Screening of Endophytic Fungi from Chlorophyta and Phaeophyta for Antibacterial Activity

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Abstract. Chlorophyta and Phaeophyta macroalgae are important sources of secondary metabolites with pharmaceutically relevant antibacterial, antifungal and antiviral bioactivities. Oftentimes, these algae-derived compounds are, in fact, produced by endophytic fungi living inside the macroalgal tissue. Numerous studies have shown that endophytic fungi can produce a broad range of active metabolites such as terpenes, alkaloids, and quinones. The aim of the present study was to screen fungal strains isolated from a variety of *Caulerpa* spp., *Halimeda* spp., and *Sargassum* spp. for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Thirteen morphologically different isolates were tested. Two of them showed pronounced activity against *S. aureus* in agar diffusion assays.
Keywords: Antibacterial activity, Antibiotics, Chlorophyta, Endophytic fungi, Macroalgae, Phaeophyta

1. Introduction

Marine macroalgae are highly biodiverse with more than 10,000 extant species [1]. They are a prolific source of secondary metabolites with antibacterial, antiviral, and antifungal activities [2]. For green algae (Chlorophyta) such as *Caulerpa* spp. phenols with antimicrobial properties against bacteria, fungi, and the DENV-2 virus have been described [3,4]. *Halimeda* spp. have been reported to yield compounds with antimicrobial activity against *Vibrio* sp. and a variety of fungal pathogens as well with antioxidant activity [5,6]. Antibacterial and antiviral activity has also been reported for polyphenolic compounds derived from brown algae (Phaeophyta) such as *Sargassum wightii* and *Turbinaria ornata* [7], and fucosterol from *Turbinaria* sp. [8,9]. It has been shown that antibacterial activity in macroalgae can be caused by the presence of sulphate polysaccharides (SPs) produced by the seaweeds themselves [10]. But there is also evidence that in some cases the ability to inhibit bacterial growth is due to the metabolic activities of fungal endophytes in the macroalgae. Oftentimes, endophytic fungi produce bioactive compounds similar in their chemical structure and their functional properties to the bioactive compounds produced by their hosts [11]. A number of studies have been conducted on bioactive compounds with antibacterial activity isolated from Chlorophyta such as

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Caulerpa spp. and *Halimeda* spp. and Phaeophyta such as *Sargassum* spp. However, the exploration of endophytic fungi from Chlorophyta and Phaeophyta has been explored in a lesser extent. The aim of the research was to explore the potential of endophytic fungi isolated from macroalgae to produce metabolites with antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.*

2. Material and Method

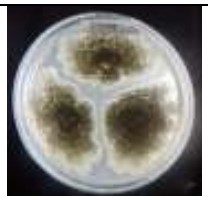
Endophytic fungi were obtained by direct isolation (direct inoculation) of seaweed *Caulerpa* spp., *Halimeda* spp., and *Sargassum* spp. that were collected off Karya Island, Kepulauan Seribu. This procedure involved taking seaweed sample and isolation of endophytic fungi using the surface sterilization method. The isolate was then inoculated on Potato Dextrose Agar (PDA) medium in fresh water and sea water (using NaCl 3% of the volume of media) and incubated at room temperature (27-29°C) for 5-7 days. The cultures were checked once per day until the fungal growth was visible. Then purification was performed to obtain single isolate. Endophytic isolates were screened for their antibacterial activity against *S. aureus* and *E. coli*. Antibacterial assays were conducted by culturing the bacteria on semisolid NA medium (NA 1.2 grams/100 ml of water) to which then the fungal mycelia were added on a disc with ± 1 cm diameter on the surface of the culture medium. The inoculated cultures were incubated for 24 hours at 37°C. Antibacterial activity was detected by the presence of inhibition zone (clear zone) around the mycelia. This inhibition zone indicates that the fungus produces compounds which are able to inhibit the bacterial growth. The inhibition zones were measured using a caliper. The extent of bacterial growth inhibition was classified in four categories based on the diameter of the inhibition zones, namely (1) weak if the inhibition zones were smaller than 5 mm, (2) intermediate for a range of 5-10 mm, (3) strong for 10-20 mm, and (4) very strong if the inhibition zones were more than 20 mm in diameter [12]. Selected fungi were then characterized macroscopically and microscopically.

3. Results and Discussions

3.1. Endophytic fungi of macroalgae Chlorophyta and Phaeophyta

Thirteen morphologically distinct endophytic fungal strains were successfully isolated from algal tissues. Of these, 6 isolates were obtained with freshwater media (sample codes KHC0003, KHC0008 A, KHC0009, KHC0016, KHC0026 A, and KHCOO26 B) and 7 isolates with seawater media (KHC0008 B, C KHC0008, KHC0012, KHC0015 A, B KHC0015, KHC0016, and KHC0026 C). The endophytic fungi isolated from *Caulerpa* sp. were written wuth the sample codes KHC0008, KHC0016, KHC0026; one strain isolated from *Halimeda* sp. written as KHC0003, and the strains derived from *Sargassum* sp. labelled as KHC0009, KHC0012, and KHC0015. The morphology of the endophytic fungi isolated from *Caulerpa* sp., *Halimeda* sp. and *Sargassum* sp. is depicted in Table 1.
Based on their morphological characteristics, the isolated endophytic fungi were tentatively identified as *Aspergillus* sp. (KHC0016, KHC0026 A, and KHC0026 C), *Fusarium* sp.(KHC0026), *Penicillium* sp. (KHC0003, KHC0009, KHC0015 A, B; KHC0015, KHC0008 A, B; KHC0008, KHC0026 P). Another isolates could not been identified yet. This identification based on the fungal characteristics such as the shape of the conidiophores, the colony color (green or yellow), and their feathery texture with straight conidiophores [13].

Table1 Endophytic fungi isolated from *Caulerpa* sp., *Halimeda* sp. and *Sargassum* sp.

Isolate	
Freshwater medium	**Seawater medium**
 KHC0003	 KHC0008 B
 KHC0008 A	 KHC0008 C
 KHC0009	 KHC0026 B
 KHC0016	 KHC0015 A
 KHC0026 A	 KHC0015 B
 KHC0012	 KHC0016
	

Isolate	
Freshwater medium	**Seawater medium**
	KHC0026 C

3.2. Antibacterial Activities of Endophytic Fungi

The screening of the endophytic fungi for antibacterial activity of their metabolites was performed by an antagonism test [14]. The screening results revealed antagonistic properties for endophytic fungi isolated from all three algal genera against the tested bacteria. The results are shown in Figure 3.

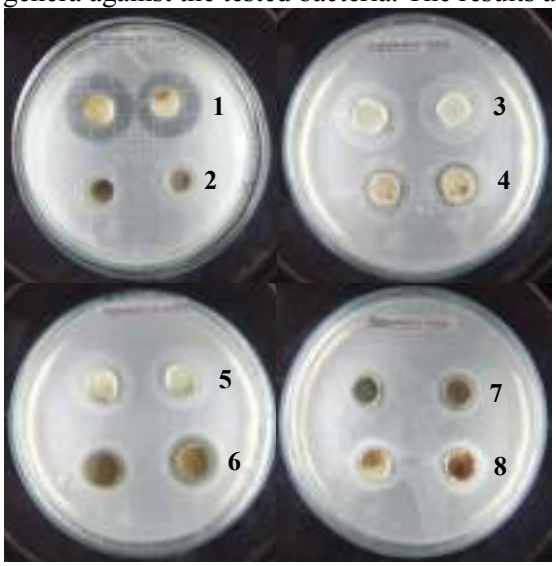


Figure 1 Antibacterial screening of endophytic KHC0003 (1), KHC0016 (2), KHC0008 A (3), KHC0012 (4), KHC0009 (5), KHC0015 A (6), KHC0026 B (7), KHC0015 B (8) against *Staphylococcus aureus*

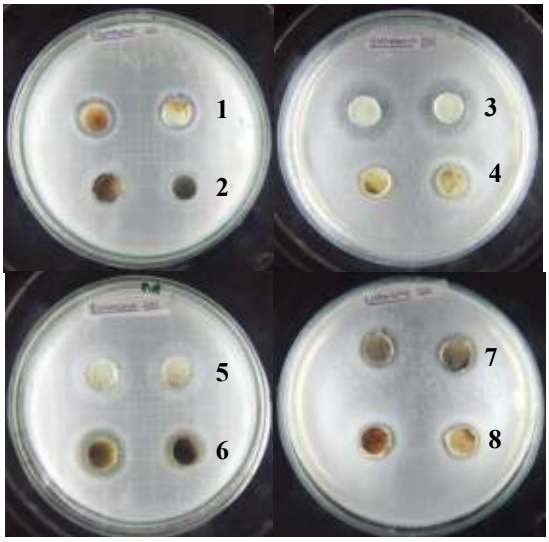
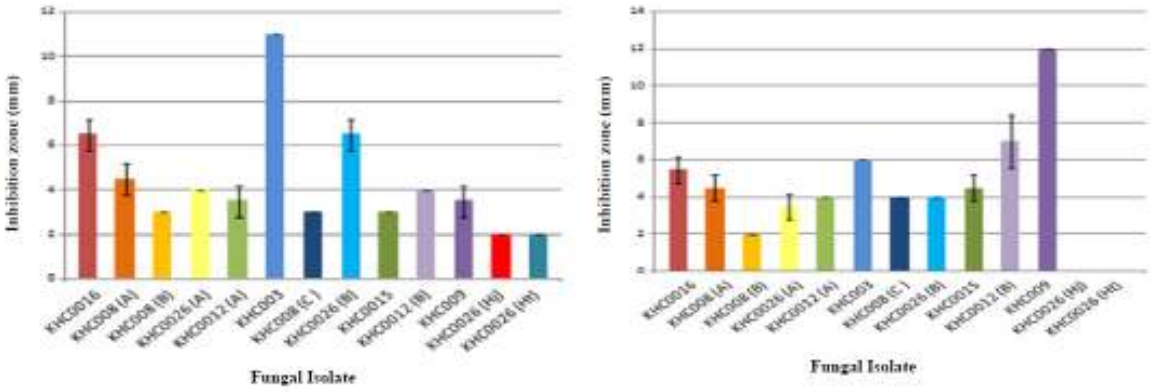


Figure 2 Antibacterial screening of endophytic KHC0003 (1), KHC0016 (2), KHC0008 A (3), KHC0012 (4), KHC0009 (5), KHC0015 A (6), KHC0026 B (7), KHC0015 B (8) against *Escherichia coli*

The results showed that fungal isolates KHC0003 and KHC0026 B had antibacterial activity against *S. aureus* and *E. coli.* Isolate KHC003 produced the diameter of inhibition zone of 11.0 ± 0.0 mm and

4.0 ± 0.0 mm against *S. aureus* and *E. coli*, respectively. For strain KHC0026 the diameter of the inhibition zone was 6.5 ± 0.7 mm and 4 ± 0.0 mm, respectively (Figure 3). It shows that both isolates inhibited the growth of *S. aureus* strongly and relatively weak in inhibiting *E. coli.*



(A) (B)

Figure 3 Diameters of inhibition zones resulting from endophytic fungal isolates tested against *Staphylococcus aureus* (A) and *Escherichia coli* (B)

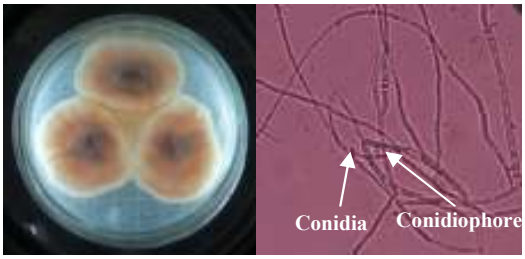
Extracellular metabolites produced by KHC0003 and KHC0026 B were suspected to be responsible for the ability of both fungi to inhibit bacteria. Observation of the inhibition zones after 20 hours incubation revealed that the fungal compounds killed the bacterial cells (bactericidal effect). The ability of the endophytic fungi to produce bioactive compounds is oftentimes beneficial to their hosts (i.e., the plants) by improving their resistance to diseases caused by microbial pathogens such as bacteria [15]. Antagonism mechanisms of fungi typically are: 1) mycoparasitism; i.e., the hyphae of antagonistic fungi twist or stick to the target microorganisms, and then penetrate the cell wall, entering the cell to take up the nutrients causing the microorganisms to die, 2) production of antibiotics that destroy microbial cells through the destruction of the target cell membrane structure, and 3) competition for living space and nutrients [16]. The extracellular compounds released by the fungal isolates KHC0003 and KHC0026 showed antibacterial activity without being extracted with inorganic solvents. This indicates that the usages of bioactive compounds may be used directly or simply extracted using water solvent. The use of water as a solvent keeps the bioactive compounds from the damage [17].

3.3. Characterization of the Selected Endophytic Fungi

The characterization of the selected endophytic fungal isolates was performed by macroscopic and microscopic observation. The results of fungal characterization are presented in Figures 4 and 5.

3.3.1. Characteristics of fungal isolate KHC0003

Fungal isolate KHC0003 cultivated on PDA medium produced pinkmycelia and orange for the reverse. The colonies had a velvety surface and whitish edges (Figure 4A). When the isolate was examined under a microscope at a magnification of 400x it showed long and septate hyphae, and rounded conidia (Figure 4B). Because of these features, the isolate was assumed to belong to the genus *Fusarium* [13].



A **B**

Figure 4. Fungal isolate KHC0003; macroscopic (A) and microscopic (B) image

3.3.2. Fungal isolate KHC0026

Fungal isolate KHC0026 cultivated on PDA medium had white mycelia and the reverse, a thin mycelia surface, flat ledges and spreading hyphae (Figure 5A). Under the microscope at a magnification of 400x it showed long conidiophores and no septa and rounded conidia at the tips (Figure 5B). This isolate has not been identified yet.

A **B**

Figure 5. Fungal isolate KHC0026; macroscopic (A) and microscopic (B) image

4. Conclusion

The endophytic fungi isolated from *Caulerpa* sp., *Halimeda* sp., and *Sargassum* sp. consisted of 13 morphologically different isolates. Isolates KHC0003 and KHC0026 were selected based on their antibacterial activity against *Staphylococcus aureus*. Based on their morphological features the isolates KHC0003 and KHC0026 were tentatively identified as *Fusarium* spp.

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