BIODIVERSITAS
Volume 22, Number 9, September 2021
Pages: 3943-3948

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220942

Diversity of endophytic bacteria and microfungi in *Syzygium cumini* fruit from West Java, Indonesia

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Manuscript received: 21 April 2021. Revision accepted: 27 August 2021.

Abstract. *Rossiana N, Fathurrohim MF, Indrawati I. 2021. Diversity of endophytic bacteria and microfungi in* Syzygium cumini *fruit from West Java, Indonesia. Biodiversitas 22: 3943-3948. Syzygium cumini* L. Skells is a native evergreen tropical tree in Southeast Asia belong to the *Myrtaceae* family, known as the Java plum, *jambul, jambolan, jamblang*, or *jamun*. The bacterial and fungal endophytes associated with fruit have not been determined and functionally characterized. The endophytic microbes live inside the surface-sterilized fruits and have no visibly harmful effects on the plants. The purposes of the study were to isolate, characterize, and determine the diversity of endophytic bacteria and fungi in *S. cumini* fruit. The endophytes from *S. cumini* fruit were observed morphologically for identification. The result of isolation and identification showed there are four bacterial isolate endophytes (*B. cereus, B. subtilis, B. megaterium*, and *Bacillus* sp.) and four fungal endophytes (*Candida guilliermondii, Penicillium* sp., *Mycelia sterilia*, and *Aspergillus* sp.) isolated from *S. cumini* fruit.

Keywords: Bacterial endophyte, biodiversity, fungal endophyte, jamblang, *Sygizium cumini*

INTRODUCTION

With its thousands of islands, Indonesia has a myriad wealth of biological resources, especially tropical fruits. Approximately 329 types of fruits, both indigenous and introduced, could be found in Indonesia. Two hundred and sixty-six species of Indonesian indigenous fruits mostly grow wild in the forest and only a small portion has been cultivated (Hermanto et al. 2013).

Syzygium cumini L. Skeels (Java plum, *jambul, jambolan, jamblang*, or *jamun*) is one of the plants that have many benefits in Indonesia. However, Indonesian people do not know much about the benefits and nutritional value of *S. cumini* fruit. *S. cumini* fruit contains flavonoids, quinones, steroids, and polyphenols (Marliani et al. 2014). So, it might be potential as a medicinal plant.

The plant is the host for various types of endophytic microbes. Natural products produced by endophytic microbes were reported to exhibit a wide range of biological activities and are categorized into various categories of chemical compounds including alkaloids, terpenoids, steroids, lactones, phenolic compounds, quinones, and lignans (Anjum and Chandra 2015).

The internal non-sterile plant tissue is inhabited by various types of fungi and bacteria known as endophytes (Goryluk et al. 2009). Endophytic microbes in plant tissues do not cause disease symptoms in their hosts (Anjum and Chandra 2015). Endophytic microbes can obtain nutrients to complete their life cycle from the host plant, while the host gets protection against plant pathogens from compounds produced by endophytic microbes (Ariyanto et al. 2013).

Endophytic microbes can have a mutualistic symbiosis with their host plant. The search for endophytic microbes from different plant species in different ecosystems will be beneficial. The diversity of endophytic microbes is under-explored although they are a great resource for application in the fields of agriculture, medicine, pharmacy, farm, and industry (Zhang et al. 2016). The diversity of microorganisms i.e., the diversity of endophytic microbes in *S. cumini* fruit is very important, to be studied.

Endophytic bacteria generally enter the plant tissues through roots and plant parts exposed to direct air such as flowers, stems, and cotyledons (Desriani et al. 2014). Endophytic bacteria can be isolated through internal plant tissue in which its outer surface has been sterilized (Munif et al. 2012).

Endophytic fungi are a group of fungi that colonize living and internal tissues of plants without causing any immediate, overt negative effects (Padhi et al. 2013). Endophytic fungi have an important role in their dead hosts to initiate biological degradation to start the nutrient recycling process (Shekhawat et al. 2010).

Studies on the diversity of endophytic bacteria and fungi in *S. cumini* fruit in Indonesia have not been conducted. This study was conducted to determine the diversity of bacteria and endophytic fungi in *S. cumini* fruit.

MATERIALS AND METHODS

Plant material

Syzygium cumini fruit was collected from the plant grow in Padjadjaran University Campus, Sumedang, West Java, Indonesia.

Isolation of endophytic bacteria

Syzygium cumini fruits were washed under running water for 5 minutes and then dipped in 96% ethanol for 2 seconds, followed by rinsing with sterile distilled water for 30 seconds and then rinsed using 1% NaOCl solution for 5 minutes. As much as 100 grams of the fruits, added with 9 ml of sterile physiological NaCl, and then finely crushed. As much as 1 ml of finely crushed fruits was diluted to 10^7 dilutions. One ml of diluted crushed fruit was inoculated on nutrient jell for the last three dilutions (10^{-5}, $10^{-6,}$ and $10^{-}7$) and incubated for 24 hours at 37°C. After incubation, one ose of each species of different morphological bacterial colonies were subcultured onto a nutrient agar slope. Pure bacterial isolate was identified.

Identification of endophytic bacteria

Pure bacterial cultures were identified microscopically, i.e., colony color, elevation, surface, and bacterial colonies. Gram-staining carried out microscopic observation to determine bacterial cell shape and biochemical characterization was performed using VITEK 2.0 compact system.

Isolation of endophyte fungi

The fruit was washed under running water for 5 minutes, then rinsed with 70% alcohol for 5 minutes, followed by soaking in 1% NaOCl solution for 5 minutes and then drained. The fruit was then rinsed with sterile distilled water for one minute twice. Then the fruit was crushed and was diluted with series from 10^{-1} to 10^{-7} in medium Potato Dextrose Agar. The last three dilutions (1ml) were added into the petri dish, as much as 1 ml and 3 last dilutions were inserted into a petri dish containing the PDA medium and were then homogenized, followed by incubation at room temperature for 48-72 hours. Emerging fungi on Petri dishes were subcultured on PDA to obtain a pure culture.

Identification of endophyte fungi

The moist chamber performed fungal identification. Macroscopic observations were carried out including colony color, colony surface, concentric circle, and radial surface lines of the colony. Microscopic observations included the morphology of spore, sporangium, and conidia.

RESULTS AND DISCUSSION

Isolation of endophytic bacteria and fungi from *S. cumini* fruit resulted in 4 isolates of bacteria (FIN 2, FIN 3, FIN 4, and FIN 11) and 4 isolates of fungi. Based on Gram-staining, the four endophytic bacteria were Gram-positive bacilli. Macroscopic characters of endophytic bacteria FIN 2 were a white colony, round shape, flat colony elevation, flat edge, and smooth surface (Figure 1.A). Microscopically, endophytic bacteria FIN 2 is a purple Gram-positive bacteria bacilli (Figure 1.B). Endophytic bacteria FIN 3 has macroscopic characteristics of spherical shaped colonies, jagged edges, flat elevation, rough surface, and slightly greenish-white (Figure 2A), and microscopic characteristics as Gram-positive bacilli (Figure 2.B). Endophytic bacterial FIN 4 has macroscopic characteristics of spherical shaped colonies, flat edges, flat elevations, smooth, and white surface (Figure 3.A) with microscopic characteristics as Gram-positive bacilli (Figure 3.B). Endophytic bacteria FIN 11 has macroscopic characteristics of spherical-shaped colonies, jagged edges, raised elevations, rough surface with white and greenish margins in the middle (Figure 4.A), and microscopic characteristics as Gram-positive (Figure 4.B).

The results of the biochemical character of four endophytic bacteria from *S. cumini* fruit using VITEK Compact 2.0 were prested Table 1.

The results of the identification of endophytic fungi from *S. cumini* fruit (MFFJB, MFFJE, MFFJF, and MFFJG) were presented in Table 2.

Discussion

***Bacillus cereus* (FIN 3)**

Bacillus cereus is a Gram-positive, aerobic, facultatively anaerobic, spore-forming, and mesophilic bacterium, with growth temperatures from 10°C to 48°C with optimal growth between 28°C and 35°C. *B. cereus* measures 1 x 3 – 4 μm. Most *B. cereus* strains are motile via peritrichous flagella, grow on solid growth media as irregular colonies, use glucose as a source of carbon (but not mannitol, arabinose, or xylose), hydrolyze starch and gelatin, show hemolytic activity, are resistant to ampicillin, and display pronounced lecithinase activity (Vilas-Boas et al. 2007). The endophytic *B. cereus* isolated from *Garcinia xanthochymus* based nanoparticles has the potential to be developed as promising antibacterial and antioxidant agents (Mujaddidi et al. 2021).

***Bacillus megaterium* (FIN 2)**

Bacillus megaterium is a Gram-positive spore-forming bacteria. As most of the spore-formers, it is usually found in the soil, from which it can easily be transmitted to the foods we consume (Periago et al. 2006). From macroscopic and microscopic observations and biochemical tests, it is shown that *B. megaterium* is concave, smooth, and milk-white. The cell morphology shows that the cell is rod-shaped, Gram-positive, and sporous (Andriani et al. 2017). Some *B. megaterium* proteins are very important in the food industry and pharmacy. *B. megaterium* secretes a variety of enzymes, ranging from amylases used in the bakery industry to penicillin amidase used to manufacture new synthetic antibiotics (Mobitec 2008). *B. megaterium* can also produce several other enzymes, such as mutarotase, glucose dehydrogenase, β-galactosidase, and cellulose (Andriani et al. 2017).

***Bacillus subtilis* (FIN 4)**

Bacillus subtilis is a Gram-positive, aerobic, non-encapsulated, mobile, and spore-bearing bacterium, a basil chain with a size of 0.8-0.7 or 2-3 um, commonly found in nature. Bacterial colonies have a white or slightly yellowish, rough, and opaque surface. Grow in the mesophilic temperature range of 25-35°C. Its active form is

usually spore-shaped. Therefore, it can survive in difficult conditions (Saleh et al. 2014). *B. subtilis* is useful for biotechnology as well as industrial and agricultural applications. *B. subtilis* can directly fight pathogens by producing a secondary metabolite of extracellular lytic enzymes to inhibit growth by quorum quenching to disrupt the communication of cell-to-cell expression from infectious expression in pathogenic bacteria (Alina et al. 2015).

***Bacillus* sp. (FIN 11)**

The genus *Bacillus* is Gram-positive bacteria, a stem cell shape with a cell size of 0.3-2.2 to 1.2-7.0 µm (Alina et al. 2015). It can also move freely and has good competence and survivability on rhizosphere and facultative anaerobes so that it can adapt to living in the soil under various environmental conditions (Yanti et al. 2018). *Bacillus* sp. can produce digestive enzymes such as proteases and amylases that can help digestion and produce short-chain organic acids with antimicrobial properties (Sumardi et al. 2012). *Bacillus* can also be used as potential biofertilizers, biopesticides, and non-pathogenic to plants.

***Candida guiliermondii* (MFFJB)**

Candida guilliermondii is the most common opportunistic fungus, a normal flora present in human skin and mucosal surfaces, but occasionally the cause of chronic onychomycosis, acutely osteomyelitis, septic arthritis, endocarditis, fungemia, and invasive infections (Girmenia et al. 2006). The yeast cells of *C. guilliermondii* are both rounded and ellipsoidal, the length is 4.21 ± 0.3 µm and the diameter is 2.29 ± 0.1 µm (Hovnanyan et al. 2019). Cells of *C. guilliermondii* are heterogeneous, mostly elongated in shape (approximately 2, 9, 10 cm). In contrast to *Candida albicans, C. guilliermondii* is unable to produce true hyphae. Nevertheless, under certain conditions, such as nitrogen or carbon deficiency, these budding yeast can efficiently switch to pseudohyphal structure harboring blast conidia circlet (Koehler et al. 1999; Papon et al. 2013).

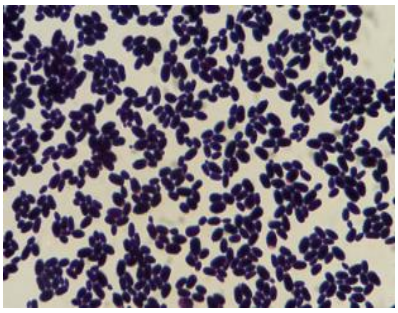
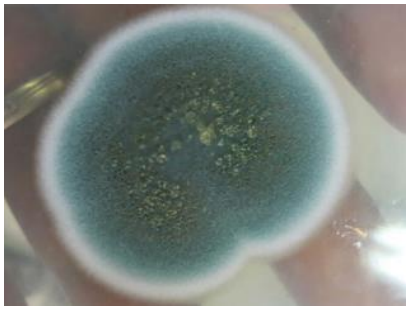
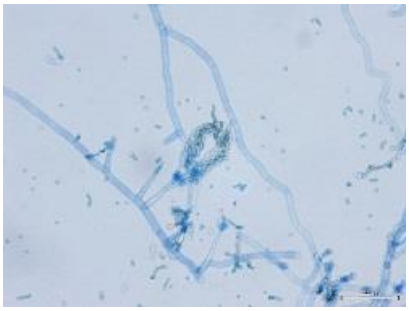
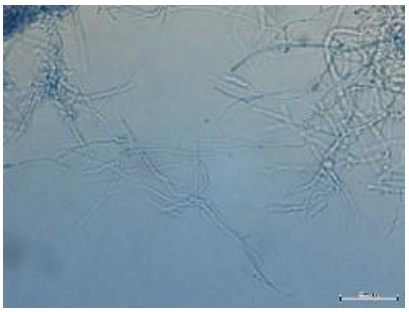
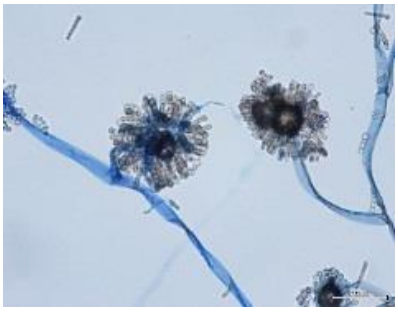
***Penicillium* sp. (MFFJE)**

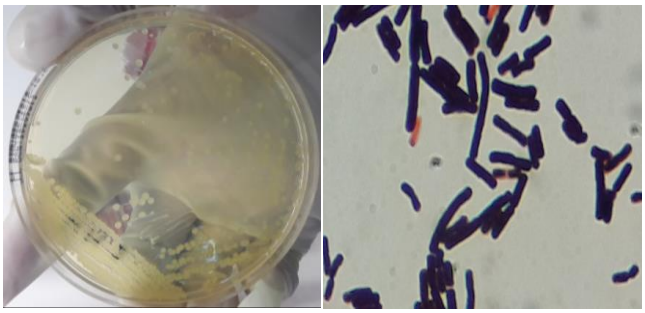
Penicillium is a genus of one the most fungi found in different environmental and suitable environmental (temperature, humidity, pH). According to Subowo (2015) *Penicillium* sp. can decompose cellulose and lignin compounds into simple carbon compounds required by microbes as an energy source (Carbon source). Penicillium species produce various secondary metabolites such as antibacterial (Petit et al. 2009). Many *Penicillium* produced the different chemical types of secondary metabolite, while some of them are important in the field of medicine. Others are used for the production of mycotoxins, important drugs, and some of the *Penicillium* species are used in industry, especially penicillin production (García-Estrada et al. 2011).

Table 1. Biochemical characters of endophytic bacteria isolated from *Sygizium cumini* fruit

Name of compound biochemical test	Endophytic bacterial isolate			
	FIN 2	FIN 3	FIN 4	FIN 11
Beta-xylosidase	-	-	-	-
L-lysine-arylamidase	-	-	-	-
L-aspartate- arylamidase	+	-	-	-
Leucine arylamidase	+	-	+	+
Phenylalanine arylamidase	+	+	+	+
L-proline arylamidase	-	+	-	-
Beta-galactosidase	+	-	+	+
L-pyrrolidonyl- arylamidase	-	-	+	-
Alpha-galactosidase	+	-	+	-
Alanine arylamidase	+	+	-	-
Tyrosine arylamidase	+		+	-
Beta-n-acetyl-glucosaminidase	-	+	-	-
Ala-phe-pro arylamidase	+	+	+	+
Cyclodextrin	-	-	+	+
D-galactose	+	-	-	-
Glycogen	+	-	-	-
Myo-inositol	-	-	-	-
Methyl-A-D-glucopyranoside acidification	-	-	+	-
Ellman	+	+	-	+
Methyl-D-xyloside	-	-	-	-
Alpha-mannosidase	-	-	-	-
Maltotriose	+	+	+	+
Glycine arylamidase	+	-	-	+
D-Mannitol	+	-	+	+
D-Mannose	-	-	+	+
D-Melezitose	-	-	-	
N-Acetyl-D-glucosamine	+	+	-	+
Palatinose	+	-	+	-
L-Rhamnose	-	-	-	-
Beta-glucosidase	+	+	+	+
Beta-mannosidase	-	-	-	-
Phosphoryl choline	-	+	-	-
Pyruvate	+	-	+	+
Alpha-glucosidase	+	+	-	+
D-tagatose	-	-	-	-
D-trehalose	+	+	+	+
Inulin	-	-	+	+
D-glucose	+	+	-	-
D-ribose	+	+	-	+
Putrescine assimilation	-	-	-	-
Growth in 6,5% NaCl	+	+	+	+
Kanamycin resistance	-	+	-	-
Oleandomycin resistance	-	+	-	+
Esculin hydrolyze	+	-	-	+
Tetrazolium RED	-	-	+	+
Polymixin_B resistance	-	-	-	+
Probabilitas	93 %	94%	87%	-
Result of identification	*B. megaterium*	*B. cereus*	*B. subtilis*	*Bacillus* sp.

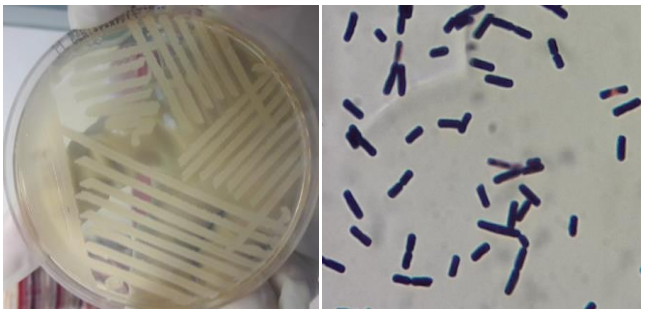
Table 2. Identification and characteristic of endophytic fungi from *Sygizium cumini* fruit using moist chamber

Isolate code	Characteristics of colonies	Characteristics of fungal cells	Identification
MFFJB			*Candida guilliermondii*
	Milk white colony, smooth rounded shape colony, corrugated, concave in the middle	Large coccus cell shape	
MFFJE			*Penicillium* sp.
	Green to grayish colonies, uneven colony edges, look like velvet	Broom-like structure	
MFFJF			*Mycelia sterilia*
	White colonies, furry and wrinkled in the middle, on the white colony-like edges of the powder	The fungal cell has the only mycelium	
MFFJG			*Aspergillus* sp.
	Colonies of green colonies, uneven colony edges, colonies shaped like cotton and growing upward.	Fungal cell resembles to fan	



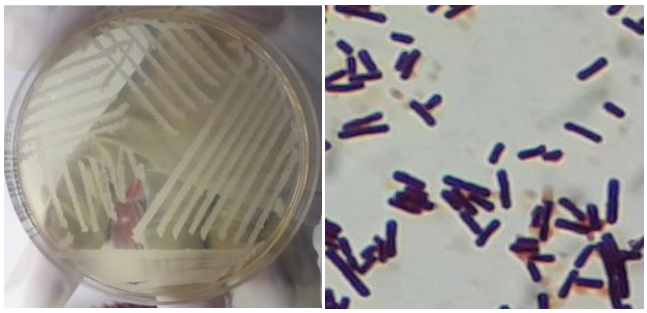
A B

Figure 1. The bacterial isolate of FIN 2 (*Bacillus cereus*) on TSA (A) and Gram Stained FIN 2 (B)



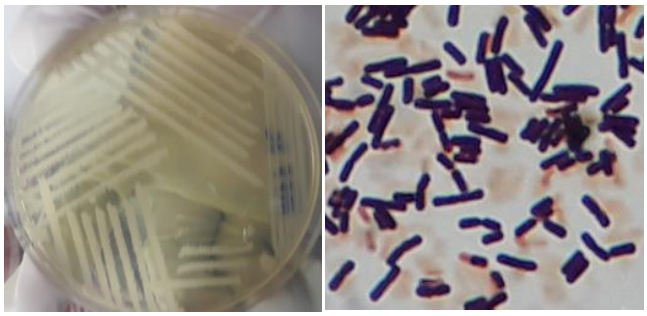
A B

Figure 2. The bacterial isolate of FIN 3 (*Bacillus megaterium*) on TSA (A) and Gram Stained FIN 3 (B)



A B

Figure 3. The bacterial isolate of FIN 4 (*Bacillus subtilis*) on TSA (A) and Gram Stained FIN 4 (B)



A B

Figure 4. The bacterial isolate of FIN 11 (*Bacillus* sp.) on TSA (A) and Gram Stained FIN 11 (B)

***Mycelia sterilia* (MFFJF)**

Mycelia sterilia is a non-forming spore fungus. Endophytic fungus *Mycelia sterilia* is often found in the bifurcated mycelium assemblies, erect conidia, globose vesicles with uniseriate sterigmata with conidia chain basipetal succession (Srinivas et al. 2015). Based on research Carbungco et al. (2017), *Mycelia sterilia* has a floccose texture, white greenish peripheral with a circular filamentous zone. *Mycelia sterilia* has hyphae with a length of 40 µ and a diameter of 8 µ and has absent spores. *Mycelia sterilia* is also found in vegetables.

***Aspergillus* sp. (MFFJG)**

Aspergillus is probably the most widespread group of fungi in the human environment. Many species are found in a variety of substrates, including soil, forage products, various types of food products, dust, organic debris, and decomposing matter. *Aspergillus* species play an essential role in the recycling of carbon and nitrogen sources. Many *Aspergillus* species, including important pathogenic species, do not have special nutritional requirements and can grow in simple media, such as glucose-asparagine-phosphate broth, which contains a single protein hydrolysate (Gugnani 2003). *Aspergillus* has the characteristics of a spore-bearing structure called a conidial head, a basal foot of a bicep. The leg cells have more or less perpendicular hyphae and a vesicular conidiophore, having one or two synchronous cell layers and asexually formed spores known as conidia produced by phialides. The head of conidia of *Aspergillus* may be uniseriate or biseriate (Nyongesa et al. 2015).

In conclusion, the isolation of endophytic bacteria and fungus from *S. cumini* fruit successfully obtained 4 species of bacteria and 4 species of fungi. Endophytic bacteria from *S. cumini* fruit were identified as *Bacillus cereus*, *B. megaterium*, *B. subtilisin*, and *Bacillus* sp. Identified endophytic fungi from *S. cumini* fruit consisted of *Candida guilliermondii*, *Penicillium* sp, *Mycelia sterilia*, and *Aspergillus* sp.

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