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Research Article

Isolation of endophytic fungi from marine algae and its bioactivity

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ABSTRACT

To isolate the endophytic fungal species from the marine macro algae and analyzed the isolation frequency and its potentials. Ten different seaweeds collected from the Mandapam and Pondicherry coastal region and endophytic fungal species were isolated**.** Isolation frequency (IF) was determined for each species of algae. 19 strains were screened for antimicrobial activity against human and fish bacterial pathogens. In this study, the higher isolation frequency was observed in *Codium sp* (80%) and least isolation frequency in *Ulva fasciata* (10%). 19 strains were screened and the antibacterial activities were expressed as zone of inhibition. Maximum bacterial inhibition >10mm was observed in strains 6, 7, 8, 9, 10 & 15. The isolation frequency was higher in *Codium sp* and among the 19 strains, 6 strains showed better antibacterial activity against human and fish pathogenic bacteria. The antibacterial activity may be due to the presence of bioactive metabolites, which are produced by the endophytic fungi.

Keywords: Antibacterial activity; Endophytic fungi; Marine algae; Seaweed associated fungi

1. INTRODUCTION

The marine environment is frequently recognized as the largest potential source of bioactivity and it is being increasingly searched for novel chemicals with bioactivity. The natural products are chemical compounds derived from primary and secondary metabolites of the organisms (Berdy, 2005). Due to diverse chemistry and biological activities, the natural products play an important role in pharmaceutical and agricultural research (Fitton, 2006). Bioactive compounds extracted from marine associated microorganisms, especially in fungi and recognized as a rich source of pharmacologically active secondary metabolites, which are different from terrestrial fungi. Marine mycology is one of the special branches of science, has evolved recently. There are 1500 species excluding those that form lichens, have been estimated although 444 formerly described higher marine fungi have been reported recently (Bharathidasan and Paneerselvam, 2011). Less than 500 filamentous higher marine fungi have been described and only 79 are associated with algae as parasites or symbionts, and 18 with animal hosts (Kohlmeyer & Volkmann-Kohlmeyer, 2003). Traditionally, microfungi isolated from soil, but in the recent search for new sources of therapeutic agents, marine fungi and endophytic fungi from higher plants proved to be greater potentials producing new biologically active secondary metabolites (Aly *et al.,* 2010; Debbab *et al.,* 2010; Xu *et al.,* 2010; Blunt *et al.,* 2011; Rateb & Ebel, 2011). Endophytes are ubiquitous in plants and the population dependent on the host species and location and they have been recognized as a potential and valuable source of the novel bioactive metabolites (Blunt *et al.,* 2006, 2007, 2008 & 2009; Suryanarayanan *et al.,* 2010; Guo *et al.,* 2008; Qiao *et al.,* 2010).

Seaweeds are well known for biological activities such as anti-viral, anti-fungal, antimicrobial, insecticidal, antifertility, anti-inflammatory and diuretic activity for a long time. The natural products chemistry of endophytes from algae is an area where few studies have been undertaken (Jones et al., 2008). The unique living conditions are thought to predestine seaweed as promising sources for the isolation of endophytic fungi and they may represent an interesting source of new lead structures for medical and agrochemical applications. The present study is under taken to isolate and identify the endophytes from red and green algae and also its antimicrobial properties against human and fish pathogens.

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2. MATERIALS AND METHODS

2.1 Collection of Sample

Seaweeds were collected from Mandapam coastal region (*Gracilaria corticata (S1GC)*, *Gracilaria edulis* (S2GE), *Gracilaria crassa (S3GG), Codium sp(S5CO), Halimeda gracilis (S6HG), Halimeda macroloba (S7HM),*

Caulerpa racemosa (S9CA) and *Gelidiella acerosa (S10GE)*) and from Pondicherry coast (*Ulva fasciata (S4UF) and Chaetomorpha antennina (S8CH)).*

2.2 Isolation of endophytic fungi from seaweeds

2.2.1 Sterilization of Seaweeds

Surface sterilization method is generally followed to ensure that all isolated fungi are endophytic (Kjer et al., 2010; Zhang et al., 2006). The chemical sterilization procedure was followed by placing the algae in the beaker containing disinfectant ethanol with 80% by specified timing and then immersed into the water.

Specific sterilization duration required for each individual algal species were performed and standardized before isolating endophytes from algal samples. After sterilization, the algae were placed in the plate of 2% Malt extract agar medium using sterile forceps, making sure that the algal sample made complete contact with the growth medium. After inoculation, the plates were incubated. A successful sterilization indicated the lack of growth of the surface on the plate, where the surface sterilized portion made contact. This verification is a clear indicator, whether the algal portion has been sterilized or not. If the growth appeared on the plate, it indicated that particular individual algae require longer sterilization procedure to eliminate the epiphytic organisms from the host.

2.3 Culturing of endophytes

The sterilized algae were cut into small pieces and placed on the petridish containing 2% of malt extract agar medium (five segments of algae per petridish). Then the plates were incubated at room temperature. After incubation period, the algal pieces were monitored for fungal hyphae growing from the cut end of the each sample. Control plates were used in the verification of the surface sterilization technique. The growth of fungal hyphae was monitored for 2 weeks. The fungal hyphae was removed from the edge of the algal portion and transferred into the 2% malt extract agar medium and allowed for growth. After incubation, the fungal samples were sub-cultured and stored for further studies.

2.4 Isolation frequency

Isolation frequency (IF) was determined for each species of algae and is defined as the number of algal pieces from which endophytes were cultured as a percentage of the total number of algal pieces prepared isolation frequency was calculated using the method of Petrini and Fisher, 1988.

Number of Algal Segments Showing fungal growth,

$$IF\% = \frac{Number\ of\ Algal\ Segments\ Showing\ fungal\ growth}{Total\ Number\ of\ Algal\ Segments} \times 100$$

2.5 Antimicrobial activity of Fungal Endophytes

Antibacterial activity was screened using dual culture technique with some modification. Suspension of 24-hours old culture of human bacterial pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Klebsiella oxytoca*, *Vibrio cholera*.) and fish bacterial pathogens (*Aeromonas hydrophila*, *Enterobacter aerogens, Flavobacterium sp*. *Micrococcus sp, Pseudomonas fluorescens*) were spread on a sterile Muller Hinton agar plate on to which five day old disc (5mm diameter) of endophytic fungi, which are isolated from the each individual was kept and incubated at 27°C for 24-48 hrs. Antibacterial activity was calculated by measuring zone of inhibition produce by endophytic fungi against pathogenic bacteria (Long *et al.,* 2003).

3. RESULTS

In the present study totally 156 fungal isolates were obtained from different algal species and 54 morphologically different species were isolated. Higher isolation frequency was observed (Fig:1) in *Codium sp* (80%) and least isolation frequency in *Ulva fasciata* (10%). Among the three *Gracilaria* species, *Gracilaria edulis* showed maximum isolation frequency of 50% whereas *Gracilaria corticata* and *Gracilaria crassa* showed 40% of isolation frequency. Similarly, *Halimeda gracilis showed* 60% whereas *Halimeda macroloba* showed 50% isolation frequency. Both *Caulerpa sp and Gelideiella acerosa* showed 30% isolation frequency. The species collected from Pondicherry coast showed minimum isolation frequency in *Chaetomorpha antennina* (20%) and *Ulva fasciata* (10%) (Fig:2).

In the present study, the antimicrobial activity of endophytic fungi isolated were tested against human bacterial pathogens viz. *Escherichia coli*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Klebsiella oxycota*, *Vibrio cholera* and fish bacterial pathogens viz., *Aeromonas hydrophila*, *Enterobacter aerogens, Flavobacterium sp*, *Micrococcus sp, Pseudomonas fluorescens*. Maximum bacterial inhibition >10mm was observed in strains 6, 7, 8, 9, 10 & 15 against both human and fish bacterial pathogens (Table 1). Similar antimicrobial activity was observed in endophytic fungi isolated from medicinal plants (Li *et al.,* 2005) and also in mangrove plants (Bharathidasan & Panneerselvam, 2012). The beneficial effect of endophytes to the host plant was clearly reported by Gangadevi and Muthumary, 2008. In the present study minimum, bacterial inhibition <5mm was observed in some of the strains, which indicate that these strains may have some resistance mechanisms such as enzymatic activation, target site modifications, and decreased intracellular drug accumulations.

4. DISCUSSION

Endophytes are microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects. The relationship between the host

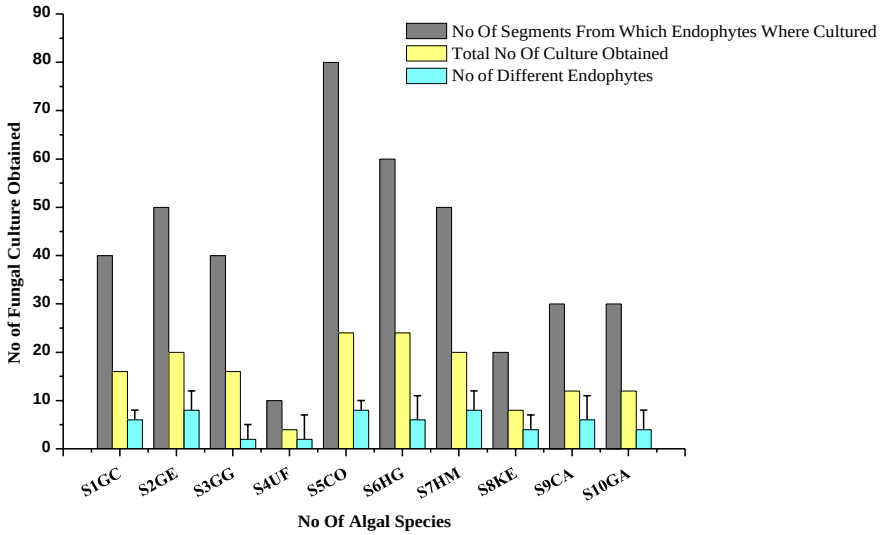

Figure 1: Isolation of endophytic fungi from marine algae

Gracilaria corticata (S1GC), *Gracilaria edulis* (S2GE), *Gracilaria crassa* (S3GG), *Codium sp* (S5CO), *Halimeda gracilis* (S6HG), *Halimeda macroloba* (S7HM), *Caulerpa racemosa* (S9CA), *Gelidiella acerosa* (S10GE), *Ulva fasciata* (S4UF) and *Chaetomorpha antennina* (S8CH).

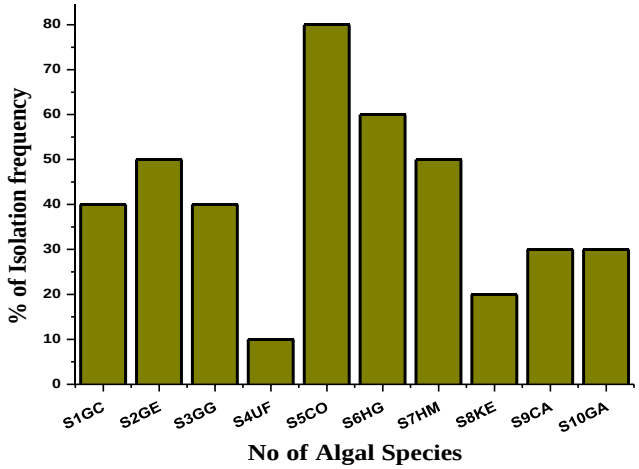

Figure 2: Illustrates the isolation frequency of seaweed endophytes

plant and its endophytes shows symbiotic characteristics as the endophytic occupant usually obtains nutrients and protection from the host plant and in return profoundly enhances the fitness of the host by producing certain functional metabolites. Biological controls or the uses of microorganisms or their secretions to prevent diseases that offer an alternative attractive supplement to disease management without negative impact of chemical controls (Gani and Ganesh, 2009).

Seaweeds are highly adapted to their environmental conditions and are able to cope with numerous physical stress factors, including sharp variation in moisture or salt concentration, changing tides, or biological stress factors such as abundant microorganisms or herbivorous insects. The present study shows greater diversity of endophytic fungi in green algae compared to red algae. Certain fungal lineages appear with greater frequency in plants representing particular families and thus denote host preference (Arnold, 2007). However, whereas in the present study, no such preference was observed.

The previous studies on medicinal plants shows high percentage frequency of fungal isolates in leaf and stem portions (Jalgaonwala *et al.,* 2010). Endophytic fungi from *Avicennia marina* mangrove plant, 11 fungal species from leafs and 9 fungal species from stem portions were isolated (Bharathidasan & Panneerselvam, 2011).

5. CONCLUSION

Endophytic organisms have received considerable attention after they were found to protect their host against insect pests, pathogens and even domestic herbivorous. Recently studies have been carried out about the endophytic bio diversity, taxonomy, reproduction, host ecology and their effort on the host (Selosse & Schardl, 2007). The seaweed harbors a sizeable portion of undiscovered potential endophytes, which may be vested with novel biochemical diversity.

Table 1: Antibacterial activity of isolated endophytic fungal strains

S.No	Strains No	Zone of inhibition against human Bacterial pathogen					Zone of inhibition against Fish Bacterial pathogen				
		1	2	3	4	5	1	2	3	4	5
1	Strain No 1	++	+++	+++	++	++	++	++	++	+	+
2	Strain No 2	++	+++	+++	++	++	++	++	++	++	++
3	Strain No 3	++	+++	+++	++	++	+	++	++	++	++
4	Strain No 4	++	+++	+++	++	++	++	++	++	++	++
5	Strain No 5	++	+++	+++	+++	++	++	++	++	++	++
6	Strain No 6	++	+++	+++	+++	+++	++	+++	++	++	+++
7	Strain No 7	++	+++	+++	++	++	++	+++	++	++	++
8	Strain No 8	+++	+++	+++	++	++	+	++	++	++	++
9	Strain No 9	+++	+++	+++	+++	++	+	++	++	+++	++
10	Strain No 10	+++	+++	+++	+++	++	+	+++	++	++	++
11	Strain No 11	++	++	+++	++	++	+	++	+++	++	++
12	Strain No 12	++	++	+++	+++	++	+	++	++	++	++
13	Strain No 13	+++	++	+++	++	++	+	++	++	+++	++
14	Strain No 14	++	++	++	++	++	+	++	++	++	++
15	Strain No 15	++	+++	++	+++	++	-	++	++	++	++
16	Strain No 16	++	+++	+++	++	++	++	++	++	++	++
17	Strain No 17	++	+++	+++	++	+++	++	++	++	+++	++
18	Strain No 18	+	+++	++	++	+++	++	++	+++	++	++
19	Strain No 19	++	++	++	++	++	+	++	+++	++	+++

Inhibition zone diameter index : **+++ >10mm, ++ 5-10mm, + <5mm, – no zone**

Human bacterial pathogen: 1.*Escherichia coli*, 2. *Staphylococcus aureus* 3*. Vibrio parahaemolyticus*, 4. *Klebsiella oxytoca*, 5*. Vibrio cholera*.

Fish bacterial pathogen: 1. *Aeromonas hydrophila*, 2. *Enterobacter aerogens,* 3. *Flavobacterium sp*, 4. *Micrococcus sp,* 5. *Pseudomonas fluorescens.*

In addition to morphological and physiological adaptations in seaweeds, the production of bioactive secondary metabolites plays an important role in the constant competition of seaweeds with other plants, animals and microorganisms for the limited resources in their habitat. The capability of seaweeds to produce a wide array of bioactive compounds is reflected in numerous publications, which describe the high chemical diversity of their metabolites.

It is concluded from the present study that the endophytic fungal isolates from seaweeds have been promising antibacterial activity against human and fish pathogenic bacteria. Further study is in progress for the identification of fungal isolates and active chemical compounds responsible for the antibacterial activity.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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