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Endophytic Fungal Species Isolated from Mangrove Trees *Rhizophora apiculata*, *Nypa fruticans*, and *Xylocarpus granatum* from Brunei Darussalam

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An attempt made to isolate the mangrove endophytes from *Rhizophora apiculata*, *Nypa fruticans*, and *Xylocarpus granatum* has shown the presence of about 28 different strains of endophytic fungi belonging to the phyla *Ascomycota*. *Rhizophora apiculata* was found to possess highly diverse endophytic species compared to *Nypa fruticans* and *Xylocarpus granatum*. Furthermore, the leaves of the mangrove trees were found to serve as a rich source of endophytic fungal species compared to other segments studied. Moreover, it was observed that the leaf acted as a major source of endophytic fungi in *Rhizophora apiculata* and *Nypa fruticans*, whereas barks were found to have a high number of species in *Xylocarpus granatum*.

Keywords: ITS, mangrove endophytes, *Nypa fruticans*, *Rhizophora apiculata*, *Xylocarpus granatum*

INTRODUCTION

Endophytic microorganisms are the group of microorganisms that are present in the intercellular spaces of the host plants' tissues with the ability to manifest a synergistic relationship with the host plants by means of symbiotic mutualistic or trophobiotic associations (Schulz and Boyle 2006; Arnold 2007; Ryan *et al.* 2008; Deivanai *et al.* 2014). Fungal endophytes, being well known for their beneficial synergistic relationship as symbionts in the host plants are abundantly available in the mangrove trees (Hamzah *et al.* 2018). These endophytes belong mostly to *Ascomycota* phyla and are found to live in the plant tissues asymptomatically (Hyde and Soytong 2008; Aly *et al.* 2011) for longer periods even throughout the life span of the plants (Rodriguez *et al.* 2009). Many studies worldwide have reported the significant beneficial role of fungal endophytes in plant health, especially as an agent accelerating seedling growth (Carlos *et al.* 2021), enhancing plant growth (Ismail *et al.* 2018; Schardl *et al.* 2008; Carlos *et al.* 2021), improving resistance to phytopathogens (de Silva *et al.* 2019), and conferring resistance against abiotic stress (Moghaddam *et al.* 2021; Zhou *et al.* 2021; Waqas *et al.* 2015; Ismail *et al.* 2021). Moreover, these endophytes are said to exist in the rhizosphere or the phyllosphere from where they enter the host plants by means of all possible openings in the host plant (Grabka *et al.* 2022). Survival, existence, and host plant selection of the endophytes depend majorly on the availability of intercellular space and the promotion of synergistic relationships by the plants (Deivanai *et al.* 2014).

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Mangroves are popularly known as the productive natural ecosystem, not only because of their role in maintaining the soil integrity in the coastal ecosystem but also due to their pervasive role in the global biogeochemical cycles like carbon dioxide, nitrogen, and sulfur cycle (Debbab *et al.* 2013; Bacal and Yu 2017; Guerrero *et al.* 2018). Mangrove lands comprise about 70 different mangrove tree species of which *Rhizophora* spp. are widely spread and are commonly observed in the tropical and sub-tropical coasts (Singh *et al.* 2014). Almost 200 species of mangrove fungal endophytes have been reported worldwide (Bibi *et al.* 2020). They are the second largest group of fungi popularly known for their different morphological structures and their ability to help plants survive in adverse and challenging environmental conditions (Zhou *et al.* 2018; Sun and Guo 2012). Most of them are known to colonize from the soil, marine, and freshwater ecosystem, which form a consortium that could help the mangrove plants to overcome and adapt to the extreme variations including the temperature, salinity, tidal activity, moisture, anaerobic soil conditions, highly fluctuating microbial diversity, and survival competition along with the dynamic interactions observed between the marine and terrestrial habitats (Ananda and Sridhar 2002; Sengputa and Chaudhuri 2002; Sridhar 2004; Jones *et al.* 2008). All the above-mentioned benefits are achieved with the help of the unique and novel natural bioactive compounds secreted by the endophytes (Debbab *et al.* 2013). Interestingly, further studies on the bioactive compounds secreted by the endophytes have proven their imperative role in the treatment and prevention of various life-threatening diseases in humans, explaining their medicinal benefits (Donayre and Dalisay 2016; Arnold *et al.* 2003; Evans 2003; Tian *et al.* 2004; Kuldau and Bacon 2008; Ting *et al.* 2008; Ahmad *et al.* 2010; Hipol 2012; Bungihan *et al.* 2013; Tan *et al.* 2015; Eskandarighadikolaii *et al.* 2015). Hence, the mangrove ecosystem is being considered a hotspot for bioactive compounds by researchers worldwide (Cadamuro *et al.* 2021).

MATERIALS AND METHODS

Sample Collection Method

This study attempted to isolate fungal endophytes of mangrove trees from two mangrove sites in Brunei Darussalam, *i.e.* Kampung Pintu Malim with 13 ppt salinity (4°52′27″N 114°57′19″E) and Kampung Batu Marang with 16 ppt salinity (4°58′55″N 115°02′01″E). Among the seven species of mangrove trees available in Brunei Darussalam, the endophytes were isolated from only three species – namely, *Rhizophora apiculata* and *Nypa fruticans* available at Kampung Pintu Malim, as well as *Xylocarpus granatum* at Kampung Batu Marang.

Sample Processing

Clean, air-dried, and sectioned (1-cm^2 fragment) leaves, barks, bark shavings, and roots were treated with 70% ethanol (10 s), followed by 4% sodium hypochlorite (90 s) and sterile distilled water to remove any contaminants present on the sample surface (Kumaresan and Suryanarayanan 2001). Surface sterility of the treated samples was ensured by culturing the imprints obtained from the sample and the final wash water in the potato dextrose agar (PDA) plates. Samples succeeding the surface sterility were then cultured (segments of the samples were placed) on PDA plates supplemented with 1% sodium chloride and 0.02% chloramphenicol at 25 °C for fungal endophytes. Colonies were isolated based on the morphological characteristics, as reported by Hamzah *et al.* (2018).

DNA Extraction, Amplification, and Identification

ITS region of the fungal DNA extracted (ZR fungal/bacterial DNA extraction mini prep kit, Zymo Research, USA) from the isolates was amplified using the universal ITS primers (Taha *et al.* 2020). Amplicons were sequenced (1st BASE Molecular Biology Services, Malaysia), which were later DNA barcoded using the online tool NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as described by Taha *et al.* (2020). Further, the sequences obtained from the mangrove endophytes were submitted to the NCBI database (https://www.ncbi.nlm.nih.gov/) with the accession numbers, as given in Table 1.

RESULTS

Figure 1 infers that the leaves of mangrove trees like *Rhizophora apiculata* and *Nypa fruticans* were found to have a highly diverse endophytic fungal species with about 28.6 and 17.9% of the total diversity, respectively. Apparently, bark was found to be the rich source of highly diverse endophytic fungal species in the mangrove tree *Xylocarpus granatum* harboring about 25% of the total diversity obtained. Similarly, from Table 1, it is inferred that the mangrove tree *Rhizophora apiculata* acted as a rich source of fungal endophytes representing the presence of about 11 different strains, followed by the other two trees – namely, *Xylocarpus granatum* with 10 different strains and *Nypa fruticans* with seven different strains.

Table 1. List of endophytes isolated from mangrove trees and their NCBI accession numbers.

Source mangrove tree	Sample type	Isolate	Fungal endophyte	NCBI accession number
Rhizophora apiculata	Leaves	MEFR02	*Colletotrichum* sp.	OP508380
		MEFR03	Hypoxylaceae	OP508381
		MEFR07	*Colletotrichum* sp.	OP508382
		MEFR09	*Penicillium* sp.	OP508383
		MEFR10	*Colletotrichum* sp.	OP508384
		MEFR11	Diaporthales sp.	OP508385
		MEFR13	*Colletotrichum* sp.	OP508386
		MEFR14	*Colletotrichum* sp.	OP508387
	Bark	MEFR16	*Aspergillus* sp.	OP508388
		MEFR17	*Aspergillus* sp.	OP508389
	Roots	MEFR11	*Metarhizium* sp.	OP508390
Nypa fruticans	Leaves	MEFN01	*Colletotrichum* sp.	OP508391
		MEFN02	*Colletotrichum* sp.	OP508392
		MEFN03	*Colletotrichum* sp.	OP508393
		MEFN04	*Colletotrichum* sp.	OP508394
		MEFN06	*Aspergillus* sp.	OP508395
	Bark	MEFN08	Xylariales	OP508396
		MEFN11	*Trichoderma* sp.	OP508397
Xylocarpus granatum	Leaves	MEFX01	*Daldinia* sp.	OP508398
		MEFX02	Hypoxylaceae	OP508399
	Bark	MEFX05	*Chaetomium* sp.	OP508400
		MEFX06	*Chaetomium* sp.	OP508401
		MEFX07	*Scopulariopsis* sp.	OP508402
		MEFX08	Chaetomiaceae	OP508403
		MEFX09	*Lasiodiplodia* sp.	OP508404
		MEFX10	*Aspergillus* sp.	OP508405
		MEFX11	*Trichoderma* sp.	OP508406
	Root	MEFX12	*Penicillium* sp.	OP508407

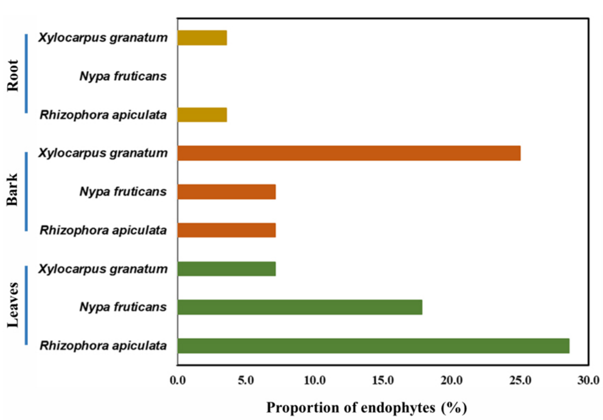

Figure 1. Fungal endophytes diversity observed in the different parts of the mangrove trees.

DISCUSSION

From the study, it was observed that the leaves acted as the major source of diverse endophytes in the mangrove trees *Rhizophora apiculata* (28.6%) and *Nypa fruticans* (17.9%) (Figure 1). Similar observations have been reported by researchers like Bayman *et al.* (1997) and Shreelalitha and Sridhar (2015). Moreover, Chaeprasert *et al.* (2010), Abraham *et al.* (2015), and Thomas *et al.* (2016) have reported that the endophytes isolated from the leaves have shown more significant bioactivity that includes antitumor, antibiotic, antimicrobial, insect repellent, and insecticidal activities. On the other hand, the bark (Figure 1) was found to be a rich source of diverse endophytes in *Xylocarpus granatum* (25%). These studies suggest that each tree species has demonstrated specificity in harboring endophytes in their tissues, in which the diversity of endophytes harbored by *Xylocarpus granatum* was high in bark compared to the other mangrove trees that showed high diversity in the leaves (Debbab *et al.* 2013). About 11, 10, and seven different endophyte strains (Table 1) were obtained from *Rhizophora apiculata*, *Xylocarpus granatum*, and *Nypa fruticans*, respectively, suggesting that *Rhizophora apiculata* acted as a rich source of fungal endophytes as stated by Hamzah *et al.* (2018). Furthermore, *Rhizophora apiculata* – a widely distributed mangrove tree occurring worldwide – has shown the presence of the highest diversity of fungal endophytes compared to the other two trees studied.

Similar observations were reported by researchers on the endophytes isolated from the mangrove trees and the beneficial effects of the bioactive compounds secreted by the endophytes (Cadamuro *et al*. 2021; Elshafie *et al*. 2020). Furthermore, the endophytes isolated in the study are capable of benefiting the ecosystem and human health with the secretion of various bioactive compounds. *Colletotrichum* spp., a widely distributed group of fungal species causing various crop diseases, is known for its numerous bioactive properties as an endophyte (Masi *et al.* 2017; Tan and Zou 2001; Zhang *et al.* 2006; Kharwar *et al.* 2011). Kim and Shim (2019) have reported the secretion of 109 secondary metabolites from the genus *Colletotrichum,* which include sterols, pyrones, nitrogenous metabolites, terpenes, phenolics, and fatty acids. Various species of *Penicillium* have also been reported as a potential source of diverse bioactive compounds (Zhu *et al.* 2018; Meng *et al.* 2017; Cao *et al.* 2019). Accordingly, Frisvad *et al.* (2004) and Leitão (2009) have stated that *Penicillium* sp. acts as the major source of antibiotics, terpenes, polyketides, and alkaloids. Moreover, most of the secondary metabolites obtained from *Penicillium* sp. have been classified as medicinal compounds and have been included in medical applications (Nicoletti and Trincone 2016; Pejin *et al*. 2013). Most notable metabolites obtained from *Penicillium* sp. are aurantiomide C, veridicatol, β-sitosterol glucoside, cerebroside A, β-sitosterol, aspterric acid, linoleic acid, 3,4-dihydroxy benzoic acid, and ergosterol which possess antitumor, antimicrobial, antioxidant, and anti-biofilm activities (Boulis *et al.* 2020). *Diaporthales*, a well-known group of phytopathogens are also known for the secondary metabolites secreted by them. According to the literature, it is estimated that they are capable of secreting about 106 bioactive natural compounds which belong to polyketides, terpenoids, steroids, ten-membered lactones, alkaloids, and fatty acids (Xu *et al.* 2021). Further, these polyketides are classified into chromones, xanthones, chromanones, furanones, pyrones, quinones, phenols, oblongolides, and unclassified polyketides (Niu *et al.* 2019). Similarly, *Aspergillus* sp. is a popular fungus possessing a variety of potential medicinal compounds that comprise terpenes, alkaloids, steroid, and polyketones, which are capable of being antioxidants, cytotoxic agents, and antimicrobial agents (Zhang *et al.* 2018; Youssef *et al.* 2021). *Metarhizium* spp. is capable of secreting a variety of chemically diverse secondary metabolites that benefit humankind (Yao *et al.* 2022). Their compounds possess rich pharmacological applications that include antiviral, antibacterial, antifungal, cytotoxic, anti-inflammatory, and antioxidant properties (Song *et al.* 2021).

The secondary metabolites secreted by *Xylariales* include diterpenoids, sesquiterpenoids, triterpene glycosides, nitrogen-containing compounds, aromatic compounds, pyrone derivatives, diterpene glycosides, polyketides, and steroids (Song *et al.* 2014). *Trichoderma* sp. is a popularly studied and well-documented species for the secondary metabolites secreted by them and for beneficial activities. They produce numerous metabolites that act as plant growth enhancers, pesticides, fertilizers, herbicides, and microbicidal agents (Stracquadanio *et al.* 2020). *Daldinia* spp. is also capable of secreting some useful secondary metabolites like EtOAc extract and polyketides that have shown a promising effect on inhibiting α-glucosidase and, thus, helping to cure diabetes (Liao *et al.* 2019). Similarly, the secondary metabolites produced by *Chaetomium* sp. like cytochalasans, indole alkaloids, terpenoids, steroids, flavonoids, azaphilones, *etc.* were found to have antiproliferative and antimutagenic activities against cancerous cells and various other medicinal benefits (Kaur *et al.* 2020; Tian and Li 2022). Likewise, *Scopulariopsis* spp. are known for the alkaloids secreted by them that are cytotoxic, antifungal, antiviral, antibacterial, and antioxidant in nature (Youssef and Simal-Gandara 2021). *Lasiodiplodia* spp. is a group of plant pathogenic fungi that are able to secrete 134 different beneficial secondary metabolites that possess phytotoxic, cytotoxic, and antimicrobial activity (Salvatore *et al.* 2020). From this, it is evident that the endophytes isolated from the mangrove trees in the study could act as a potential pharmacological, medicinal, or bioactive compound that could be used for a wide range of applications.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

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