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Original Article

Antimicrobial potential of few marine derived fungi against dermatophytes, moulds and fouling bacteria

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ABSTRACT

Marine derived fungi were screened for antimicrobial activity against dermatophytes, food spoilage *Aspergillus* species and fouling marine bacteria. The *Aspergillus* and *Fusarium* species showed moderate to strong activity against one or one more strains dermatophytes, moulds and fouling bacteria. However, *Populospora* sp. and *Cirrenalia* sp. exhibited moderate to significant activity against fouling bacteria. The present finding identifies few marine derived fungi for an antimicrobial potential against dermatophytes, food spoilage *Aspergillus* species and fouling marine bacteria.

Keywords: Bioactivity, Marine fungi, food spoiling, biofouling, *Aspergillus sp.*, *Porteresia*

INTRODUCTION

Marine fungi are highly potent producer of bioactive molecules with antibacterial, antifungal, antiviral and cytotoxic properties. The various biological activities make them of valuable potential source of pharmaceutical application. Fungi derived from marine environment are considered to represent a huge potential of biological active molecules [1]. The unique physico-chemical properties of the marine environment are likely to have conferred marine fungi with special physiological adaptations. Large number of natural products from various types of marine biota have served as rich sources of drugs as well as provided clues for new biomolecules of medicinal importance [2]. During recent years the chemical ecological studies pertaining to marine organisms led to the discovery of novel biologically active compounds. Several compounds have been identified from marine organisms, exhibiting different medicinal properties such as antitumor, antiviral, antibiotic, antimalarial, anticoagulant etc. [3]. However, fungi from various marine environments and in association with other marine organisms have been explored for their bioactive potential [4].

The Dermatophytes are a group of filamentous fungi that have capacity to invade keratinized tissue such as skin, hair, nail of human and referred to as the ringworm fungi. Hot and humid climate of India makes dermatophytosis a very common superficial fungal infection of skin [5]. The genus *Aspergillus* is one of the most common and wide spread mould associated with food spoilage in the tropics on a variety of substrates [6]. *Aspergillus* species is a dominate spoilage in the tropics in the way *Penicillum* species do in temperate. They contaminate foodstuffs by producing mycotoxins (aflatoxins) which are harmful to mankind [6]. These species have been reported to be potential opportunistic pathogens to a number of important marine organisms. Fouling bacteria cause enormous damage to the marine structures raised for commercial purposes. It has been observed that adhesive microbes, such as *Bacillus* and *Pseudomonas* species, provide the substrate for fouling organisms by forming a biofilm of exopolymer [7]. The settlement of foulers in the marine environment depends on the biofilm produced by these types of microorganisms. Therefore, the present investigation was undertaken to identify bioactive potential marine fungi against dermatophytes, food spoilage *Aspergillus* species and fouling marine bacteria. The data generated in the present investigation would be of importance in

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follow up studies in the field of marine natural products, particularly in identifying potential bioactive molecules from marine derived fungi.

MATERIALS AND METHODS

Test Organisms: The antifungal activity was tested against the commonly occurring food spoilage *Aspergillus* strains e.g. *A. niger*, *A. japonicus*, *A. fresenii* and dermatophytes (*Candida* sp., *Cryptococcus neoformans, Trichophyton mentagrophytes*, *T. tonsurans*, *T. violaceum*, *Microsporium gypsum* and *Fusarium* sp.) from India. The antibacterial activity was tested against the fouling bacterial species *Bacillus cereus*, *B. circulans*, *B. pumilus*, *Pseudomonas versiculari* and *P. putida*, commonly occurring in marine environments of India.

Isolation of Aspergillus species: Three strains of *Aspergillus* that were reported to cause food spoilage [6] were isolated from wheat bread, with sterile needles. They were grown in mycological agar (HiMedia Laboratories Ltd. India) and sub-cultured for purification, following standard procedure [8]. The taxonomic identification of *Aspergillus* species was done by using various relevant keys provided elsewhere [9]. The identifications were further confirmed from the recognized experts in the relevant field of fungal taxonomy, from Agarkar Research Institute, Pune, Maharashtra, India. Pure cultures of these species were used for the testing of antifungal properties after making appropriate methanol extracts from (facultative-remove) marine derived fungi.

Dermatophytes: Pathogenic fungal strains of yeast (*Candida* sp. *Cryptococcus neoformans*) and filamentous fungi (*Trichophyton mentagrophytes*, *T. tonsurans*, *T. violaceum*, *Microsporium gypsum* and *Fusarium* sp.) were obtained from Goa Medical College, Bambolim, and used for the antifungal test. Single colonies of yeast strains i.e. *Candida* species and *Cryptococcus neoformans* were transferred on mycological agar and maintained in (3-5°C), for further studies. Filamentous fungi were maintained on czapek malt agar (HiMedia Laboratories Ltd India.) slants at (3-5 °C), until needed for further assay.

Bacterial strains: Bacterial strains of *Bacillus cereus*, *B. circulans*, *B. pumilus*, *Pseudomonas versiculari*, *P. putida* species were obtained from the stock cultures maintained in the Microbiology Laboratory of National Institute of Oceanography, Goa. These strains were isolated from the open ocean (Arabian Sea), and maintained in the refrigerator at 3-5 °C, by transferring single colony on zobell marine agar (HiMedia Laboratories Ltd. India). The adhesive nature of bacterial strains were confirmed by inoculating them in to the sterilized filtered seawater in 50 ml beaker and floating glass cover slips on the seawater in the beaker. After 6 hours, the cover slips were removed and stained with Gentian violet to check the adherence of bacteria. Those bacteria, which formed a slimy layer on the cover slips, were selected for the bioassay tests.

Isolation marine derived fungi: The vegetative parts such as leaves, stem and root of *P. coarctata* were collected from estuarine environment. The sample were washed immediately after collections, with sterile seawater to remove adhering debris, and brought to the laboratory in sterile polythene bags separately. The plant materials were immediately transferred individually to the moist chamber and kept at room temperature (29°C ± 0.5°C). The humidity in the moisture chamber was maintained by adding ~ 5ml of sterile seawater every alternate day, till the end of the experiments. The fungal species were isolated, purified and identified using standard methods. These species further confirmed from the recognized experts in the relevant field of fungal taxonomy, Agharkar Research Institute, Pune Maharashtra, India.

The pure cultures of these species were transferred onto the culture media containing nutrient agar (HiMedia Laboratories Ltd. India) in sterile plates. These pure cultures were maintained on nutrient agar slants at 3-5°C until taken up for further experimental studies.

Mass culture marine derived fungi: Isolated pure fungal strains were cultured in five liter conical flasks in the culture media. *Aspergillus niger*, *A. sulphureus*, *A. versicolor*, *Trichothecium roseum*, *Populospora* sp. and *Cerrenalia* sp. were grown in the mycological broth. While *Fusarium solani* and *F. nivale* were grown in the nutrient broth with 50% seawater. Mass cultures of these strains were obtained by growing them at 29 ° C ± 1 in the culture laboratory. The periods of optimum growth and biomass production of individual strains were noted. The biomass from individual culture was removed at the optimum growth. This biomass was freeze dried using (lyophillizer Heto lyolab3000).

Preparation of extracts: The entire lyophilized biomass of individual strain was extracted in 90% aqueous methanol at ambient temperature of 29 °C ± 1. These extracts were dried by condensing them under reduced pressure using Laborota 4000 (Heidolph, Germany). The concentrated methanol extracts were stored in amber coloured sterilized glass bottles and used for studying their antibacterial and antifungal properties. Individual

culture of specimens used for the extraction has been deposited in the taxonomic reference center at National Institute of Oceanography (CSIR), Goa, India.

Antimicrobial activity: Antibacterial and antifungal studies were carried out using the disc diffusion method in agar plated petri dishes. Whatman (GF/F) filter paper discs of 6 mm diameter were prepared and sterilized in autoclave for ~15 minutes under 15 lbs pressure. The stock solution for the test was prepared by dissolving 0.5 gm of each extract in 1 ml of solvent depending upon the solubility of extracts either in methanol, acetone or dimethyl sulfoxide (DMSO). Final concentration of 500 µg $disc^{-1}$of each stock solution was obtained by spreading 10 µl of stock solution on the paper disc. The discs were placed in zobell marine agar and mycological agar (pH - 7.3), and plates were seeded with different bacterial and fungal strains. The cultures were incubated for 24-148 hours at room temperature, to obtain maximum growth in the culture media. The zones of inhibition around the discs were measured as No inhibition (inactive); + - 2-3 mm inhibition (mild); ++ - 4-5 mm inhibition (moderate); +++ - 6-8 mm inhibition (significant); ++++ - 9-11 mm inhibition (strong). Standard discs of antibacterial agent, Penicillin - G (1 unit $disc^{-1}$), Penicillin - G (10 unit $disc^{-1}$), Streptomycin (10 m cg $disc^{-1}$) and Ampicillin (10 m cg $disc^{-1}$) and antifungal agent Amphotericin - B (100 units $disc^{-1}$) and Nystatin (100 units $disc^{-1}$) were used to check the sensitivity. Control tests with the solvents loaded on to discs were performed in triplicate, for every bioassay of a set, in triplicate of each sample extract, and the results were expressed as average of an inhibition zone in mm.

RESULTS

The crude methanol extract of *Aspergillus niger* exhibited mild to significant activity against the food spoilage fungal strains such as *A. japonicus*, *A. niger*, as well as dermatophytes like *Candida* sp., *T. mentogrophytes* and *Fusarium* sp (Table 1). Similarly, the same species showed moderate to significant activity against *Bacillus cereus*, *B. circulans*, *B. pumilus*, *Pseudomonas versiculari* and *P. putida* (Table 2). The extract of *A. sulphureus* was active against the *Bacillus cereus*, *B. circulans*, *P. versiculari* , *P. putida*. But the same was observed to be inactive against all strains of food spoilage *Aspergillus* species and dermatophytes (Table 1). *Aspergillus versicolor* expressed moderate to significant activity against fouling bacteria (Fig 1). However, this species was moderately active against the food spoilage *A. japonicus* and *C. neoformans*. The *T roseum* was totally inactive against all the fouling bacteria, molds and dermatophytes (Table 1& 2).

Fusarium solani, a commonly occurring fungus, was observed to be strongly active against the *B. circulans.* However, it exhibited moderate to significant activity against the *Bacillus cereus*, *B. pumilius*, *P. versiculari* and *P. putida*. Similarly, *F. solani* exhibited antifungal activity against food spoilage *Aspergillus* species and dermatophytic fungi except *Candida sp.* (Fig 1). *Fusarium nivale*, a pathogen of *P. coarctata* plant showed moderate to significant activity against fouling bacteria (Table 2). This species also exhibited moderate to significant bioactivity against food spoilage *Aspergillus* (Fig 1). An obligate marine fungus *Populospora* sp. was moderately active against the *B. circulans*, *B. pumilus* and *P. putida*. *Cirrenalia* species was commonly occurred on vegetative parts as well as on roots of *P. coarctata* plant. This fungus was active against *Bacillus cereus*, *B. circulans*, *B. pumilus* and *Pseudomonas putida* (Table 2).

DISCUSSION

Aspergillus species grows saprotropically on numerous substrates and different environmental condition such as temperature, pH, and microbial competition. The present investigation revealed that all the three strains of *Aspergillus* such as *A. niger, A. sulphreus and A. versicolor* strongly inhibit the growth of marine bacteria, which are responsible for developing favorable substrate for the infestation of fouling organisms. Any material exposed to marine seawater environment experiences a sequence of discrete physical, chemical and biological events that result in the formation of complex layer for the attachment of marine bacteria. Marine bacteria such as *Pseudomonas* and *Bacillus* species serve as precursors in biofouling which cause tremendous damage to the man raised marine structures. These bioactive marine derived *Aspergillus* species would be potential source antifouling agents. Besides, *A. niger* also exhibited significant antifungal activity (Table 1) against food spoiling mould such as *A. japonicus* and dermatophyte *M. gypsum* which causes ring worm of scalps and nails to human. Recently, marine-derived *Aspergillus* species reported for novel and bioactive compounds from different marine environment [4, 9, 10]. *Fusarium* species associated with *P. coarctata* inhibited growth of most of the tested organisms except *Candida* sp. (Table 1 & 2), indicating the presence of potential an antimicrobial metabolite. These *Fusarium* species exhibited significant antibacterial and antifungal activities against dermatophytes (*M. gypsum* and *T. rubrum*) and *Aspergillus* spp. (food spoilage). Though, earlier *Fusarium solani* has

been reported for antifungal properties and other *Fusarium* species also forms a great source of commercially important chemicals [11, 12, 13]. The antibacterial and antifungal properties of *Fusarium* spp., particularly against 15 test organisms, indicated their great potential against dermatophytes, food spoilage *Aspergillus* species and marine bacteria.

Marine fungus *Cirrenalia* species was commonly occurred on vegetative part during monsoon season. Earlier this *Cirrenalia* species was reported for antifungal activity against plant pathogen [14], but in our studies this species was inactive against *Aspergillus* species and dermatophytes. This would be because of they used different species of fungal plant pathogen for their antifungal property. But marine anamorphic *Cirrenalia* species exhibited significant to moderate antibacterial activity. Marine derived fungus *Populospora* sp. mostly grows on decomposing *Porteresia* plant parts, this species inhibited the growth of *B. circulans, B. pumilius and P. putida.* Earlier same *Populospora* species was reported to be cytotoxic against human tumor cell line [15].

The fungal strains particularly species of *Aspergillus, Fusarium, Cirrenalia* and *Populospora* species exhibiting, antibacterial activity against marine bacteria in the present investigations could be the potential source of antifouling compounds.

CONCLUSION

In the present investigation marine derived (fungus-remove) *Fusarium* species exhibited both antibacterial and antifungal activity. *Aspergillus* species, *Cirrenalia and populaspora* species exhibited either one or both antibacterial and antifungal activity against dermatophytes, food spoilage *Aspergillu*s species and marine bacteria respectively. The antifungal and antibacterial bioactive fungal species reported in this study would be an importance in medicinal and biotechnological application.

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Table 1: Antifungal bioactivity of marine derived fungi against food spoiling Aspergillus species and dermatophytes

Name of species	Moulds			Dermatophytes							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Fungi											
A. niger	-	+++	+		++	+++	-	-	-	++	-
A. sulphureus	-	-	-	-	-	-	-	-	-	-	-
A. versicolor	-	++	-	++	-		-	-	-	-	-
Trichothecium roseum	-	-	-	-	-	-	-	-	-	-	-
Populospora sp.	-	-	-	-	-	-	-	-	-	-	-
F. solani	++	+++	+++	++	-	+++	++	+++	++	++	++
F. nivale	++	++	+++	++	-	+++	++	++	++	++	++
Cirrenalia sp.	-	-	-	-	-	-	-	-	-	-	-
Standard Antibiotics											
Amphotericin –B 100 unit disc^{-1}	++	++++	++++	+++	+++	+++	+++	+++	+++	+++	+++
Nystatin 100 unit disc $^{-1}$	++	+++	-	++++	++++	+	++	-	++	++	++

Legends:

Molds

1. *Aspergillus niger*
2. *A. japonicus*
3. *A. fresenii*

Dermatophytes

4. *Cryptococcus neoformans*
5. *Candida sp.*
6. *Microsporum gypsum*
7. *Trichophyton mantagrophytes*
8. *T. rubrum*
9. *T. violaceum*
10. *T. tonsurns*
11. *Fusarium sp.*

Table 2: Antibacterial activity of marine derived fungi against marine bacteria

Name of species	Marine Bacteria				
	B. cereus	***B. circulans***	***B. pumilius***	***Pseudomonas versicularis***	***P. putida***
Fungi					
Aspergillus niger	++	++	++	+++	+++
A. sulphureus	+++	++	-	+++	++
A. versicolor	+++	+++	+++	+++	++
Trichothecium roseum	-	-	-	-	-
Populospora sp.	-	++	++	-	++
Fusarium solani	+++	++++	++	++	+++
Fusarium nivale	++	+++	++	++	+++
Cirrenalia sp.	+++	++	++	-	+++
Standard Antibiotic					
Penicillin – G 1 unit disc $^{-1}$	-	++++	-	-	+++
Penicillin – G 10 unit disc $^{-1}$	-	++++	-	-	++++
Streptomycin 10 mcg disc $^{-1}$	+++	++++	++++	++++	++++
Ampicillin 10 mcg disc^{-1}	-	++++	++++	-	++++

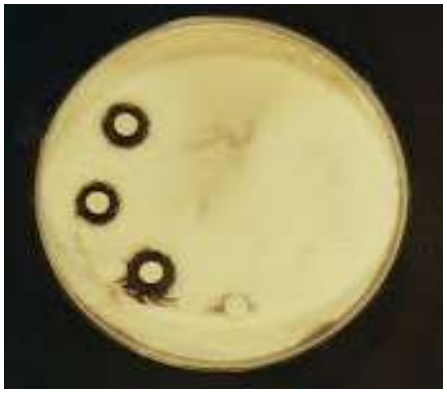
Fig 1: Antifungal & Antibacterial bioactivity of marine derived fungi against dermatophytes, food spoilage *Aspergillus* spp. and marine bacteria

***A. versicolor* (marine bacteria)**

***F. nivale* (moulds)**

***Populospora sp.* (marine bacteria)**

***F. solani* (Dermatophytes)**



REFERENCES

1. Xu L et al. Antibacterial and antifungal compounds from marine fungi. Mar Drugs 2015; 13: 3479-3513.
2. Molinski TF et al. Drug development from marine natural product. Nat Rev Drug Discovery 2009; 8: 69-85.
3. Meyer AMS et al. Marine Pharmacology in 2009 -2011: Marine compounds with Antibacterial, Antifungal, Anti-Inflammatory, Anti-protozoal, Antituberculosis, and Antiviral activities; Affecting the Immune and nervous system, and other Miscellaneous Mechanism of action. Mar drugs 2013; 11:2510-2573.
4. Bugni TS, Ireland CM. Marine- derived fungi: a chemically and biologically diverse group of micro-organisms. Nat Prod Rep 2004; 21:143-163.
5. Niranjan HP et al. Study of onychomycosis at a tertiary care hospital in South India. J Evol Med Dent Science 2012; 1(5):823–829.
6. Pitt JI, Hocking AD. Fungi and food spoilage. 3rd ed.; Springer: New York, 2009.
7. John A et al. Composition of *Pseudomonas putida* biofilms, accumulation of proteins in the biofilm matrix. Biofouling 1999; 14: 49-57.
8. Collins, CH, Tayler, CED. Microbiological Methods. Plenum Press: New York, 1967
9. Liao L et al. Alkaoidal Metabolites from a marine-derived *Aspergillus sp*. fungus. J Nat Prod 2015; 78: 349-354.
10. Saraiva NN et al. Cytotoxic compounds from the marine-derived fungus *Aspergillus* sp. recovered from the sediment of the Brazailian coast. Nat Prod Res 2015; 29: 1545-1550.
11. Chakravarthi BVSK et al. Production of paclitaxel by *Fusarium solani* isolated from *Taxus celebica*. J of Bioscien. 2008; 33: 259-267.

12 Deng BW et al. *Fusarium solani*, Tax-3, a new endophytic taxol-producing fungus from *Taxus chinensis*. World J Microbiology Biotech 2009; 25: 139-143.

13 Bhosale S et al. Process for the isolation of pharmaceutical compound Cyslosporin A from fungus *Fusarium nivale* U. S. Patent 7,335,495, Feb 26, 2008.

14 Cuomo V el al. Antimicrobial activities from marine fungi J. Mar Biotech 1995; 2: 199-204.

15 Margareth BCG et al. Chemical constituents of *Papulospora immersa*, an endophyte from *Smallanthus sonchifolius* (Asteraceae) and their cytotoxic activity. Chem & Biodiversity 2010; 7: 2941-2950.