

ANTIMICROBIAL PROPERTIES OF SOIL FUNGI ISOLATED FROM SEA BEACH AND DRY FISH AREA OF PUDUCHERRY COAST

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ABSTRACT

Fungi from different sources regularly soil provide pharmaceutical products and other valuable substances, including organic acids, enzymes that are used in varied ways for the mankind. In the present study, Beach soil and Dry Fish area soil of Muthialpet coast, Puducherry were studied for the isolation and enumeration of fungi based on their availability. Dry Fish area soil (73%) was dominated with the fungal flora than Beach soil (27%) in our study. *Aspergillus niger* was found as the dominant one in Dry Fish area soil but *Penicillium chrysogenum* was the dominant one in Beach soil. The results obtained clearly indicated that *Aspergillus flavus*, *A. terreus*, *Aspergillus niger*, *A. terreus* and *Penicillium verruculosum* were recorded at different concentration in both the soils. Among the isolates, aspergillus and penicilli were dominant in all the soil conditions due to high sporulation capacity. *Penicillium chrysogenum* and *Aspergillus flavus* were found to be more effective fungi in order to prevent the growth of the pathogenic bacteria and *Candida albicans* than other isolated fungi in our present study. The frequency of mycoflora in soil samples in beach area were found to be regulated by many factors like temperature, humidity, vegetation, organic and inorganic materials, soil type and texture. *Pseudomonas* sp. and *Candida albicans* were found as more susceptible pathogens towards the fungal active plugs in our study. The present would be helpful to the enthusiastic people who have great interest to find out stressful microbe in particular with fungi from these types of environments to find any bioprospecting fungi.

KEYWORDS: Antimicrobial properties, Soil fungi, Sea beach and Dry fish area, Puducherry Coast, fungal flora, *Candida albicans*

INTRODUCTION

Fungi are very successful microbial inhabitants of soil based on their high elasticity and their capacity to accept various forms in response to adverse or uncomplimentary conditions.^[1] Because of their aptitude to produce a wide variety of extracellular enzymes, they are gifted to break down all kinds of decomposing soil components, organic matter and thus regulating the balance of carbon and nutrients.^[2,3] Fungi convert dead organic matter into biomass, carbon dioxide, and organic acids. Many species of fungi own the ability to act as an effective bio sorbent of toxic metals such as cadmium, copper, mercury, lead, and zinc, by accumulating them in their fruiting bodies. Though these elements may inhibit their growth and affect their reproduction.^[4,5] The diversity and activity of fungi is regulated by various biotic (plants and other organisms) and abiotic (soil pH, moisture, salinity, structure, and temperature) factors.^[6,7] Fungi can be found in almost every environment and can live in wide range of pH and temperature.^[8,9] The aim and objectives of the present investigation is to record the

fungal flora of Sea beach soil and Dry sea fish soil in the coastal area of Puducherry. It will be finding out the percentage contribution of different fungal species in above said soil. Further the fungal extracts and their active culture agar plug were evaluated for the antimicrobial properties of the culturable fungi against pathogenic bacteria and fungi.

MATERIALS AND METHODS

In our present study period, isolation and identification of soil fungi was done from Beach soil and Dry sea fish soil near Muthialpet beach area of Puducherry district.

Study site

Pondicherry is the Union Territory of India and one of the major districts of Puducherry state situated in the coromandel coast of Bay of Bengal and around 160 km south of Chennai metropolis. Puducherry is located on the tributary of the River bank of Kaveri at 11.9139° N, 79.8145° E. The climate of the district is generally characterized by high humidity almost all round the year,

oppressive summer and retreating seasonal rainfall during October to December. The temperature varies between 20 and 40°C and average annual rainfall is within 1000 mm. The nature of the soil are generally red ferrallitic, black clay and coastal alluvial. Paddy, Gingelly, Maize, Ground nut, Cotton are main kharif crops while Sugarcane, Maize and Pulses are cultivated in Rabi season are prevailing in the Union territory of Puducherry. In addition to these crops, other cash crops and vegetable crops are plenty in the district.

Collection of soil samples

The soil samples from the beach soil and dry fish soil situated at Muthialpet Beach of Puducherry were collected during January 2020 to March 2020 at different intervals. The soil samples were collected from the areas just 5-10 cm depth by small sterilized polythene bags and brought to the Microbiology laboratory, Department of Botany, K. M. Govt. Institute for Postgraduate Studies and Research (Autonomous), Pondicherry-605008 with utmost care and stored at 4° C in the refrigerator for further studies.

Isolation of fungi from the soil samples

The soil micro-fungi were isolated and enumerated by two methods, namely Soil Dilution^[10] and soil plate method^[11] on different media such as Potato Dextrose Agar and Sabouraud Dextrose Agar.

Methods of isolation of Beach soil fungi

1gram of each soil sample was suspended in 10ml of double distilled water to make microbial suspensions (10^1 to 10^5). Dilution of 10^{-2} and 10^{-3} were standardized in order to get countable number of colony forming units (CFUs) and 10^{-3} dilution was employed to isolate fungi in the present study.^[9] 1 ml of microbial suspension of each concentration were added to sterile Petridishes (triplicate of each dilution) containing 15-20 ml of sterile Potato Dextrose Agar and Sabouraud Dextrose Agar. One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth. The petridishes were then incubated at $25 \pm 3^{\circ}\text{C}$ in dark condition. The plates were observed after second day up to seventh day with routine check-up. On third day onwards, the plates were counted for the total number of colony forming units (CFUs) and followed by identification of fungi up to species level.

Identification of the soil fungi

Fungal morphology was studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lactophenol cotton blue and observed under compound microscope for the conidia, conidiophores and arrangement of spores. The fungi were identified with the help of available literature and monographs present in the laboratory and with the expertise of the research scholars.^[12,13,14,15,16,17,18]

Antibacterial activity of the active agar plugs of beach soil fungi

The active agar plugs of 6mm size were taken carefully from the three-day pure culture plates of the beach soil and dry fish area soil fungi and were studied for antimicrobial activity against pathogenic bacteria (clinical isolates) using agar plug assay method. The test organisms used were MTCC cultures i.e., *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas fluorescense*, *E. coli*, *Pseudomonas* sp., *Vibrio cholera*, *Bordetella pertussis*, *Salmonella typhi*, *Klebsiella* sp. and *Candida albicans*. The microbes were grown in nutrient broth for 12 h. Lawns of pathogenic bacteria and *Candida albicans* were prepared on nutrient agar plates using sterile cotton swabs. Active agar plugs were placed on nutrient agar plates and each plug was placed inside the wells prepared earlier by cork borer. The plates containing bacteria and active plugs of endophytic fungi discs were incubated at 37°C for 24 to 48 hours in the BOD incubator. The plates were examined for the zone of inhibition after 24 hrs, which appeared as clear area around the wells. Inhibition zone diameter was measured in mm by the HI-Media scale.

RESULTS AND DISCUSSION

During the study period, isolation and identification of soil fungi was made from sea beach and dry fish area soil in Muthialpet coast of Puducherry during January to March 2020. The growth of soil fungi (CFUs) on agar plates isolated from both the soil samples by serial dilution method (Waksman 1922) is given in Fig 1a & 1b respectively. In concentration and composition, dry sea fish area soil was found to be the highest contributor of soil fungi per gram soil than sea beach soil (Fig 2).



Fig. 1a: Growth of fungi (CFUs) on agar plates isolated from Beach soil.

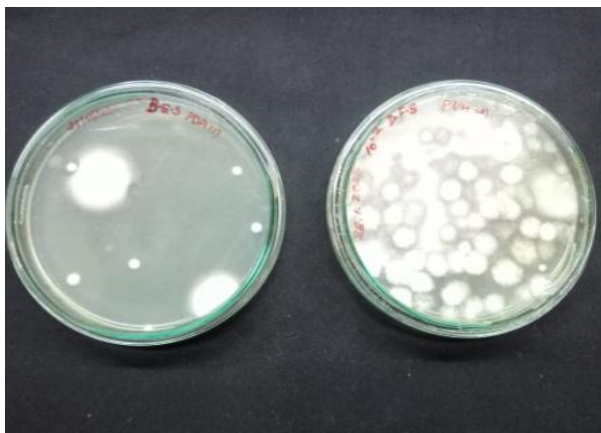


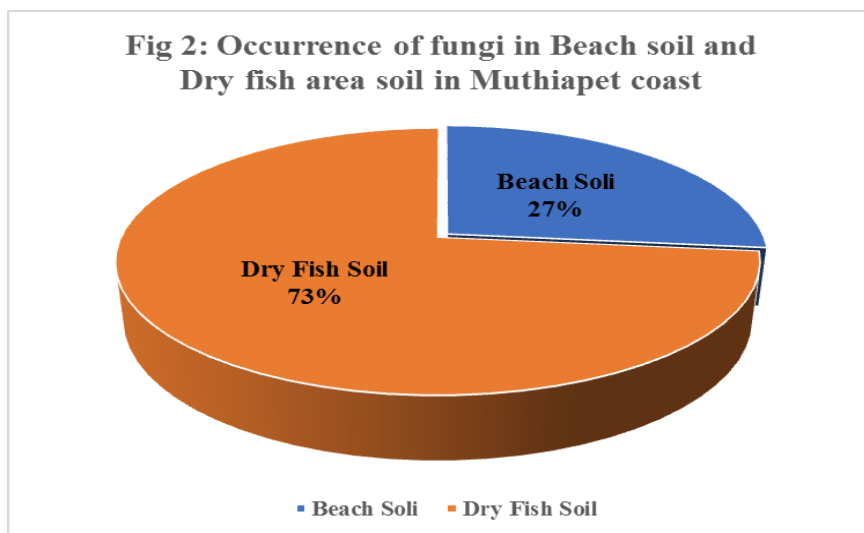
Fig. 1b: Growth of fungi (CFUs) on agar plates isolated from dry fish area soil.

In our soil mycoflora study, altogether 162 fungal colony forming units (CFUs) were isolated from the soil samples of Sea beach and Dry fish area soil in Muthialpet coast. In fungal composition, a total of 8

species under 2 genera were recorded from both of the soils. Total number of fungal colony forming units (CFUs) isolated from different soil samples of each area is given in Table 1. Beach area soil contributed the maximum (27%) of fungal population followed by Dry fish area soil (73%) (Fig 2). It was found that the fungal diversity of the soils was dependant on a large number of factors of the soil such as organic content mostly from the dead fishes available in the attached soil, their pH as well as the moistures prevailing in that soil environments³. Most of the reports on the same study like us opined that the physicochemical parameters like, soil pH and their textures are also determining the fungal population in different fields of varied places. The prevalence of soil fungal flora in our work was found equivalent to Gaddeyya and his coworkers^[19] who reported 173 fungal colonies under 15 fungal species from the crop fields.

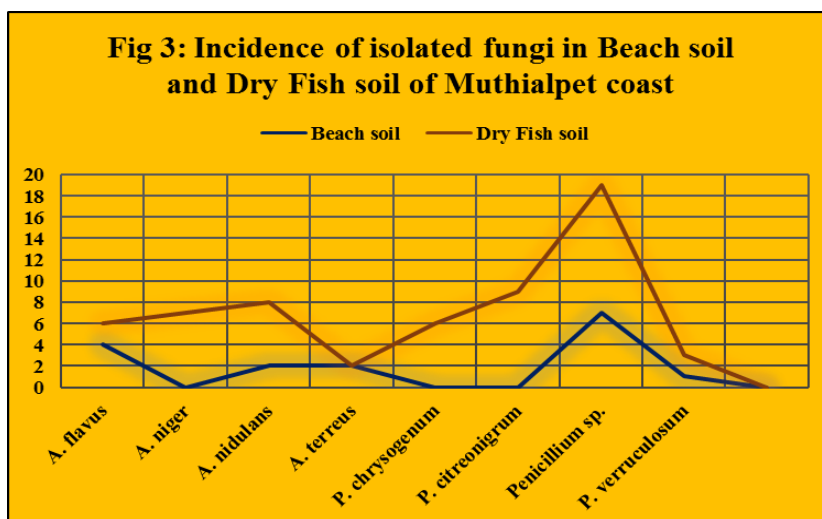
Table 1: Percentage occurrence of soil fungi isolated from Sea Beach and Dry sea fish area of Muthialpet coast, Puducherry.

Sl. no.	Name of the fungi	Beach soil	Dry fish area soil
1	<i>Aspergillus flavus</i>	25	4.5
2	<i>Aspergillus niger</i>	0	15.9
3	<i>Aspergillus nidulans</i>	12.5	13.6
4	<i>Aspergillus terreus</i>	12.5	0
5	<i>Penicillium chrysogenum</i>	0	13.6
6	<i>Penicillium citreonigrum</i>	0	20.4
7	<i>Penicillium sp.</i>	43.75	27.3
8	<i>Penicillium verruculosum</i>	6.3	4.5



Among the fungal isolates, aspergilli were dominant followed by penicilli. It may be ascribed that the biomaterials available in the field promoted the growth and proliferation of mould fungi like aspergilli and penicilli in the soil samples on the beach area.^[4]

Diversity of soil fungi was found to be higher in the Dry Fish area soil as compared to the Beach soil, where the humidity, shade as well as the nutrients secreted from dead fishes aggregated with soil particles might have enhanced the mycoflora. The fungal variation in two types of soils and their percentage frequency are given in Table 1.



Aspergillus were isolated with four species like, *Aspergillus. flavus*, *A. niger*, *A. nidulans* and *A. terreus*. *Penicillium* spp like *P. citreonigrum*, *Penicillium chrysogenum*, *Penicillium sp.* and *Penicillium verruculosum* were recorded from Dry fish area soil but *A. flavus*, and *A. nidulans* and *A. terreus*, *Penicillium sp.* and *Penicillium verruculosum* were recorded from Beach soil only. White sterile mycelia were not recorded in any numbers from both the soils. Other Dematiaceous fungi were isolated sporadically from the soil samples.

During the study we found most of the isolated fungi are preventing the growth of pathogenic bacteria studied by well plug method by employing active grown fungal cultures on agar (Table 2). *Penicillium chrysogenum* and *Aspergillus flavus* were found to be more effective fungi in order to prevent the growth of the pathogenic bacteria and *Candida albicans* than other fungi studied in our present work (Table 2).

Table 2: Antimicrobial potency (inhibition zone) of agar plug from the active culture of soil fungi against MTCC pathogens studied by well diffusion method.

MTCC strains	Fungi from Beach and Dry sea fish area soil/ Inhibition zone in mm					
	<i>Penicillium chrysogenum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Aspergillus niger</i>	<i>Penicillium citreonigrum</i>	<i>Aspergillus nidulans</i>
<i>Staphylococcus aureus</i>	15	25	20	11	12	12
<i>Bacillus</i> sp.	14	21	12	13	15	15
<i>Pseudomonas fluorescense</i>	20	15	13	17	13	13
<i>E. coli</i>	18	13	15	14	12	12
<i>Pseudomonas</i> sp.	12	22	16	15	21	21
<i>Vibrio cholera</i>	20	21	13	18	13	13
<i>Bordetella pertussis</i>	20	18	12	22	16	16
<i>Salmonella typhi</i>	27	15	11	12	14	14
<i>Candida albicans</i>	23	14	12	16	13	13
<i>Klebsiella</i> sp.	15	14	13	15	15	15

Relative occurrence of soil fungi isolated from the two soils are given in Fig 2 and distribution and composition of fungal isolates are given in Fig 3, which showed the different pattern of their distribution in the soil. Huda²⁰ during 2010 described in his work about the bacterial and fungal filtrates as well as the isolated species for their antimicrobial activities against some pathogenic microbes causing dermatological diseases (*Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA) and *Aspergillus niger*). In their work, the bacterial filtrate showed significant antagonistic effect against *S. aureus* and methicillin resistant *S. aureus* (MRSA), whereas showed non inhibitory action

on the pathogenic fungus. In contrast, the fungal filtrate antagonized the growth of the pathogenic fungus (*A. niger*) and did not produce any inhibitory effect on the two tested pathogenic bacteria. Sethi and his team²¹ worked on Antibiotic production by microbes isolated from soil and found that among all the screened isolates, *Penicillium chrysogenum* metabolite showed the maximum inhibition against both gram positive as well as gram negative bacteria. Production of antibiotic by microorganisms from soil is affected by many factors including nitrogen and carbon source. Therefore, there is a great need to optimize with different substrates that

provides maximum production of antimicrobial substance.

Earlier workers on their work on soil fungi described that the Soil pH, organic and moisture contents are the main factors affecting the fungal population and diversity⁴. Further it was documented that the organic phosphorus, carbon, potassium and nitrogen are important for the growth of soil fungi. They observed that the absence of the abovesaid growth parameters, the growth and sporulation of moulds as well as other microbes are not possible in the soil.^[22]

Anitha and Nayak^[23] reported that the density of fungal population occurred during the monsoon period when the sand dune soil moisture was significantly high. They^[23] also supported and elaborated that the environmental factors such as temperature, organic carbon, organic nitrogen, pH and moisture, play an important role in the distribution of mycoflora and it was supported by Frac *et al.*^[24]

CONCLUSION

In our recent study, Beach soil and Dry Fish area soil of Muthialpet coast, Puducherry was studied for the isolation and enumeration of fungi based on their availability. Dry Fish area soil (73%) was dominated with the fungal flora than Beach soil (27%) in our study. *Aspergillus niger* was found as the dominant one in Dry Fish area soil but *Penicillium chrysogenum* was the dominant one in Beach soil. The results obtained clearly indicated that *Aspergillus flavus*, *A. terreus*, *Aspergillus niger*, *A. terreus* and *Penicillium verruculosum* were recorded at different concentration in both the soils. *Aspergillus flavus* and *Penicillium chrysogenum* were found to be more effective fungi in order to prevent the growth of pathogenic bacteria than other fungi isolated and studied in our present study. The frequency of mycoflora in sand dunes in beach area were found to be regulated by many factors like temperature, humidity, vegetation, organic and inorganic materials, soil type and texture. *Pseudomonas sp.* and *Candida albicans* were found as more susceptible pathogens towards the fungal active plugs in our study. The present will be helpful to the people who have boundless interest to find out stressful microbes particularly fungi from these type of environments for their bioprospecting.

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