

Diversity and antibacterial activity of endophytic fungi isolated from the medicinal plant of *Syzygium jambos*

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Abstract. 'Aini K, Elfita, Widjajanti H, Setiawan A. 2022. Diversity and antibacterial activity of endophytic fungi isolated from the medicinal plant of *Syzygium jambos*. *Biodiversitas* 23: 2981-2989. Currently, antimicrobial resistance is one of the most important threats to global public health. This is due to the various mechanisms of antimicrobial resistance. Thus, the need for new sources of bioactive compounds outside conventional antibiotics is a top priority. This investigation evaluated the diversity and antibacterial activities of endophytic fungi isolated from the leaves and root bark of jambu mawar (*Syzygium jambos* (L.) Alston). The endophytic fungi were grown on PDA media, and their antibacterial activities were tested using the Kirby-Bauer method on two Gram-positive and two Gram-negative bacteria. A total of 10 (SJD1-SJD10) and 11 isolates (SJA1-SJA11) of endophytic fungi were identified from the leaves and root bark of *S. jambos*, respectively. Among them, SJA8 isolate exhibited strong antibacterial activity. Based on morphological characterization and phylogenetic tree analysis, SJA8 was identified as *Pleiocarpon livistonae*. Isolation and identification of pure compounds from the fungi may reveal potential candidates for new antibiotic substances.

Keywords: Antibacterial activity, diversity, endophytic fungi, *Pleiocarpon livistonae*, *Syzygium jambos*

Abbreviations: PDA: Potato Dextrose Agar; PDB: Potato Dextrose Broth; PCR: polymerase Chain Reaction; DNA: Deoxyribonucleic Acid

INTRODUCTION

During the last two decades, endophytic fungi have been highlighted as the most important source of bioactive compounds, with novel and highly bioactive structures that offer a perfect backbone for developing potential new antibiotic drugs (Mbekou et al. 2021). Endophytes are those microorganisms that live in various tissues or spaces between plant cells, which generally do not indicate infection in these plants. Endophytes generally include fungi, bacteria, and actinomycetes, commonly found in nature. Among them, endophytic fungi are most often isolated. Endophytic fungi have been isolated from mosses, fern plants, pteridophyta, herbs, and other woody plants that live in the tropics to the poles. These fungi have unique physiological and metabolic mechanisms and encode various bioactive materials that enable them to adjust to specialized environment within the plant (Khan et al. 2021).

Endophytic fungi evolve together with the host plant over a long time to produce secondary metabolites that are similar to those of the host plant and are useful as medicinal drugs. Some endophytic fungi can aid the host medicinal plants inefficiently synthesizing active compounds. This contrivance is a breakthrough in providing a new method for producing bioactive

compounds that have same drug-like effects and are directly isolated from medicinal plant tissues. This can be a solution to the scarcity of natural plant resources and the ecological damage caused by the late growth of some natural plants and their artificial exploitation in large numbers (Zheng et al. 2021). Many endophytic fungi produce secondary metabolites, such as terpenoids, polyketides, derived compounds of shikimic acid, and terpenes. Endophytic fungi play an important role in the pharmaceutical and drug industries, such as production of alcohol, antibiotics, enzymes, and other medicinal ingredients (Yan et al. 2018; Rana et al. 2019; Devi et al. 2020; Slama et al. 2021). The diversity of endophytic fungi has been described in typical Egyptian medicinal plants, *Pelargonium graveolens* (Yasser et al. 2020), *Litsea cubeba* Pers. in medicinal plants common in Northeast India (Deka and Jha 2018), and in the Western Ghats' ethnomedicinal plants *Tabernaemontana heyneana* which are used as antioxidants (Bhavana et al. 2020). Apart from being an alternative source of medicinal ingredients, endophytic fungi can also help in the decomposition process of dead plants (Al-Nasrawi and Hughes 2012). The diversity and bioactivity of endophytic fungi have also been extensively researched and explored to find new sources of metabolites as alternative medicinal ingredients. Endophytic fungi are found in typical plants that grow in

the Sumatra, such a *Syzygium cumini* (Aceh, jambang) (Nurhaida et al. 2019), from mangroves *Sonneratia griffithii* Kurz (West Sumatra) (Handayani et al. 2017), from medicinal plants that grow in Toba and Samosir (North Sumatra), such as *Taxus sumatrana*, *Styrax sumatrana*, *S. benzoin* and others (Ilyas et al. 2019), from *Coleus amboinicus* Lour. (Astuti et al. 2021) and from brotowali (*Tinaspora crispa*) (Elfita et al. 2014). Some are isolated from the plants of Java region, such as pacar cina (*Aglaia odorata* Lour) (Sugijanto and Dorra 2016), and *Alyxia reinwardtii*, which is used as jamu ingredient in Indonesia (Hartanti et al. 2016), which belongs to the family *Zingiberaceae* (Central Java) (Praptiwi et al. 2016; Lutfia et al. 2020), purwoceng (*Pimpinella alpina* Molke), a medicinal plant that grow in the mountains of West Java, Central Java, and East Java (Aminin et al. 2020).

Several techniques have been described to isolate and identify endophytic fungi from the medicinal plants. *Syzygium jambos* (L.) Alston, known as jambu mawar, belongs to the genus *Syzygium*, a medicinal plant widely used to treat various diseases by the people of South Sumatra, Indonesia, and different parts of the world. *S. jambos* contains some chemical compounds, such as flavonols, polyphenols, and phenolic acids, which shows antifungal, antiviral, antibacterial, antiprotozoal, analgesic, antidiarrheal, antidiabetic, antimalarial, anti-inflammatory, anticancer, antioxidant, anti-allergic, anti-inflammatory, anticancer, and antihypertensive activities (Cock and Cheesma 2018; Mikłasińska-Majdanik et al. 2018). Other *Syzygium* genera, such as stem bark *Syzygium samarangense* are also known to have antioxidant activity (Metasari et al. 2020). Previous studies have been conducted on the diversity and bioactivity of endophytic fungi isolated from the bark of *S. jambos* (Roux et al. 2020; Aini et al. 2022). This study was designed to determine the diversity and antibacterial activity of endophytic fungi isolated from the leaves and root bark of *S. jambos*.

MATERIALS AND METHODS

Sampling

Samples of leaves and root bark of *Syzygium jambos* (L.) Alston were obtained in March 2021 from the different multipurpose fields of Jalan Sakura 3 Kompleks Kencana Damai, Kelurahan Sukamaju, Kecamatan Sako, and Palembang, Indonesia. Identification of the samples were performed at Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Palembang, Indonesia with number: 233/UN9.1.7/4/EP/2021.

Isolation of endophytic fungi

Endophytic fungi were isolated from leaves and root bark samples of *S. jambos*, according to the modified method of Caicedo et al. (2019). Fresh samples of leaves and root bark were washed with running water for 5-10 min. Then, they were immersed in 70% ethanol for 1-2 min, followed by a 3% NaClO solution for 5 min. The samples were rinsed thrice with sterile aquadest. Small

plant pieces (1 x 1 cm²) were placed on potato dextrose agar (PDA, Merck®) media, supplemented with chloramphenicol (0.2 mL/100 mL) and then incubated at 28 ± 2°C until fungi appeared. Fungal colonies grown on PDA medium were subsequently purified. Purification was performed by transferring the colonies to fresh PDA medium by the single spore isolation method.

Identification of endophytic fungi

Pure isolates of endophytic fungi were identified macroscopically and microscopically. Morphological characterization was determined by the growth patterns, colony color, texture, and other characteristics. The slide culture method was used to observe the microscopic view of the fungus. A small culture of endophytic fungi was placed on the slide and then a drop of lactophenol blue reagent was added prior to observation by microscope (Hirox MXB-2500REZ). The characteristic data were compared to the fungal identification books (Watanabe 2010; Walsh et al. 2018) and other relevant articles. If the morphology of the isolated endophytic fungi was not to match one of known identities, the specimen was marked as 'unknown' and assigned a number (Idris et al. 2013).

Cultivation and extraction

Cultivation and extraction methods were performed with modifications as described by Nagarajan (2019). All endophytic fungal isolates were cultured in 5 × 300 mL PDB medium by placing one block each of pure culture agar of approximately 6 mm in diameter into six different Erlenmeyer flasks. The cultures were incubated for 28 days under static conditions at room temperature. The mycelia were separated, and the liquid culture was portioned with ethyl acetate at a ratio of 1:1. The ethyl acetate extract was separated from the liquid culture and evaporated using a rotary evaporator until a thick extract was not obtained (Nagarajan 2019).

Antibacterial activity assay

Antibacterial analysis was performed using the disc diffusion method. Each fungal extract sample was dissolved in dimethyl sulfoxide (DMSO, Merck, Germany) at a concentration of 10%. Antibacterial activity test was done against two Gram-positive bacteria - *Staphylococcus aureus* (InaCCB4) and *Bacillus subtilis* (InaCCB4) and two Gram-negative bacteria - *Escherichia coli* (InaCCB5) and *Salmonella typhi* (ATCC 1408). The concentration of extract tested was 400 µg/disc, and 30 µg/disc of tetracycline antibiotic served as a positive control. The inhibition zone was measured by measuring the apparent area formed around the paper disc after being incubated at 37 °C for 24 h. The determination of antibacterial activity of the test sample and the criteria for the diameter of clear zone was calculated using the following equation, (A = clear zone of sample (mm); B = clear zone of antibiotics (mm)) (Elfita et al. 2019).

$$\text{Weak} = \frac{A}{B} \times 100\% < 50\%; \text{Moderate} = 50\% < \frac{A}{B} \times 100\% < 70\%; \text{Strong} = \frac{A}{B} \times 100\% > 70\%$$

Molecular identification

Endophytic fungi with the strongest antibacterial activity were identified using molecular assays to determine the species. Molecular identification was performed at the Genetics Laboratory, LIPI, Bogor, Indonesia. Fungal DNA extraction was performed using the Quick-DNA Fungal Miniprep Kit by Zymo Research, D6005. PCR amplification was conducted using 2x MyTaq HS Red Mix by Biorline and PCR primers using ITS 1 and ITS 4 (Singha et al. 2016). The DNA structure was analyzed using Molecular Evolution Genetics Analysis Version 11 (Tamura et al. 2021).

RESULTS AND DISCUSSION

Isolation of endophytic fungi

A total of 21 endophytic fungal isolates were obtained from the leaves and root bark of *S. jambos*, with 10 isolates from the leaves (SJD1 - SJD10) and 11 isolates found in the root bark (SJA1 - SJA11). The isolates were identified macroscopically and microscopically. Morphological traits, such as colony color, texture, and other characteristics are presented in Figure 1.A and Table 1. Microscopic observation included the hyphae, shape, type of spores, and other characteristics (Figure 1.B and Table 2). The isolated endophytic fungi were identified as the members of the genus *Aspergillus*, *Talaromyces*, *Cladophialophora*, *Phialophora*, *Arthrographis*, *Acremonium*, *Penicillium*, *Emmonsia*, *Microsporium*, *Madurella*, *Mucor*, *Botrytis*, *Graphium*, *Pleioacarpon*, *Malbranchea*; and two 'unknown' species.

Antibacterial activity assays

Endophytic fungi isolated from the leaves and root bark of *S. jambos* exhibited antibacterial activity against *S. aureus*, *S. typhi*, *E. coli*, and *B. subtilis* (Figure 2). Antibacterial activity was determined based on the measured zones of inhibition against the tested bacteria, based on the strong, moderate, and weak criteria.

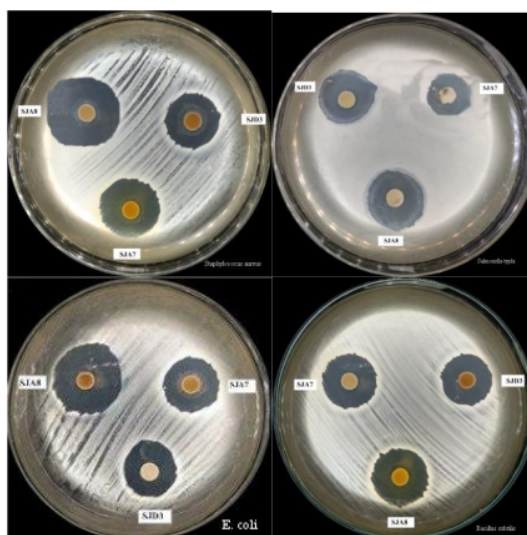


Figure 2. Inhibition zone of *Acremonium* sp. (isolate code SJD3), *Graphium* sp. (isolate code SJA7), *Pleioacarpon livistonae* (isolate code SJA8), against *S. aureus* (A), *S. typhi* (B), *E. coli* (C), *B. subtilis* (D)

Table 1. Macroscopic characterization of endophytic fungi isolated from leaves¹⁾ and root bark²⁾ of *S. jambos*

Isolates	Surface color	Reverse color	Texture	Topography	Pattern
SJD1 ¹⁾	Black	Black	Cottony	Raised	Radiated
SJD2 ¹⁾	Dark grey	Nonpigmented	Velvety	Raised	Zonate
SJD3 ¹⁾	Light grey	Light grey	Powdery	Raised	Zonate
SJD4 ¹⁾	White	White	Powdery	Umbonate	Zonate
SJD5 ¹⁾	White cycle	Cream	Cottony	Raised	Zonate
SJD6 ¹⁾	Pale brown	Brownish	Velvety	Umbonate	Radiated
SJD7 ¹⁾	Yellow around white	Yellowish	Velvety	Umbonate	Zonate
SJD8 ¹⁾	Grey around white	Dark	Cottony	Raised	Radiate
SJD9 ¹⁾	Black	Dark	Cottony	Raised	Zonate
SJD10 ¹⁾	White	White	Granular/powdery	Raised	Flowery
SJA1 ²⁾	Black	Black	Powdery	Umbonate	Radiate
SJA2 ²⁾	White around black	White	Powdery	Umbonate	Flowery
SJA3 ²⁾	Tan with white edge	Brownish	Powdery	Umbonate	Flowery
SJA4 ²⁾	Dark brown	Dark brown	Velvety	Umbonate	Radiate
SJA5 ²⁾	Brownish grey	Dark	Velvety	Raised	Flowery
SJA6 ²⁾	Darkgrey	Dark	Woolly	Umbonate	Flowery
SJA7 ²⁾	Gray	Dark	Cottony	Umbonate	Flowery
SJA8 ²⁾	Yellowish	Pale yellow	Cottony	Raise	Radiate
SJA9 ²⁾	Black	Cream	Granular	Umbonate	Radiate
SJA10 ²⁾	White	Light	Wolly	Raised	Radiate
SJA11 ²⁾	Dark mouse grey	Dark mouse grey	Wrinkled	Umbonate	Radiate

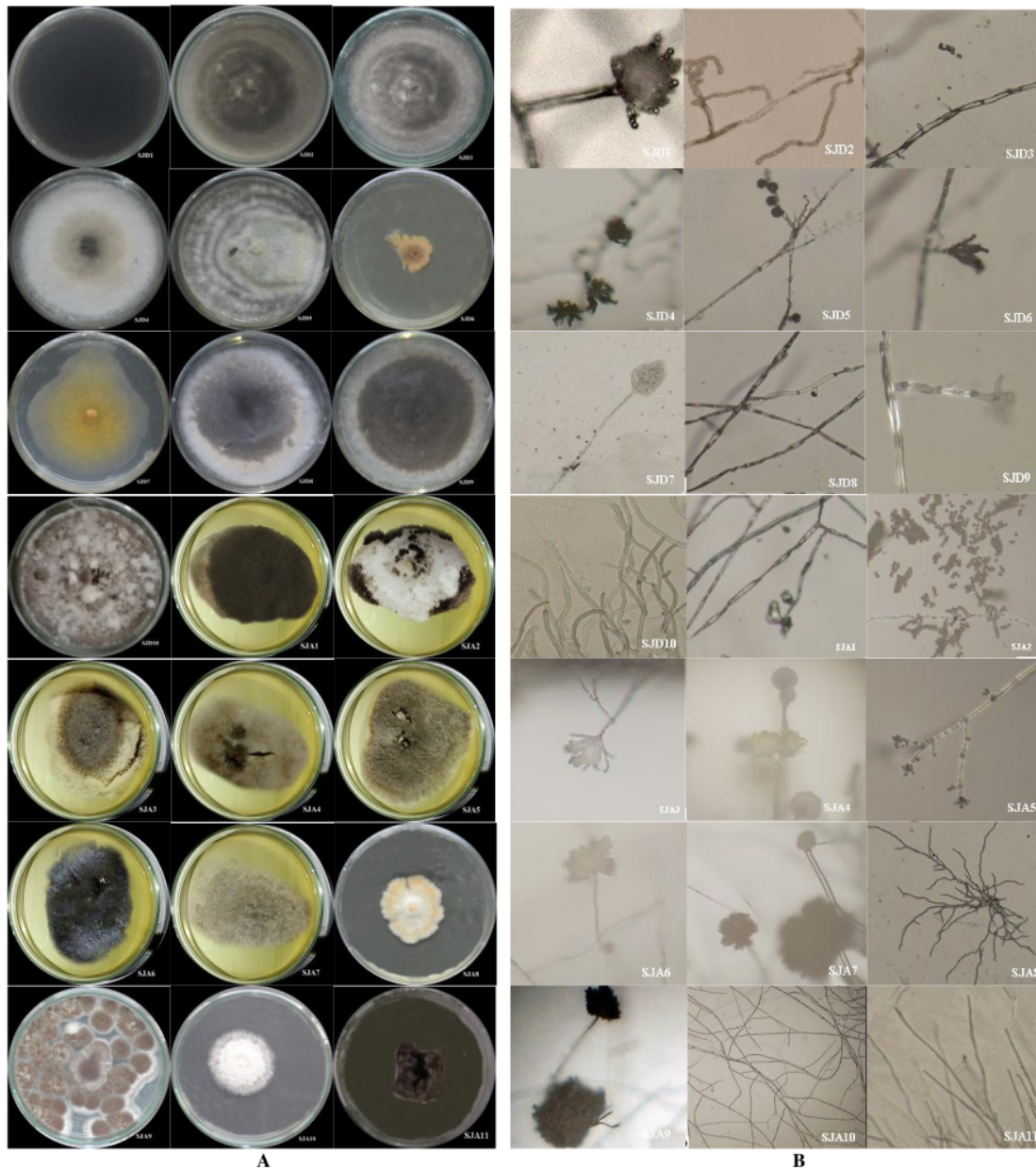


Figure 1. Endophytic fungal colonies (A) macroscopic view (B) microscopic view of isolates SJD1- SJD10 from leaves and SJA1- SJA11 isolates from root bark of *S. jambos*

Four endophytic fungal isolates, namely SJA8, SJA7, SJD2 and SJD3 showed strong antibacterial activity against the four bacteria (Table 3). The percentage of inhibition zone of each fungus on *S. aureus* were 76.6, 74.7, 72.8, and 70.9%, respectively. For *S. typhi*, the zones were 72.5, 70.1, 72.5, and 71.1%, respectively. The inhibition zone against *E. coli* were 76.9, 74.6, 71, and 74.6%,

respectively. The zone of inhibition against *B. subtilis* were 76.2, 73.8, 73.8, and 74.3%, respectively. Of the four endophytic fungal isolates, SJA8 showed the highest level of antibacterial activity. Thus, only SJA8 was selected for identification at the molecular stage, as it showed strong antibacterial activity and yielded the highest extract quantity.

Table 2. Microscopic characterization of endophytic fungi isolated from leaves¹⁾ and root bark²⁾ of *S. jambos*

Isolates	Spore type	Spore forms	Hyphae	Specific characteristic	Genus / species
SJD1 ¹⁾	Conidia	Globose	Septate	Uniseriate, contiguous (compact) phialides	<i>Aspergillus</i> sp.
SJD2 ¹⁾	Conidiospore branched	Globose	Septate	Conidiophores form a chain of arthroconidia	<i>Arthrographis</i> sp
SJD3 ¹⁾	Conidia	Globose	Septate	Hyaline, simple conidiophores (phialides)	<i>Acremonium</i> sp.
SJD4 ¹⁾	Conidia	Ellipsoidal	Septate	Branched conidiospores, hyaline, erect	<i>Penicillium</i> sp
SJD5 ¹⁾	Conidia	Globose	Septate	The apex of conidiophores swelled, and produced three conidia. Conidia formed directly with short stalks along the hyphae.	<i>Emmonsia</i> sp.
SJD6 ¹⁾	Conidia	Globose	Septate	Conidia form hyphal elements, and fragmented in septa, resulting single arthroconidia	<i>Talaromyces</i> sp.
SJD7 ¹⁾	Conidia	Globose	Septate	Egg shaped with truncate base, single macroconidia with rough walls	<i>Microsporium</i> sp.
SJD8 ¹⁾	-	-	Septate	Hyphae, branched cylindrical, thinner and dark in color.	<i>Madurella</i> sp.
SJD9 ¹⁾	Conidia	Globose	Septate	Branched, conidiospore lateral	<i>Cladophialophora</i> sp.
SJD10 ¹⁾	-	-	-	Like sterile mycelia	Unknown
SJA1 ²⁾	Conidia	Oval	Septate	Conidia round to oblong, piled on top of phialides. Phialides vase-shaped, spreading like cups.	<i>Phialophora</i> sp.
SJA2 ²⁾	Conidia	Globose	Aseptate	Sporangium dissolve in the wall, spores spread around or slightly oval, no rhizoid are formed.	<i>Mucor</i> sp.
SJA3 ²⁾	Conidia	Globose	Septate	Conidia formed hyphal elements, and fragmented in septa.	<i>Talaromyces</i> sp.
SJA4 ²⁾	Conidia	Globose	Septate	Conidiophores erect, head bearing the conidia.	<i>Aspergillus</i> sp.
SJA5 ²⁾	Conidia	Globose	Septate	Phialides have a distinct septal base, conidia nearly spherical in shape. Phialides simple, short, not wide along the hyphae.	<i>Phialophora</i> sp.
SJA6 ²⁾	Conidia	Globose	Septate	Hyphae broad, conidiophores dark, septate, long branching at the apex. The branches have swollen ends and round to oval in shape	<i>Botrytis</i> sp.
SJA7 ²⁾	Conidia	Globose	Septate	Hyphae septate, conidiophores simple, dark, fused to form synnemata	<i>Graphium</i> sp.
SJA8 ²⁾	Conidia	Globose	Septate	Conidiophores simple, solitary, branched, forming conidia in false chains	<i>Pleiocarpon</i> sp.
SJA9 ²⁾	Conidia	Globose	Septate	Conidiophores long, simple, erect, thick-walled, with leg cells at the base	<i>Aspergillus niger</i>
SJA10 ²⁾	No spores	No spores	Septate	No conidiophores formed. Arthroconidia vary in length but having the same width as hyphae and quite narrow	<i>Malbranchea</i> spp.
SJA11 ²⁾	-	-	Septate	Like sterile mycelia	Unknown

Note: (-) = characteristic doesn't appear.

Table 3. Comparison of the percentage of antibacterial activity of endophytic fungal isolates

Samples	Genus/species	Ethyl acetate extract (g)	Antibacterial activity (%)			
			<i>S. typhi</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>
Tetracycline	-	-	100.0	100.0	100.0	100.0
SJD1 ¹⁾	<i>Aspergillus</i> sp.	3.3	63.2**	61.9**	56.7**	68.0**
SJD2 ¹⁾	<i>Arthrographis</i> sp	3.5	72.5***	71.0***	73.8***	72.8***
SJD3 ¹⁾	<i>Acremonium</i> sp.	4.0	71.1***	74.6***	74.3***	70.9***
SJD4 ¹⁾	<i>Penicillium</i> sp	4.2	56.8**	54.2**	55.2**	68.3**
SJD5 ¹⁾	<i>Emmonsia</i> sp.	3.7	45.2*	36.9*	40.5*	36.3*
SJD6 ¹⁾	<i>Talaromyces</i> sp.	4.0	49.4*	44.6*	47.9*	45.6*
SJD7 ¹⁾	<i>Microsporium</i> sp.	4.7	59.1**	55.1**	61.6**	69.4**
SJD8 ¹⁾	<i>Madurella</i> sp.	3.0	49.8*	50.5**	58.9**	56.6**
SJD9 ¹⁾	<i>Cladophialophora</i> sp.	4.0	52.6**	42.8*	65.5**	43.3*
SJD10 ¹⁾	Unknown	3.8	53.5**	46.9*	55.4**	41.4*
SJA1 ²⁾	<i>Phialophora</i> sp.	4.4	70.6***	64.6**	67.1**	71.8***
SJA2 ²⁾	<i>Mucor</i> sp.	4.5	67.8**	65.1**	54.4**	71.3***
SJA3 ²⁾	<i>Talaromyces</i> sp.	4.5	56.8**	61.9**	71.3***	55.6**
SJA4 ²⁾	<i>Aspergillus</i> sp.	4.0	47.5*	62.4**	49.8*	41.4*
SJA5 ²⁾	<i>Phialophora</i> sp.	4.3	70.1***	68.7**	71.8***	77.5***
SJA6 ²⁾	<i>Botrytis</i> sp.	3.8	44.8*	54.2**	55.2**	36.5*
SJA7 ²⁾	<i>Graphium</i> sp.	4.0	70.1***	74.6***	73.8***	74.7***
SJA8 ²⁾	<i>Pleiocarpon</i> sp.	6.8	72.5***	76.9***	76.2***	76.6***
SJA9 ²⁾	<i>Aspergillus niger</i>	4.1	40.1*	41.3*	57.6**	63.2**
SJA10 ²⁾	<i>Malbranchea</i> spp.	3.5	56.3**	63.3**	51.8**	69.9**
SJA11 ²⁾	Unknown	3.5	60.9**	56.9**	59.1**	64.7**

Note: (***) strong; (**) moderate; (*) weak; ¹⁾ leaves, ²⁾ root bark

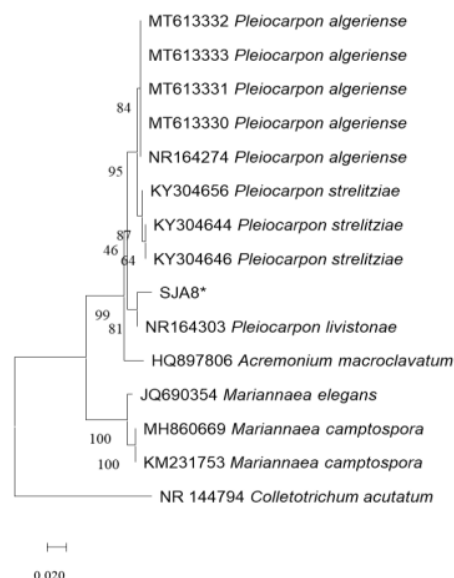


Figure 3. Phylogenetics tree of SJA8

The endophytic fungal isolate SJA1 exhibited strong activity against *S. aureus* (71.8%) and *S. typhi* (70.6%), and moderate activity against *E. coli* (64.6%) and *B. subtilis* (67.1%). Isolate SJA5 showed strong activity against three bacteria, but moderate activity against *E. coli* (68.7%). Endophytic fungal isolate SJA2 had strong activity against *S. aureus* (71.3%) and moderate activity against the other three bacteria. The endophytic fungi SJA3 had strong activity toward *B. subtilis* (71.3%) and medium activity toward the other three bacteria. Endophytic fungal isolates, namely SJD1, SJD4, SJD7, SJA10, and SJA11 showed moderate activity against the four test bacteria. SJD5 and SJD6 isolates had a weak activity against all the four bacteria.

Molecular identification

The results of antibacterial assay revealed that fungal isolate SJA8 showed strongest antibacterial activity and was selected for molecular identification. Molecular identification by PCR amplification showed a sequence assembly of 566b. Based on the results of molecular tests SJA8 was identified as *Pleiocarpon livistonae* (Figure 3).

Discussion

Recently, endophytic fungi have been widely probed to elucidate their diversity and identify new resources for secondary metabolite production (Barrios-González 2018; Avalos and Limón 2021; Zheng et al. 2021). Secondary metabolites generated by endophytes linked with medicinal plants can be used to treat various diseases. Thus, endophytic fungi are potent sources of new drugs. Secondary metabolites are produced by fungi as an adaptation to environmental stress (Rajamanikyan and Vadlapudi 2017). These metabolites come from a group of

compounds formed through various biosynthetic pathways, so they have diverse chemical structures (Meena et al. 2019). Several secondary metabolites produced by endophytic fungi, such as terpenoids, ketones, alkaloids, phenylpropanoids, lactones, anthraquinones, and steroids, have antibacterial activity (Zheng et al. 2021).

The endophytic fungus SJD2 (*Arthrographis* sp.) exhibited antibacterial activity against the four tested bacteria. Xi et al. (2004) reported that *Arthrographis kalrae* cause panophthalmitis and invasive sinusitis infections in humans (Xi et al. 2004) and lung infections in patients with AIDS (Samal et al. 2021). However, other species of *Arthrographis* *curvata* produce lipases that has physicochemical properties that can be utilized for industrial or bioremediation purposes (Aamri et al. 2020). The genus *Arthrographis* is still related to *Malbranchea* (SJA10) due to the presence of arthroconidia, resembling *Oidiodendron*, but the conidiophores of *Arthrographis* do not have characteristic pigmentation (Sainaghi et al. 2015; Hernandez-Restrepo et al. 2020). Three analogs of ardeemin and sartoryglabrin, namely albolutein A-C, isolated from *Malbranchea albolutea*, function as protein tyrosine phosphate inhibitors (Díaz-Rojas et al. 2021). Compounds of gymnoascolide A isolated from *Malbranchea dendritica* have activity in inhibiting yeast-glucosidase (Rebollar-Ramos et al. 2021).

The endophytic fungus SJD3 (*Acremonium* sp.) exhibited antibacterial activity against the all four bacteria. Previous studies indicate that compounds isolated from *Acremonium* have antibacterial activity (Yu-heng et al. 2019). Acremonidin A-C compounds isolated from *Acremonium camptosporum* showed antibacterial activity (Martins et al. 2021). The gancidin W compound found in the genus *Acremonium* has antibacterial (Khan et al. 2021). Endophytic fungi SJA1 and SJA5 belong to the genus *Phialophora* and have strong antibacterial activity against *S. aureus* and *S. typhi*, endophytic fungi SJA5 also showed strong antibacterial activity against *B. subtilis* but not against *E. coli*. *Phialophora* isolated from mangrove leaves have antibacterial activity against *S. aureus* and *Micrococcus luteus* (Ramirez et al. 2020). *Phialophora mustae* species contain bioactive metabolite Phialomustin A-D, which has antibacterial activity (Nalli et al. 2015). However, *Phialophora richardsiae* and *Phialophora verrucosa* species are pathogenic, causing chromoblastomycosis, phaeohyphomycosis, and mycetoma (Son et al. 2010; Granato et al. 2021). The fungi SJD4 (*Penicillium*), SJD6, and SJA3 were identified as *Talaromyces*. *Talaromyces* is a teleomorph of *Penicillium* (Nicoletti and Trincone 2016). Several species of *Talaromyces* produce compounds in the form of derivatives of cyclopentenone, phenolic ethers, and itaconic acid, namely talarocyclopenta A-C, isolated from *Talaromyces assiuensis*, which has antibacterial and anti-inflammatory activity (Cai et al. 2020). Secondary metabolites, such as steroids, terpenoids, isocoumarins, polyketides, alkaloids, peptides, and quinones, have antifungal and antimicrobial activity (Zhai et al. 2016; Lan and Wu 2020). *Penicillium* is also known to produce compounds that are anti-migratory

and antifungal, namely terretrion C-D (Shaala and Youssef 2015).

Endophytic fungal isolates SJD1, SJA4, and SJA9 were identified as the members of genus *Aspergillus*. Species of *Aspergillus* are able to produce secondary metabolites, such as alkaloids that have antibacterial and antioxidant activity (Youssef et al. 2021). Metabolite compounds eicosane, eicosane 2-methyl, phenol, 2,6-bis(1,1-dimethyl ethyl)-4-methyl, hexadecane 2, 11-octadecenoic acid, and methyl ester isolated from *A. fumigatus* have antibacterial activity against *S. aureus* dan *E. coli* (Octarya et al. 2021). Compounds such as secalonic acid F, variecolin, and ergosterol isolated from *Aspergillus aculeatus* have antimalarial activity (Yodsing et al. 2018). Species of the genus *Mucor* (SJA2), namely *Mucor circinelloides*, are also known to produce phenolic compounds that have high antioxidant potential (Hameed et al. 2017). The endophytic fungus SJA7 was identified as *Graphium*. The genus *Graphium* is known to cause traumatic wound infection in immunocompetent patients (Wu et al. 2018). It also causes mycotic keratitis, which causes visual disturbances (Palanisamy et al. 2015). The endophytic fungus SJA6 (*Botrytis*) is also one of the pathogenic fungi that cause disease in grapes (Ammad et al. 2014). However, *Botrytis allii* can produce polyketides ramulosi compounds that have antimicrobial activity (Collado and Viaud 2016).

Antibacterial mechanisms can occur in four ways: synthesis of cell wall inhibitors, inhibitors of membrane function, synthesis of protein inhibitors, and inhibitors of nucleic acid. Bacterial cell walls are composed of a polysaccharide called peptidoglycan, which consists of N-acetylglucosamine and N-acetylmuramic acid. β -lactam drugs such as penicillin, are antibiotics that prevent the synthesis of the bacterial cell wall. The mechanism of inhibition of cell wall synthesis begins with the recruiting of penicillin-binding proteins that bind to cell receptors. Bacterial cells recognize β -lactams as incorrect D-alanine-D-alanyl transpeptidase molecules, resulting in the inhibition of the transpeptidase response and peptidoglycan synthesis (Bugg et al. 2011). Gram-negative bacteria have lower sensitivity because they fail antibiotics, allowing the synthesis of the cell wall, which is blocked by the external membrane (Ullah and Ali 2017).

In this study, endophytic fungal isolate SJA8 showed the most potent antibacterial activity and further selected for molecular identification. DNA sequences obtained from SJA8 were analyzed using MEGA-11 with the neighbor-joining method. The neighbor-joining method reconstructs the phylogeny tree from evolutionary distance data (Saitou and Nei 1987). Bootstrap test was carried out to measure the consistency of the internal set of molecular data by analyzing the modified alignment supporting the same clade. To generate each repeat alignment, the algorithm samples the site from the original alignment column (Russo and Selvatti 2018). Evolutionary distances are calculated using the p-distance method and the number of units based on differences per site (Nei and Kumar 2000). There are 254 positions in the final dataset of evolutionary analysis using MEGA 11 (Tamura et al. 2021). The results of the evolutionary analysis showed that SJA8 was in the same

clade of *Pleiocarpon livistonae* species. Based on the results of molecular tests SJA8 was identified as *Pleiocarpon livistonae*. The genus *Pleiocarpon* is known to be pathogenic by causing rot in plant parts, such as roots and stems (Aiello et al. 2017, 2020).

In the present study 21 isolates of endophytic fungi were isolated from leaves and root bark of *S. jambos*, 10 of which were isolated from leaves, and 11 fungi from the root bark. Morphological and microscopic results identified 15 different genera and two unknown species. The result showed that three species belonged to the genus *Aspergillus*, two species of the genus *Phialophora*, and *Talaromyces*, respectively. The results of antibacterial activity test showed that all isolates had antibacterial activity. The endophytic fungus *Pleiocarpon livistonae* showed greater potential as a candidate source for new antibacterial compounds because it had strong antibacterial activity. Further research is needed to isolate purified compounds from *Pl. livistonae* and to identify bioactive substances that may substitute for current resistant-inducing antibiotics.

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