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Variation in antibacterial properties of endophytic fungi isolated from Phaeophytes and Rhodophytes of Johor, Malaysia

N.F.A. Zainee¹, N. Ibrahim², N. Hidayah¹ and M. Rozaimi^{1*}¹Department of Earth Sciences and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Malaysia²Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Malaysia*Corresponding Author Email : mdrozaimi@ukm.edu.my

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Abstract

Aim: To identify endophytic fungi of tropical macroalgae and analyse variation in antibacterial activity.**Methodology:** Endophytic fungi were aseptically isolated from macroalgae tissue, identified by macroscopic and microscopic observations, screened for the presence of antibacterial activity using cross streak and disc diffusion methods against six human pathogenic bacteria.

Results: A total of 27 endophytic fungi were isolated and identified from 7 species of macroalgae collected from the Johor coast, Malaysia. Twenty-three fungal isolates belonged to Ascomycota, while two belonged to Zygomycota and Oomycota, respectively. *Aspergillus niger* was the most common and abundant endophyte found in macroalgal samples. Preliminary screening determined 13 species (48.15%) having positive antibacterial activity. Quantitative analysis of antibacterial activity showed significant differences ($p < 0.01$) against six human pathogenic bacteria. Two fungal isolates indicated strong and broad-spectrum antibacterial activity namely, *Pythium* sp. and *Trichoderma viride*.

Interpretation: The findings demonstrate that certain macroalgae-derived endophytic fungi from Johor possess antibacterial properties and can be potentially new antibiotic sources, which are hitherto under-reported.

Key word: Antibacterial activity, Endophyte, Microfungi, Seaweed

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Introduction

Antibiotic resistance is a global threat and widely recognised as one of the biggest risks to healthcare industry (Frieden, 2013; O'Neill, 2016). The World Health Organization (2019) has reported that the number of people requiring interventions against the risk of infectious disease is increasing up to 1582.9 million. In this sense, the current problem we are facing is the resistance of microorganisms towards antibiotics, which not only reduces the effectiveness of treatment, is also increasing the cost of treatment and mortality (O'Neill, 2016). This has led to the need for exploring new sources of antibacterial substances from natural environment, which is still under-explored for the treatment of modern infections (Wohlleben et al., 2016).

Endophytes from natural environment are potential sources of bioactive metabolites for new antibiotic substances (Radic and Strukelj, 2012; An et al., 2013). Metabolites from the natural environment are valuable sources of therapeutic agents due to their effectiveness, low toxicity and environmental impacts (Bagchi and Banerjee, 2014; Strobel, 2002). Endophytic fungi in terrestrial and ocean ecosystems have attracted researchers to exploit them as alternative source for pharmaceuticals (Al-Saif et al., 2014; Ariffin et al., 2011, 2014; Zainuddin et al., 2010).

Symbiosis between fungi and macroalgae produces a mutualistic relationship. The presence of endophytic fungi within the host has various functions depending on the microhabitat of fungi within the host (Chai et al., 2016). Endophytic fungi are able to promote the growth of their host (Bilal et al., 2018). Endophytes provide a variety of defence systems to the host such as reducing herbivory levels and increasing resistance to pathogens and pests (Bamisile et al., 2018). Endophytic fungi increases the resilience of host from extreme marine environmental conditions such as desiccation and drought, particularly during low tides when intertidal macroalgae are exposed for 8 hrs (Wicklow et al., 2005). The ability to survive in a high-stress environment is one of the factors that allows endophytic fungi to have a high degree of resistance and tolerance to extreme environmental changes (Waqas et al., 2015). Moreover, the endophytic fungi colonised in macroalgal thallus has the ability to mimic and improve the quality of copied metabolite compounds produced by its host (Radić and Štrukelj, 2012). In addition, endophytic fungi help the host in terms of nutrient absorption and increase resistance to predators and herbivores (Suryanarayanan, 2012).

Most of the microbial diversity studies have been conducted on the endophytic fungi of higher plants (Maadon et al., 2018), especially those that have ethnobotanical values (Nalini et al., 2014). Seagrasses recorded low frequency of colonisation but produced secondary metabolites that are capable of inhibiting pathogenic bacterial growth (Supaphon et al., 2013). In Malaysia, the studies on microfungi associated with

macroalgae include testing for their anticancer (Ariffin et al., 2011), antioxidant (Ariffin et al., 2014) and antibacterial potentials (Zainee et al., 2018, 2019a, 2019b). However, very limited reports are available on the variation of endophytic fungi of macroalgae. This study, in line with the quest to discover new alternatives from natural sources for antibiotic production, explores the effectiveness of endophytic fungi isolated from macroalgae as antibacterial agents against human pathogenic bacteria.

Materials and Methods

Collection of macroalgae and seawater: Macroalgal specimens (including *Dictyopteris divaricata*, *Gracilaria blodgettii*, *G. salicornia*, *Padina minor*, *Ulva lactuca*, *Sargassum oligocystum* and *S. polycystum*) were collected during sampling in June 2015 at four different locations of Johor coast: Pulau Merambong; Nusajaya; Tanjung Dato and Tanjung Lompat (Fig. 1). The specimens were aseptically hand-collected, transferred into zip-lock plastic bags and kept at 4°C in an ice box. Seawater was collected at 0.5 to 1 m depth from the surface to prevent cross-contamination of microorganism from sediment and benthic organisms. The collected seawater was filtered, autoclaved and used for cleaning macroalgae thallus during surface sterilisation protocols.

Isolation of endophytic fungi: The macroalgal specimen was washed with sterilised seawater for 1 min and then was square-cut into 1 cm × 1 cm using sterilised surgical blades. Surface sterilisation was done by soaking the specimen as follows: 75% ethanol (30 sec), 5% sodium hypochlorite (30 sec) and 75% ethanol (10 sec). The specimen was blot-dried using tissues to remove alcohol residue and transferred onto potato dextrose agar (PDA; Oxoid, USA). Six replicates of macroalgae specimens were incubated for 7-14 days at 30°C. Inspection for any evidence of fungal growth was done periodically.

Identification of endophytic fungi: Endophytic fungi were prepared for microscopic observation using Henrici slide technique with mycelium stained using lactophenol cotton blue dye. Morphological characteristics of endophytic fungi such as spore structure, hyphae and mycelium were observed using light microscope (Leica, US). Macroscopic observation was done to differentiate the endophytes' morphology for species identification. This include features of ascospore and peridium morphology, odour, colour, and zoning, which were compared against morphological traits in published observations (Zainee et al., 2019a; CLSI, 2015)

Antibacterial screening: Cross streak method was performed to screening antibacterial potentials of endophytic fungi. Fungal mycelium at late exponential phase of fungal growth (20-30 days old), were cut into 6 cm x 1 cm portions using sterile surgical blades and transferred onto the middle of Mueller Hinton Agar (MHA; Oxoid, USA). Screening was done against six bacterial

Fig. 1 : Location of sampled macroalgae along the Johor coast: (1) Pulau Merambong; (2) Nusajaya; (3) Tanjung Dato and (4) Tanjung Lompat.

species obtained from Microbiology Laboratory, UKM including Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria, namely *Serratia marcescens*, *Salmonella typhi*, *Shigella* sp., *Escherichia coli* and *Klebsiella pneumoniae*. Turbidity of bacterial suspension was adjusted to 0.5 McFarland standard solution (equal to 10^8 colony forming unit (CFU) ml^{-1}). Bacterial suspensions were streaked (3 cm in length) using a sterile cotton swab on MHA perpendicular to fungal-cut agar. Triplicate of each fungal species were incubated at 30°C for 24 hrs. Fungi that were able to inhibit the growth of bacteria were later tested for antibacterial activity using disc diffusion assay.

Disc Diffusion Assay: The assay was performed as per the Guidelines of the Clinical Laboratory Standards Institute (CLSI, 2015). Interpretative charts were used to classify the sensitivity level of antimicrobial agents into susceptible (inhibition zone, ≥ 22 mm), intermediate (inhibition zone, 15-21 mm) and resistant (inhibition zone, <15 mm) (CLSI, 2015).

Statistical Analysis: The differences in the antibacterial activity of endophytic fungi species on each bacterial strain were tested by One-way PERMANOVA test followed by *post hoc* tests (PERMANOVA pairwise comparisons). The data were analysed according to single factor design (endophytic fungi) using Euclidean distance similarity matrices generated from transformed square-root data and was tested by the Monte Carlo tests using 999 per mutations under unrestricted permutation of raw data. This analysis was conducted using Primer-E (Version 6).

Results and Discussion

Fungal endophytes colonising marine plants are reservoirs of novel bioactive secondary metabolites, potentially

for the pharmaceutical industry (Zainee et al., 2018). In this study, the effectiveness of endophytic fungi isolated from macroalgae as antibacterial agents has been reported. The endophytic fungi species studied responded differently to human pathogenic bacteria and it was found that some fungal species were relatively more effective as agents for exerting antibacterial activity. Furthermore, a new occurrence of *Pythium* sp. in the marine environment is reported herein, and this endophyte represents the most effective antibacterial agent against human pathogenic bacteria among the other fungi studied.

Twenty-seven endophytic fungi species were isolated from 7 species of macroalgae collected from Johor coastal areas (Fig. 1). Twenty-three fungal isolates belonged to Ascomycota with two each belonged to Zygomycota and Oomycota, respectively. Green algae, *Ulva lactuca* recorded the highest number of fungal isolates (7 species), followed by *Sargassum polycystum* (6 species), *Gracilaria salicornia* (5 species), *Sargassum oligocystum* (4 species), *Gracilaria blodgettii* (2 species), *Padina minor* (2 species) and *Dictyopteris divaricata* (1 species). *Aspergillus niger* was dominant in almost all the macroalgal host, whilst *Pythium* sp. was a rare species and recorded only once in *Ulva lactuca*.

Screening of antibacterial activity against six tested human pathogenic bacteria revealed that 48.15% (13 out of 27 species) of the isolated macroalgal-endophytic fungi displayed antagonism activity against pathogenic bacteria (Table 1). Nine species showed active antibacterial activity against all human pathogenic bacteria. Four species namely *Aspergillus niger*, *Penicillium notatum*, *P. rubens* and *Cunninghamella elegans* showed active antibacterial activity up to five strains of human pathogenic bacteria only (Table 1).

The antibacterial activity was further analysed by disc diffusion assay. Pairwise one-way PERMANOVA tests indicated that variation endophytic fungi antibacterial activity was significantly different ($p < 0.01$) on each of the bacterial strains (Table 2). Our results indicated strong and broad-spectrum antibacterial activities of two fungal isolates (*Pythium* sp. and *Trichoderma viride*) isolated from *Ulva lactuca* and *Sargassum polycystum*, respectively. These endophytes were able to destroy pathogenic bacteria by forming susceptible type of inhibition against tested bacterial strains. Statistical analysis showed significant difference ($p < 0.01$) between endophytic fungi species (Supplementary Material S1) to inhibit the pathogenic bacteria.

In this study, *Pythium* sp. isolated from *Ulva lactuca* produced the largest inhibition zone against three bacteria species, namely *Shigella* sp., *E. coli* and *S. aureus* (66.17 mm, 49.83 mm and 45.75 mm), respectively, followed by *S. marcescens* (32.00 mm), and *S. typhi* (29.00 mm) (Table 3). No inhibition was shown against *K. pneumoniae*. The strong antibacterial activity of *Pythium* has been reported as a key ability

of these fungi in resisting pathogenic bacteria (Herrero et al., 2020).

The microbial diversity associated with *Ulva* has been reported (Esthela et al., 2012). *Ulva lactuca* extract itself is capable of exhibiting a broad-spectrum and strong antibacterial activity against various pathogenic bacteria (Deveau et al., 2016; Kim et al., 2007). Bacterial endophytes of *Ulva* are implicated in the presence of various bioactive compounds (Hollants et al., 2013). Compounds from marine algae may play an important role in the formation of many other bioactive secondary metabolites, which exhibit inherent antibacterial activity (Al-Saif et al., 2014). Endophytic fungi have been reported to produce similar compounds produced by the host (Tayung et al., 2011). This suggests that *Pythium* sp. isolated may have adapted to produce same secondary metabolite compounds found in *Ulva*. Interestingly, there was a rare observation of green pigment formed around the inhibition zone of *Pythium* disk (Fig. 2). According to Tan et al. (2018), the pigment resulted from the antagonistic response of fungi in the presence of bacteria, likely

Table 1 : Antibacterial screening of macroalgae-derived endophytic fungi using cross-streak method (+ active; - : inactive)

Fungal endophyte isolate	Macroalgae (Host)	Inhibition zone					
		<i>S. aureus</i>	<i>S. typhi</i>	<i>S. marcescens</i>	<i>K. pneumoniae</i>	<i>Shigella</i> sp.	<i>E. coli</i>
Ascomycota							
<i>Fusarium solani</i>	<i>Gracilaria salicornia</i>	+	+	+	+	+	+
<i>F. oxysporum</i>	<i>Padina minor</i>	+	+	+	+	+	+
<i>F. semitectum</i>	<i>Ulva lactuca</i>	-	-	-	-	-	-
<i>Paecilomyces lilacinus</i>	<i>Ulva lactuca</i>	-	-	-	-	-	-
<i>P. variotii</i>	<i>Gracilaria salicornia</i>	-	-	-	-	-	-
<i>Trichoderma viride</i>	<i>Sargassum polycystum</i>	+	+	+	+	+	+
<i>Aspergillus flavus</i>	<i>Ulva lactuca</i>	+	+	+	+	+	+
<i>A. fumigatus</i>	<i>Gracilaria blodgettii</i>	+	+	+	+	+	+
<i>A. oryzae</i>	<i>Sargassum polycystum</i>	-	-	-	-	-	-
<i>A. terreus</i>	<i>Sargassum oligocystum</i>	+	+	+	+	+	+
<i>A. nidulans</i>	<i>Gracilaria salicornia</i>	+	+	+	+	+	+
<i>A. niger</i>	<i>Sargassum polycystum</i>	+	+	+	-	+	+
<i>A. versicolor</i>	<i>Sargassum oligocystum</i>	-	-	-	-	-	-
<i>Penicillium citrinum</i>	<i>Padina minor</i>	+	+	+	+	+	+
<i>P. chrysogenum</i>	<i>Gracilaria salicornia</i>	-	-	-	-	-	-
<i>P. digitatum</i>	<i>Sargassum oligocystum</i>	-	-	-	-	-	-
<i>P. expansum</i>	<i>Ulva lactuca</i>	-	-	-	-	-	-
<i>P. notatum</i>	<i>Sargassum polycystum</i>	+	-	+	+	+	+
<i>P. rubens</i>	<i>Sargassum polycystum</i>	+	+	-	+	+	+
<i>P. roqueforti</i>	<i>Ulva lactuca</i>	-	-	-	-	-	-
<i>Trichophyton rubrum</i>	<i>Sargassum polycystum</i>	-	-	-	-	-	-
<i>Pseudallescheria boydii</i>	<i>Gracilaria blodgettii</i>	-	-	-	-	-	-
<i>Trichothecium roseum</i>	<i>Gracilaria salicornia</i>	-	-	-	-	-	-
Zygomycota							
<i>Cunninghamella elegans</i>	<i>Sargassum oligocystum</i>	+	+	+	-	+	+
<i>Rhizopus</i> sp.	<i>Ulva lactuca</i>	-	-	-	-	-	-
Oomycota							
<i>Pythium</i> sp.	<i>Ulva lactuca</i>	+	+	+	+	+	+
<i>Phytophthora</i> sp.	<i>Dictyopteris divaricata</i>	-	-	-	-	-	-

by producing secondary metabolite substances. Therefore, this is the first report of *Pythium* forming secondary metabolite substances with green pigmentation, which may be associated with the virulence factor of this fungus.

Trichoderma viride is grouped as a wide-spectrum fungus and has been reported as an endophytic fungi in the brown algae *Sargassum* (Zainee et al., 2019b). The fungus has an ability to produce or release a variety of compounds that induces localised or systemic resistance responses, explaining their lack of pathogenicity towards plants (Leelavathi et al., 2014). It shows effectiveness as an antibacterial agent that produces strong antibacterial activity second to *Pythium*. Sadykova et al. (2015) and Saleh et al. (2011) have reported that *Trichoderma* produced wide-spectrum antibacterial activity with strong bacterial susceptibility. Isolates of *Trichoderma* from another brown algae (*Padina* sp.) showed inhibition zone against *S. aureus* (12.8–17.09 mm) and *E. coli* (13.60 mm) (Handayani et al., 2019). However, this study had recorded larger inhibition zones with susceptible-type of inhibition than those, achieving 48.58 mm against *S. aureus*, followed by *E. coli* (37.33 mm), *S. marcescens* (31.17 mm), *Shigella* sp. (24.00 mm) and *S. typhi* (22.42 mm),

while eliciting intermediate susceptibility from *K. pneumoniae* (17.50 mm) (Table 3).

Aspergillus isolates were most commonly found in the tissues of six macroalgal (Zainee et al., 2018). According to Barakat and Gohar (2012), *Aspergillus* is the largest fungal group producing metabolite compounds with antibacterial activity. In this study, all tested fungal isolates produce broad-spectrum antibacterial activity, except for *Aspergillus fumigatus*. *A. fumigatus* showed activity towards Gram-negative bacteria only and may be classified as narrow-spectrum activity. Zainuddin et al. (2010) stated that Gram-positive bacteria were easily inactivated (86.7%) by marine-derived fungi compared to Gram-negative bacteria (13.3%). In contrast to terrestrial *A. fumigatus* isolates, inhibition (20.67 mm) against *S. aureus*, was smaller than the positive control (33 mm) (Furtado et al., 2002). Overall, *Aspergillus* generally produced resistant-type inhibition except for *A. terreus*. This species, among other *Aspergillus* species, formed the strongest inhibition towards *Shigella* sp. (31.58 mm, Table 3). A study by Barakat and Gohar (2012) recorded *A. terreus* successfully inhibited fish pathogenic bacteria with an inhibition zone of 15–30 mm, which contrasted from the findings

Table 2 : One-way PERMANOVA test for variation of endophytic activity on selected bacterial strains. ** = $p < 0.01$

Bacterial strain	df	SS	MS	F	p
<i>S. aureus</i>	12	150.74	12.56	11.19	**
<i>S. typhi</i>	12	126.23	10.52	35.42	**
<i>S. marcescens</i>	12	148.17	12.35	49.30	**
<i>K. pneumoniae</i>	12	73.27	6.10	4.05	**
<i>Shigella</i> sp.	12	137.51	11.46	11.20	**
<i>E. coli</i>	12	221.10	18.43	1179	**

Table 3 : Antibacterial activity of endophytic fungi isolated from macroalgae using Kirby-Bauer Method. The fungal disk diameter was 6.00 mm; data that showed this value means no zone of inhibition was observed.

Endophytic fungi isolates	Inhibition zone (mm)						Control	
	<i>S. aureus</i>	<i>S. typhi</i>	<i>S. marcescens</i>	<i>K. pneumoniae</i>	<i>Shigella</i> sp.	<i>E. coli</i>	Positive	Negative
<i>Aspergillus flavus</i>	13.50±0.33	6.00	6.00	16.83±0.27	17.16±0.26	13.17±0.23	27.27±0.13	0.00
<i>A. fumigatus</i>	6.00	16.83±0.41	6.00	6.00	14.58±0.25	14.50±0.29	29.55±0.52	0.00
<i>A. nidulans</i>	16.16±0.78	6.00	8.83±0.10	11.67±0.19	17.50±0.80	6.00	12.31±0.17	0.00
<i>A. niger</i>	6.00	6.00	6.00	6.00	6.00	6.00	27.92±0.31	0.00
<i>A. terreus</i>	9.50±0.09	6.00	6.00	16.58±0.36	31.58±1.36	16.5±0.26	37.58±0.29	0.00
<i>Cunninghamella elegans</i>	25.83±0.97	16.83±0.37	9.42±0.08	6.00	24.33±0.54	21.67±0.42	24.23±0.12	0.00
<i>Fusarium oxysporum</i>	21.92±0.25	9.42±0.08	15.75±0.32	11.67±0.19	30.83±0.50	6.00	31.10±0.21	0.00
<i>F. solani</i>	22.58±0.65	17.50±0.26	16.00±0.31	16.25±1.26	11.67±0.26	32.50±1.38	29.17±0.39	0.00
<i>Penicillium citrinum</i>	20.17±1.02	6.00	6.00	6.00	8.92±0.08	6.00	23.04±0.41	0.00
<i>P. notatum</i>	13.00±0.17	6.00	25.92±2.50	17.25±0.29	14.58±0.27	6.00	23.13±0.29	0.00
<i>P. rubens</i>	17.76±0.32	14.53±0.17	18.17±0.31	14.75±0.08	19.50±0.37	6.00	27.33±0.37	0.00
<i>Pythium</i> sp.	45.75±2.96	29.00±1.35	32.00±1.21	6.00	66.17±4.47	49.83±4.01	31.57±0.32	0.00
<i>Trichoderma viride</i>	48.58±1.30	22.42±0.47	17.33±0.38	17.50±0.42	24.00±0.61	37.33±1.17	33.35±0.17	0.00

mean of inhibition zone ± S.E.M (n=3)

Fig. 2 : (Left). *Aspergillus terreus* mycelial growth around the disc on *Shigella* sp. lawn. (Right). Green pigment formation around *Pythium* sp. disc on *Serratia marcescens* lawn.

showing that isolates have high susceptibility levels. Interestingly, a rare observation of mycelial growth were observed around *A. terreus* disk on MHA of *Shigella* lawn (Fig. 2). According to Mueller *et al.* (2004), this may be a result of the fungus producing secondary metabolites during the lag phase of growth.

Kalyani and Hemalatha (2017) reported that *A. niger* produced small inhibition zones towards *S. aureus*, *K. pneumoniae* and *E. coli*, which contrasted our findings. Although *A. niger* was recorded in this study as the dominant endophyte in macroalgae, this fungus did not show antibacterial activity (Table 1). On the other hand, *A. flavus* and *A. nidulans* recorded weak antibacterial activity towards all tested pathogen (Table 3). There was no significant differences ($p > 0.05$) in size of inhibition zone for *A. flavus* and *A. nidulans* against all bacterial strains except for *E. coli*. This is congruent with isolation studies of marine fungi from the South China Sea (Zhang *et al.*, 2013), which showed that 56% of fungi (including *Aspergillus* and *Penicillium*) had strong antibacterial activity against pathogenic bacteria.

In previous research, penicillin-like substances were isolated from *P. notatum* associated with the brown algae *Sargassum ringgoldianum* (Amagata *et al.*, 1998). In addition, Leitão and Enguita (2016) stated that sodium chloride ions present in marine environment enhances the production of high quality antibacterial substances in *Penicillium*. In this study *S. marcescens* was susceptible to the growth of *P. notatum* (25.92 mm), while *K. pneumoniae*, *Shigella* sp. and *S. aureus* showed relatively more resistant responses (17.25 mm, 14.58 mm, and 13.00 mm, respectively). Pairwise analysis showed significant

difference ($p < 0.01$) of *P. notatum* and *P. rubens* against *S. marcescens*. *P. rubens* exerted weak inhibition against all tested pathogen, with a slight inhibition compared to the positive control (Table 3). Compaore *et al.* (2016) reported that, *P. citrinum* was capable to produce citrinin, which is a broad spectrum antibiotic, especially against Gram-positive bacteria. However, in this study *P. citrinum* elicited an intermediate susceptibility response towards Gram-negative bacteria *S. aureus* (20.17 mm), and a weak response towards Gram-negative bacteria *Shigella* sp. (8.92 mm). Whilst, *S. typhi*, *S. marcescens*, *K. pneumoniae* and *E. coli* showed no formation of inhibition zones around the disk for *P. citrinum* (Table 3).

Fusarium solani was effective against *E. coli* (32.50 mm) and formed intermediate susceptibility towards *S. aureus* (22.58 mm), whilst exhibiting weak antibacterial activity against *S. typhi* (17.5 mm), *S. marcescens* (16.00 mm), *K. pneumoniae* (16.25 mm) and *Shigella* sp. (11.67 mm) (Table 3). This differs from the findings of Kyekyeku *et al.* (2017), wherein *F. solani* colonies did not produce any inhibition towards *S. aureus*. Similar to Rahaweman *et al.* (2016), *Fusarium* isolates cultivated had produced inhibition zone of 11.0 mm and 4.0 mm against *S. aureus* and *E. coli*, respectively. These comparisons indicate that the endophytes in this study produced stronger inhibition compared to other studies. There was a significant difference in the pairwise test between *F. oxysporum* and *F. solani* against *E. coli*, where $p < 0.01$. *F. oxysporum* showed no inhibition against *E. coli*, however, *Shigella* sp. (30.83 mm) formed susceptible inhibition, showing weak inhibition against *S. aureus* (21.92 mm), *S. marcescens* (15.75 mm), *K. pneumoniae* (11.67 mm) and *S.*

typhi (9.42 mm) (Table 3), respectively. It is worth noting that *Fusarium* showed antimicrobial activities against pathogenic and multidrug-resistant bacteria, and these isolates were from environments with extreme conditions, including high nutrient pollution (Deshmukh et al., 2014), temperature and pH (Liu et al., 2012). *Fusarium* has been found in terrestrial sediment and proved as a good source of biologically active secondary metabolites (Jayasinghe et al., 2006; Schneider et al., 2008). Several studies have reported that *Fusarium* spp. isolated as plant endophytes play an important role in protecting plants (De Felício et al., 2015; Sondergaard et al., 2016). Zhang et al. (2016) reported that *Fusarium* had strong antimicrobial effects on *in-vitro* *Pseudomonas aeruginosa* by increasing the permeability of this bacterium's cell membranes. This suggests that *Fusarium* has the potential as a producer of antibacterial agent and can be a valuable source for the discovery of new drugs or agents for antimicrobial purposes.

Marcellano et al. (2017) had reported that *Cunninghamella* was among five isolated endophytes with strong antibacterial activity against *S. aureus* and *E. coli*, with inhibition zones of 10.4 mm and 9.7 mm, respectively. In contrast, a larger inhibition zone elicited by *Cunninghamella elegans* was displayed in this study with classifications as susceptible against *S. aureus* (25.83 mm) and *Shigella* sp. (24.33 mm), intermediate response towards *E. coli* (21.67 mm), resistance towards *S. typhi* (16.83 mm) and *S. marcescens* (9.42 mm), and no antibacterial activity against *K. pneumoniae* (Table 3). The trend of this findings is similar to those of Ali et al. (1995), where *Cunninghamella* isolates from terrestrial plants produced effective antibacterial activity with inhibition zones of approximately 20-25 mm.

Overall, this study demonstrates that endophytic fungi isolated from macroalgae exhibited strong inhibitory activity against a number of bacterial strains that are pathogenic to human health. This research may lead to the exploration of novel sources of antibacterial agents from the natural marine environment, which will fulfil the objectives of bioprospecting new sources of antibiotics. In this respect, the endophytic fungi isolated from the macroalgae that produced antibacterial agents should be further identified biochemically. It is suggested the evaluation of secondary metabolite compound produced by the macroalgal-derived endophytic fungi, this can be done by performing further phytochemical screenings for potential uses in the pharmaceutical industry, which is essential to fulfil the quest for new antibiotic compounds from natural sources.

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Add-on Information

Authors' contribution : N.F.A. Zainee: Conceptualization, data curation formal analysis, investigation, methodology, validation, visualization, writing: original draft, review and editing, N. Ibrahim, M. Rozaimi: Conceptualization, funding acquisition, project administration, resources, supervision, validation, visualization, writing: review and editing, N. Hidayah: Investigation, validation, visualization, writing: review and editing.

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