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Research Article **ISSN: 2321-5038**

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND LIFE SCIENCES
www.pharmaresearchlibrary.com/ijrpls

Isolation and characterization of fresh water fungal metabolites against Clinical pathogens

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Received: 25 February 2014, Accepted: 5 April 2014, Published Online: 24 May 2014

Abstract

Fresh water samples were collected from Cauvery River in Thanjavur district, Tamilnadu, India. Five sampling stations (Kallanai, Thirukkatupalli, Thiruvaiyar, Papanasam, Kumbakonam) were selected for sample collection. The water samples were examined for fungi by plating method culturing in Rose Bengal agar and Potato Dextrose agar medium. The isolated fungal strains were identified by Lactophenol Cotton Blue staining. A total number of 25 species of fungi belonging to 2 genera were recorded. Thus the water samples from Cauvery river of Tamil Nadu yield impressive diversity of fungi. Among 25 species, *Aspergillus* was selected for screening and production of metabolites. The extracted fungal metabolites were tested against *E.coli* and *Pseudomonas* for antimicrobial activity.

Key words: *E.coli*, *Pseudomonas*, antimicrobial activity, *Aspergillus*

Contents

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PAPER-QR CODE

1. Introduction

Freshwater -derived fungi have been widely studied for their bioactive metabolites and have proven to be a rich and promising source of novel anticancer, antibacterial, antiplasmodial, anti-inflammatory and antiviral agents. Considering the number of novel bioactive compounds that have been isolated from freshwater- derived fungi and the fact that the number of the compounds is increasing rapidly. The introduction of penicillin and other antibiotics ushered in an era of effective treatment of microbial infections. However, with the overuse of antibiotics, resistance of pathogens to antimicrobial agents has become an escalating problem. In the past several years, only a few antibacterial agents have received approval by the US Food and Drug Administration. Therefore, there is an urgent need for effective new antimicrobial agents. Fungi have an excellent record in producing novel bioactive compounds and many of these presently have important medicinal and other uses. (JE Lazaro *et al.*, 2001). Nowadays the

researchers realizing the capability of microorganisms such as fungi to produce diverse bioactive molecules and the existence of an explored microbial diversity, research is underway to isolate and screen fungi from diverse habitats and unique environments for discovery of novel metabolites. Fungi are an indispensable part of the life in the biosphere for they have a functional role in ecosystems. Most if the species reported are found to be saprobic or pathogenic on higher plants. One such group is *Coelomycetes* (mitosporic fungi) representing 7000 species recorded from widest range of ecological niches (K Tayung *et al*., 2011). *Coelomycetes* are known to be imperfect fungi in which conidia are formed within a cavity lined by either fungal tissue, host tissue or a combinational of both (PM Kirk *et al.,* 2001 and WB Grove, 1935). Such organisms are the asexual reproducing states of ascomycetes (BC Sutton, 1980). They are commonly found and reported from cultivated and uncultivated soil of different types leaf litter and other organic debris from both natural (HJ Hudson, 1968 and R Senthilkumaran *et al.,* 2009). and manufactured sources from saline and fresh water (GC Hughes, 1975).

Until recently, the search for new fungal metabolites concentrated mainly on the random screening of isolates. In optimizing the search for new bioactive secondary metabolites, it is relevant to consider that the secondary metabolites a fungus synthesizes may correspond with its respective ecological niche, e.g. the mycotoxins of plant pathogens that metabolic interactions may enhance the synthesis of secondary metabolites. Thus, the fungi screened should originate from which fungi have not been previously isolated for biochemical purpose and they should have metabolic interactions with their environment. This is an example of intelligent screening and is a strategy for exploiting the untapped potential for secondary metabolites that fungi offer

2. Materials and Methods

Study area: Five different stations from the Cauvery River were selected for water sample collection for fungal diversity analysis from Thanjavur city viz., Station-1.Kallanai, Station-2.Trirukkatupalli, Station-3.Triruvaiyaru, Station-4.Papanasam Station-5.Kumbakonam.

Collection and analysis of fungi: The water samples for fungal diversity analysis were collected in the river. The samples were collected from each collection site. Water sample for fungal diversity analysis was collected in sterile plastic bottles from three different locations from each site and brought to the laboratory.

Isolation of pure culture of fungi

Isolation of fungi was out by serial dilution method (JG Cappuccion, *et al.,* 2006). Cultures were maintained on potato Dextrose agar (PDA) and Rose Bengal agar medium.

Identification of fungal strains: The isolated fungal strains were identified by using Lacto phenol cotton blue stain and fungal slide culture technique. The standard plate count is a reliable method for enumerating fungi (JG Cappuccion, *et al.,* 2006). Identification of pure cultured fungi by direct mount from culture and its slide was help of aquatic fungi manual (RD Khuble, 2001 and MW Dick, 1990).

Test bacteria: Two clinical bacterial pathogens such as *Pseudomonas sp, E.coli,* were obtained from Dept of Microbiology, Urumu Dhanalakshmi College, Trichy, Tamil nadu.

Primary screening of antimicrobial substance from fresh water fungi

Aspergillus cultures were inoculated into fermentation broth supplemented with filtered fresh water and incubated at 28°C for 3 weeks. After incubation, the mycelial growth was removed by using centrifugation. After collecting the supernatant, used for primary screening of antibacterial activity against test organisms. The supernatant were load in a sterilized disc placed over the MHA plate inoculates with 2 test organisms. All the plates were incubated at 37°C for 24 hours.The zone of inhibition was measured and expressed in diameter in millimeter.

Production of antimicrobial substance from fresh water fungi

Aspergillus isolates were inoculated into each nutrient agar plates and incubated at 28°C for 3 weeks. Then the mycelia growth was remove aseptically using sterile spatula. The agar medium was cut to pieces and extracted using (1:2 ratio) acetone and ethyl acetate respectively, for 24 hours. The acetone and ethyl acetate portion was collected and concentrated by evaporation. Quantity of crude extract was measured and the stock solution was prepared in the concentration of 10mg/ml of acetone and ethyl acetate respectively.

Antimicrobial activity of fungal extracts

Antimicrobial activity of fungal extracts was tested by using paper disc diffusion method (NCCLS, 2003). 10 µ 1 of crude extract from stock solution was added in to sterile filter paper disc (5 mm in diameter) and allowed to dry. Final concentration of crude extract is 100 µ g/disc. The crude extract impregnated discs were placed over MHA plates inoculated with test organisms. All inhibition was measured and expressed in diameter in millimeter.

3. Results and Discussion

In the study, totally 25 species of fungi were isolated by dilution plating technique (Table-1). Out of 25 species recorded, the maximum number of organisms recorded belonged to Deuteromycetes followed by zygomycetes. The fungi that were more frequently isolated for these studies were *Rhizopus nigricans, Aspergillus terreus, Aspergillus*

niger, *Penicillium sp.*, *Fusarium sp*., *Alternaria*, *Gliocladiopsis*, *Cladosporium* respectively. Besides these, 4 unidentified fungal forms were isolated and recorded. Among 25 species, *Aspergillus* was selected for screening and production of metabolites. The extracted fungal metabolites were tested against *E.coli & Pseudomonas* for antimicrobial activity.

Fungi are ubiquitous achlorophyllous and heterotrophic organisms, which are directly influenced by environmental factors. They are cosmopolitan in occurrence and are found in rivers, oceans and occur commonly on decomposing organic matter. Excessive levels of nutrients and other chemicals lead to changes in aquatic life (D Dudgeom *et al.,* 2006). Heterotrophic organisms are usually present in natural water in direct proportion to the physicochemical nature of the aquatic environment (D Prasad *et al.,* 2009). Fungi play an important biological process in an aquatic ecosystem. Aquatic fungi contribute significantly in aquatic ecosystem as decomposers of animal and plant remains (TW Jonshonn, 1956 and FK Sparrow, 1968). Aquatic fungi contribute to the energy flow and productivity of ecosystem by their active role in the utilization and biodeterioration of organic materials (RD Khuble, 1991). These fungi also process the ability to paradise aquatic plants and animals including fishes under certain condition (CT Ingolg 1964). New trends in drug discovery from natural sources emphasize on investigation of the freshwater ecosystem to explore numerous complex and novel chemical entities. These entities are the source of new lead for treatment of many diseases such as cancer, AIDS, inflammatory condition, arthritis, malaria and large variety of viral, bacterial, fungal diseases. Because of the fresh water contamination, the organisms produce a variety of molecules with unique structural features and exhibit various biological activities. Infectious diseases are leading health problems with high morbidity and mortality in the developing countries. The development of resistance to multiple drugs is a major problem in the treatment of these infectious diseases caused by pathogenic microorganisms.

Table 1. Fungi isolated from five sampling stations

S.No	Species	No. of Colonies				
		S_1	S_2	S_3	S_4	S_5
	Deuteromycetes					
1	*Alternaria alternate*	+	-	-	-	+
2	*Aspergillus awamori*	-	-	+	+	-
3	*A.clavatus*	+	+	+	-	-
4	*A.flavus*	+	+	+	-	-
5	*A.fumigatus*	+	+	+	+	+
6	*A.humicola*	+	+	+	+	+
7	*A.luchuensis*	+	+	+	+	+
8	*A.nidulans*	+	+	+	+	+
9	*A.niger*	+	+	+	+	+
10	*A.sulphureus*	-	+	+	+	+
11	*A.variecolor*	+	+	-	-	+
12	*A.versicolor*	+	-	-	-	-
13	*A.wentii*	+	-	-	+	-
14	*Cladosporium sp.*	-	+	+	+	+
15	*Fusarium oxysporum*	--	+	-	+	+
16	*Gliocladiopsis sp.*	+	-	-	+	+
17	*Penicillium funiculosum*	+	+	-	+	-
18	*Penicillium sp.*	+	+	+	+	+
	Zygomycetes					
19	*Rhizopus nigricans*	+	+	+	+	+
20	*Black sterile mycelium*	+	-	-	-	-
21	*White sterile mycelium*	-	-	+	+	+
22	*Unidentified fungi*	-	+	-	-	-
23	*Unidentified fungi*	-	+	-	-	-
24	*Unidentified fungi*	-	+	-	-	+
25	*Unidentified fungi*	+	-	+	+	+

(+) = Present; (-)=Absent;
s_1= Kallanai; s_2= Thirukkatupalli;
s_3= Thiruvaiyar; S_4= Papanasam;
s_5= Kumbakonam

4. Conclusion

A total of 25 fungal species were isolated and identified from Cauvery River at Thanjavur district of Tamilnadu. *Aspergillus sp* were dominant than other fungal species. The antibacterial activity of *Aspergillus* against fungal metabolites was tested. This multidrug resistance is presently an urgent focus of research and new bioactive compounds are necessary to combat these multidrug resistance pathogens. On this view point, attempts have been made to develop novel drugs against infectious diseases for the mitigation of suffering of the vast masses of humanity.

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