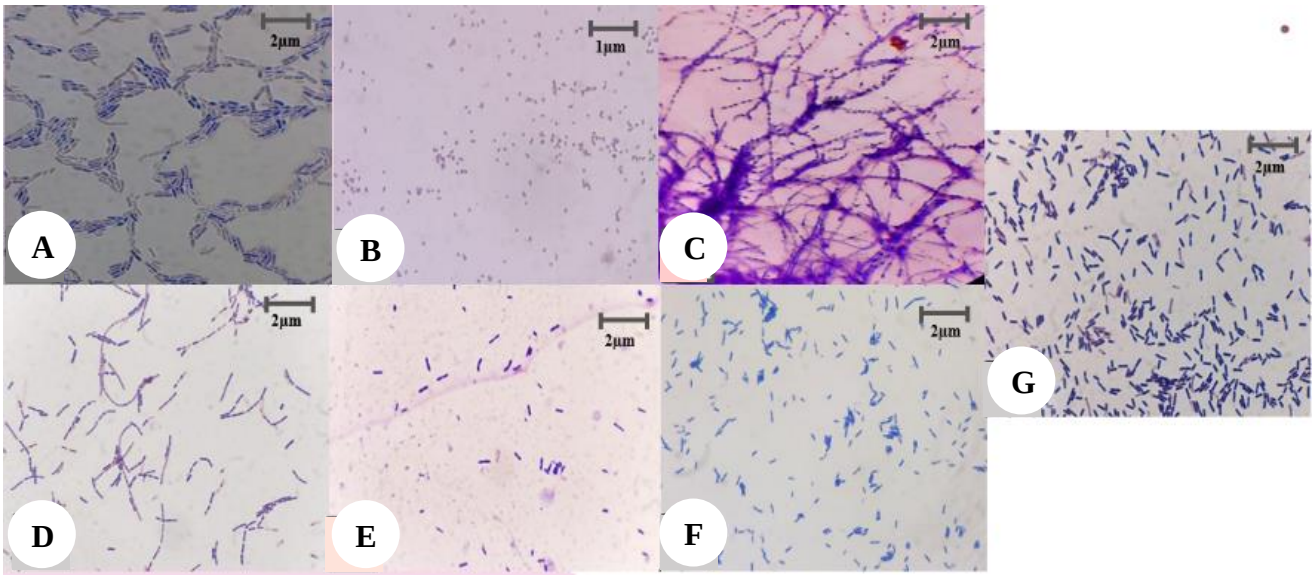

Figure 3. Cell morphology of endophytic bacteria A. 6D; B. 5R; C. 2R; D. 3D1; E. 10D; F. 4R2; G. 2D

Identification of the most potent endophytic bacteria based on 16S rRNA gene sequence analysis

Molecular identification of the endophytic bacterial species was carried out on isolates that produced the largest inhibition zone, namely isolates 5R and 3D1. Isolate 5R has the highest similarity with *Micrococcus luteus* strain SGAir0127, while isolate 3D1 has the highest similarity with *Bacillus cereus* strain PJA4.2 (Table 4). After knowing the percentage identification sequence owned by isolates 5R and 3D1 with the NCBI database, it followed by phylogeny tree construction to determine the relationship of bacterial isolates 5R and 3D1 with the five isolates of ginger endophytic bacteria (*E. balikpapanensis*) and three external isolates (Figure 4).

Based on Table 3, it can be seen that during the 24-hour incubation period, the seven endophytic isolates of Balikpapan ginger (*E. balikpapanensis*) showed antibacterial activity against *E. coli* with various inhibition diameters during 24-48 hours incubation period. Isolate 3D1 has the largest mean inhibition zone diameter (48 hours), which is 8.72±0.47 mm. However, isolate 5R has a slightly smaller mean inhibition zone diameter of 8.57±0.08 mm. When comparing the two, isolate 5R has a lower standard deviation, indicating more homogeneous data than isolate 3D1. Isolate 4R2 has the smallest inhibition zone, measuring 6.00±0.00 mm (24 hours). In comparison, the positive control, ciprofloxacin, has a mean inhibition zone diameter of 26.23±0.40 mm (24 hours).

Discussion

Endophytes are naturally found in healthy plants and are defined as microbes that live inside plant tissues without causing adverse effects. Approximately 300,000 plant species are known to host endophytes in the form of mutualistic symbiosis (Strobel et al. 2002; Aly et al. 2011). Endophytic microorganisms can be bacteria or fungi (Shankar Naik et al. 2014; Khamwan et al. 2018). Endophytic bacteria with their unique ability to help their hosts tolerate stressful conditions and produce allelopathic effects on other competing plant species, are a fascinating area of study. This research highlights the significant role of endophytic bacteria in enabling their hosts to better survive in facing biotic and abiotic challenges as well as competition with other plants (Afzal et al. 2019). It's particularly surprising to note that the most commonly isolated endophytic bacterial genera included *Bacillus, Burkholderia, Microbacterium, Micrococcus, Pantoea, Pseudomonas,* and *Stenotrophomonas,* with *Bacillus* and *Pseudomonas* emerging as the most dominant genera (Chaturvedi et al. 2016; Afzal et al. 2019).

Seven pure isolates were obtained from the sterilized Balikpapan ginger plant (*E. balikpapanensis*). The effectiveness of surface sterilization was confirmed by the absence of bacterial growth in the final wash rinse, which was inoculated on culture media (Eevers et al. 2015). The obtained isolates were coded 6D, 5R, 2R, 3D1, 10D, 4R2, and 2D. The results of the macroscopic and microscopic characterization of endophytic bacteria are presented in Figures 1 and 2. All endophytic bacterial isolates from *E. balikpapanensis* exhibited diverse colony morphologies: white colonies (except for the red colony of 5R), irregular shape (2D, 4R2, 3D1, and 6D), circular (5R and 10D), and filamentous (2R); undulate margins (6D, 3D1, 10D, and 4R2), filiform (2R and 2D), and entire (5R); flat elevation, except for 5R (convex) and 10D (raised). In the same genus, 11 endophytic bacterial isolates were successfully isolated from *the Etlingera elicitor* (Jack) RM Smith, and 19 endophytic bacterial isolates were successfully isolated from the rhizomes of the Zingiberaceae family, including *Etlingera* sp., *Globba patens* Miq., *Globba pendula* Roxb., and *Zingiber multibracteata* Holttum (Suryanto et al. 2016; Mamangkey et al. 2020). Microscopic characterization revealed that six of the seven obtained endophytic bacterial isolates were bacilli, while isolate 5R was coccus-shaped. All isolated endophytic bacteria (6D, 5R, 2R, 3D1, 10D, 4R2, 2D) were Gram-positive. Previous studies on the isolation of endophytic bacteria have shown that endophytic bacteria can be either Gram-positive or Gram-negative and exhibit diverse morphologies (Suhandono et al. 2016; Ying et al. 2016).

Table 3. Antimicrobial activity of endophytic bacteria *Etlingera balikpapanensis* against *E. coli*, *S. aureus* and *C. albicans*

Isolate code	Diameter of the inhibitory zone (mm)					
	E. coli		*S. aureus*		*C. albicans*	
	24 (h)	48 (h)	24 (h)	48 (h)	24 h	48 h
6D	7.50±0.39	8.23±0.08	7.03±0.50	7.07±0.45	0.00±0.00	0.00±0.00
5R	8.45±0.07	8.57±0.08	7.93±0.32	8.14±0.49	0.00±0.00	0.00±0.00
2R	8.09±0.39	8.23±0.24	6.81±0.76	7.10±0.73	0.00±0.00	0.00+0.00
3D1	8.14± 0.08	8.72±0.47	7.27±0.38	7.60±0.39	0.00±0.00	0.00±0.00
10D	7.83±0.45	8.38±0.18	7.28±0.75	7.43±0.74	0,00±0,00	0.00±0.00
4R2	6.00±0.00	6.00±0.00	6.07±0.12	7.00±0.53	0.00±0.00	0.00±0.00
2D	7.68±0.58	7.58±0.71	6.48±0.48	6.78±0.44	0.00±0.00	0.00±0.00
K+	24.97±0.72	26.23±0.40	23.23±0.40	24.35±0.30	22.63±3.90	22.66±3.88
K-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Notes: K+: Ciprofloxacin; K-: Sterile distilled water

Table 4. Identification of 5R and 3D1 sequences based on the NCBI database with BLASTn

Isolate code	Strain	Access code	Max value	Query cover	E- value	Percent identity
5R	*Micrococcus luteus* strain SGAir0127	CP025616.2	2067	99%	0.0	100%
3D1	*Bacillus cereus* strain PJA4.2	MT275698.1	2008	100%	0.0	99.55%

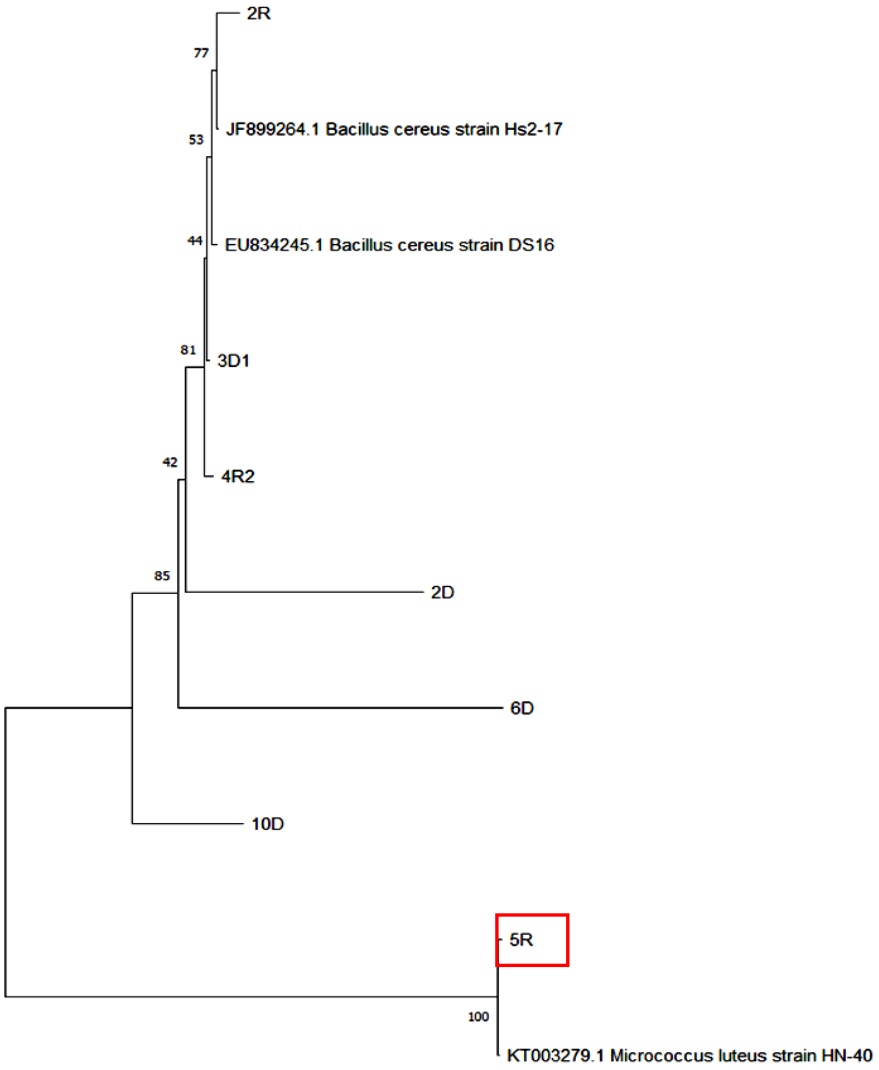

Figure 4. Phylogenetic tree of endophytic bacterial isolates from Balikpapan ginger (*Etlingera balikpapanensis*). Isolates with red boxes are those with the highest potential antibacterial activity

The antimicrobial tests performed in this study used the disc diffusion method with the test bacteria *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231. According to Rahman et al. (2010), the disc diffusion method is highly effective for testing natural antibiotics. The most potential endophytic isolate in inhibiting *E. coli* growth was isolate 5R, with an average inhibition diameter of 8.45±0.07 mm after 24 hours. The diameter of the inhibition zone was increased after 48 hours of incubation; isolate 3D1 had the largest average inhibition zone diameter (8.72±0.47 mm), followed by isolate 5R (8.57±0.08 mm). Thus, the two isolates were analyzed and identified to determine their species name as potential isolates producing antibacterial compounds. Isolate 5R had the largest inhibition zone diameter against *S. aureus* ATCC 25923 at 24 24-hour incubation period (7.93±0.32 mm), then increased to 8.14±0.49mm. The seven endophytic bacterial isolates from Balikpapan ginger (*E. balikpapanensis*) showed antagonistic activity against *S. aureus* and *E. coli* but no antifungal activity against *C. albicans*. This is in contrast with the findings of Gos et al. (2017), which revealed the potential of the endophytic *Micrococcus* sp. isolated from the medicinal plant *Vochysia divergens* Pohl to produce antimicrobial activity against *C. albicans*. Endophytic bacteria with an inhibition zone diameter of 9-14 mm against pathogens are declared effective in the production of antimicrobial compounds (Kumar et al. 2016). Another opinion states that endophytic bacteria from Zingiberaceae group plants, which can show the diameter of the inhibition zone against pathogens of 8-9 mm, have been declared as a promising source for antimicrobial development (Mamangkey et al. 2020).

Endophytic bacteria exhibit varying capabilities in producing secondary metabolites that can inhibit pathogen growth (Indrawati et al. 2018). Endophytic bacteria can inhibit pathogens by several mechanisms, including the production of antimicrobial compounds, hydrolytic enzymes, and volatile organic compounds; disruption of quorum sensing; and competition for nutrients and space (Ali et al.

2024). Endophytic bacteria isolated from the medicinal plant *Curcuma longa* L. (*Bacillus* sp. and *Clavibacter michiganensis*) did not exhibit antagonistic activity against the fungi *Byssochlamys fulva* and *Aureobasidium pullulans* but did demonstrate antibacterial activity against *Escherichia coli* (Kumar et al. 2016). Antibacterial activity has been reported for endophytes within the Zingiberaceae family, including *Stenotrophomonas maltophilia*, *Bacillus safensis*, *Bacillus pumilus*, and *Brevibacterium halotolerans*, which were successfully isolated from *Curcuma longa* (Deshmukh et al. 2018). Most endophytic bacteria occupy ecological niches similar to those of pathogens on their host plants. Consequently, endophytes employ various biocontrol mechanisms against pathogens, such as competing for habitats and substrates, producing antibacterial compounds, and inducing systemic resistance in host plants (Compant et al. 2005; Chaudhry et al. 2017). The leaf extract of *E. balikpapanensis* has been shown to contain alkaloids, phenolics, flavonoids, and steroids, suggesting that endophytic bacteria in *E. balikpapanensis* may also produce similar antimicrobial compounds. Therefore, further research is needed to investigate this hypothesis (Manurung et al. 2019). Ciprofloxacin has a mechanism to inhibit the replication of DNA by inhibiting DNA topoisomerase and DNA gyrase of pathogenic bacteria (Hangas et al. 2018). The mechanism of action of nystatin is to bind to steroids in the cell membranes of susceptible fungi, disrupting cell membrane permeability and causing cytoplasmic contents to leak out of the cell (Francisconi et al. 2020). The diameter of the inhibition zone produced by the endophytic bacteria *E. balikpapanensis* is much smaller than that of ciprofloxacin. This is because the antibiotics produced by the endophytic bacteria are not pure compounds but rather culture supernatants of bacterial isolates. However, antibacterial purification by extraction of endophytic bacterial cells holds great potential to achieve maximum results, making it a promising method for further research (Suryanto et al. 2016).

Based on the average diameter of the inhibition zone produced, isolates 5R and 3D1 have the greatest potential to inhibit the bacteria *S. aureus* and *E. coli*. After comparison with the NCBI database, it was determined that isolate 5R has a 100% identity and 99% query cover with *Micrococcus luteus* strain SGAir0127. This is consistent with the biochemical characteristics of *M. luteus*, which shows negative responses in catalase and oxidase tests and is immotile (Mohana et al. 2013). Isolate 3D1 has a 99.55% identity and 100% query cover with *Bacillus cereus* strain PJA4.2 (Table 4). The query coverage value indicates the percentage of 16S rRNA gene sequences of sample isolates that were successfully aligned to sequences in the NCBI database. The similarity of the two sequences is expressed as a percent identity (NCBI News 2006).

Bacillus sp. has been reported as endophytic bacteria in several plants, such as maize (Gond et al. 2015), cassava (Feng et al. 2023), *Dodonaea viscosa* L. (Afzal et al. 2016), and *Curcuma longa* (Kumar et al. 2016). *B. cereus* isolated from *Dodonaea viscosa* L. was able to produce ammonia, HCN, siderophore, and IAA (Afzal et al. 2016). *B. cereus* was also able to solubilize phosphate and exhibited enzyme activities of cellulase, protease, pectinase, and chitinase. *Micrococcus* sp. has been reported as an endophytic bacterium successfully isolated from *Vitis vinifera* L. (Asghari et al. 2019), *Catharanthus roseus* (Ranjan and Jadeja 2017), *Oryza sativa* L. (Raweekul et al. 2016), *Cajanus cajan* (L.) Huth and *Lablab purpureus* (L.) Sweet (Riskuwa-Shehu and Ismail 2018). *Micrococcus* sp. strains isolated from the *Plectranthus tenuiflorus* (Vatke) Agnew herbal plant were able to produce extracellular hydrolytic enzymes, including esterase, protease, xylanase, and cellulase. They showed antagonistic activity against the pathogenic bacterium *Proteus mirabilis* (El Deeb et al. 2013). Most of the literature on *Micrococcus* species focuses on pigments with potential antioxidant, antibiotic, antifungal, and anticancer properties (Tizabi and Hill 2023). Crude exopolysaccharides derived from *M. luteus* exhibited varying degrees of activity against *Escherichia coli*, *Klebsiella* sp., *S. typhi*, *Staphylococcus* sp., and *Pseudomonas* sp. (Nisha et al. 2019).

In conclusion, seven isolates were successfully isolated from roots, stems, and leaves of endemic Balikpapan ginger (*E. balikpapanensis*), namely isolates 6D, 5R, 2R, 3D1, 10D, 4R2, and 2D. These isolates showed antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 but were negative against *Candida albicans* ATCC 10231. The two most potent isolates were identified as *Bacillus cereus* strain PJA4.2 (3D1) and *Micrococcus luteus* strain SGAir0127 (5R) and prospective for development as an antibacterial agent.

ACKNOWLEDGEMENTS

The authors would like to thank Universitas Airlangga, Surabaya, Indonesia for funding this research through the Research Group Scheme Universitas Airlangga, Surabaya, 2020 (contract number 347/UN3.14/PT/2020).

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