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Aquatic Fungi in Polluted Marine Water and their Relationship with Dried Fish

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The fungal flora of the polluted water and mud samples of Pasni and Ormara coasts were investigated using baiting and plating techniques. Most of the isolated fungi were the members of Mastigomycotina, Zygomycotina and Deuteromycotina. Fungi isolated from dried fish were the same as isolated from mud and water samples. *Saprolegnia parasitica, S. declina* and an unidentified lower fungi recorded exclusively from dried fish samples.

Key words: Aquatic fungi, Marine pollution, Marine fish.

Introduction

Fungi in polluted marine water have important role in the biological breakdown and conversion of organic material in marine ecosystem (Cuomo and Pagano 1987). Cook (1994) isolated different groups of fungi from polluted water by using different isolation techniques. Fish is an important source of protein for the entire world. Like the terrestrial plants and animals, fish also suffer from fungal diseases (Wood 1988). Fungi in fish may occur as secondary parasites, facultative parasites as well as primary pathogenic causative agents of the diseases. Fungal spores as well as hyphae found in fish externally show no pathological signs (Roberts 1972). *Aspergillus* and its teliomorph *Eurotium* are among the most encountered fungi which are of great significance in the spoilage of food because of their ability of producing mycotoxins. This study was undertaken to establish the microfungal flora present in polluted water and compare them with the mycoflora present in dried fish samples.

Materials and Methods

Polluted water and mud samples were collected from Pasni and Ormara coasts of Baluchistan, Pakistan during the period of 31-01-1990 to 24-06-1990. The samples of dried fish include Cat fish (*Arius thalassinus, A. dussumieri*), Frigetetuma (*Auxis thazard*),Scad (*Alepes djedaba*), Trigatooth Croaker (*Otolithes riber*), Shark (*Scolidon laticandius*), Queen fish (*Scomberoides lysan*) and yellow fish tuna (*Thumus calbacares*).

Sampling and isolation. Samples of water and mud were collected at random. On each occasion three replicate samples of benthic mud were taken from near the edge using a 0.5 cm alcohol-sterilized cork borer and placed in the polyethylene bags for transport to the laboratory. Water samples were collected in three sterile 250 ml flasks from below the surface about 0.5 cm out from the edge. In the laboratory 0.1 mud sample was placed at the center of ten 8 cm dia, petri dishes containing 2% water agar and incubated in the dark at 18°C. Two methods were used to isolate fungi from the water samples. First six 1.0 ml portions were pipetted out from each flask into separate sterile petri dishes and mixed with about 15 ml molten tap water agar cooled to 45°C, then 50 boiled hemp seeds were added to the water remaining in each flask and allowed to stand for 24 h in the dark at room temperature. Five seeds were then transferred to petri dishes containing 20 ml of a sterilized mixture of tap water and distilled water (1:1) with Penicillin and Streptomycin added @ 20 units per ml to suppress bacterial growth. A total of 30 dishes with hemp seed baits were prepared each month. Half of the inoculated culturese were maintained at 4°C and the remainder at 18°C. Fungi from the dried fish samples were isolated by cutting the infected portion of each fish at 5 different sites and placed into flasks containing 250 ml sterile distilled water; then 45 sterilized hemp seeds were added to each flask. The flasks were kept in dark for 5 days and the colonized seeds were transferred to the petri dishes (15 seeds per dish) containing 15 ml sterilized mixture of distilled and tap water in a 1:1 ratio and observed under microscope at 40x. Infected portion of the fish was cut into small segments (1 cm) and 5 such pieces were plated onto PDA plates containing Penicillin and Streptomycin @ 20 units per ml. Plates were incubated at room temperature at 28°C for 15 days and observed for fungal growth under compound microscope with 40x objective.

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Results and Discussion

More than 30 different species of microfungi were isolated from the mud and water samples of Pasni and Ormara coasts (Table 1). There were minor differences for example *Verticillium* sp and unidentified Phycomycetes were found only in Ormara while *Alternaria alternata* was isolated from Pasni; but on the whole the lists from the two coasts are broadly similar. Most of those found may be regarded as terrestrial forms. Baiting the water with hemp seeds closely isolated a different group of fungi, mainly those with zoospores. There was not much difference between fungal flora isolated from the water and the mud samples of the two coasts and dried fish. The range of fungi isolated from the two coasts was broadly similar to that found in natural water in this country (Mehdi and Saifullah 1992) and abroad (Hyde and Jones 1988; Jones and Kutbutheen 1989). Many of the species found are common in the soil and on the vegetation and were presumably imported on allochthomous plant detritus (Barlocher and

Table 1
Fungal species isolated from Ormara and Pasni coasts of Baluchistan

	Ormara					Pasni				
	Water		Mud	Dried fish		Water		Mud	Dried fish	
Fungal species	Plated	Baiting	Plated	Plated	Baiting	Plated	Baiting	Plated	Plated	Baiting
Achiya americana	-	+	-	-	+	+	+	-	-	+
Alternaria alternata	-	-	-	-	-	+	-	+	-	-
Aternaria sp.	-	-	+	+	-	-	-	+	+	-
Aspergillus flavus	+	-	-	-	-	+	-	+	+	-
A glaucus	+	-	+	-	-	+	-	+	+	-
A niger	+	-	+	+	-	+	-	+	-	-
A sulphurus	-	-	-	+	-	+	-	+	-	-
Aspergillus sp.	+	-	+	-	-	+	-	-	-	-
Candida sp.	+	-	+	-	-	-	-	+	-	-
Cephalosporium sp.	+	-	-	-	-	+	-	+	-	-
Chaetomium globosum	-	-	-	+	-	+	-	+	+	-
Cladosporium sp.	+	-	-	+	-	+	-	+	+	-
Drechslera hawiiensis	+	-	+	-	-	+	-	+	-	-
Drechslera haiodes	+	-	+	-	-	+	-	-	-	-
Fusarium equisetti	+	-	+	-	-	+	-	-	-	-
F moniliforme	+	-	-	-	-	+	-	-	-	-
F oxysporum	+	-	+	-	-	+	-	-	+	-
F solani	+	-	-	-	-	+	+	-	-	+
Fusarium sp.	+	-	-	-	+	+	+	-	-	-
Mucor sp.	+	-	-	-	-	+	-	-	-	-
Penicillium notatum	-	-	-	-	-	+	-	-	+	-
Penicillium sp.	+	-	-	+	-	+	-	+	+	-
Pythium aphanidermatum	+	-	-	-	+	+	-	-	-	+
Rhizopus nigricans	-	-	-	+	-	-	-	+	-	+
Rhizopus stoloniter	+	+	-	-	-	-	-	-	-	-
Saprolegnia diclina	-	-	-	-	+	-	+	-	-	-
S terex	-	+	-	-	+	-	+	-	-	+
S mixta	-	+	-	-	-	-	+	-	-	-
S parasitica	-	-	-	-	+	-	-	-	-	+
Verticillium sp.	+	-	-	-	-	-	-	-	-	-
Unidentified hyphomycetes	-	-	-	-	+	-	-	-	-	+
Total Species=31	19	4	9	7	7	21	6	13	8	7

+, present; -, absent

Kendrick 1974), in run off from the surrounding banks or from the air (Richards 1956). The uptake and survival of the spores of the fish pathogens reflect the similar aquatic and terrestrial fungi mycoflora of their aquatic surroundings (Wood *et al* 1988; Wheeler *et al* 1988). It is recognized that list of microfungi recorded here is limited by the range of methods used as for example zoosporic fungi (*Saprolegnia* and *Achlya*) were isolated only when hemp seeds were used as baits. In the present study, species of *Aspergillus* were the fungi most frequently isolated from water and mud samples of the two coasts. The on-going studies of the role of micro fungi in dry fish spoilage at Korangi creek-Karachi demonstrated that the species of *Aspergillus* are very common particularly on dried fish (Mehdi and Saifullah 1992). *Eurotium rubrum, Aspergillus flavus, A. wentii and A. penicillioides* were among the most frequently isolated Aspergilli during a study of fish spoilage in the tropics (Wheeler *et al* 1986). Mehdi and Saifullah (1992) also noted various yeasts in different oceanic creeks. It is interesting to note that *Saprolegnia parasitica* and an unidentified Phycomycetes were isolated from fish of both coasts. The secondary zoospore cyst coat of *S. parasitica* is characterized by bundles of long, hooked hair in contrast to saprophytic species which have short, single, hooked hair or no hair at all (Pickering *et al* 1979; Beakes 1983). It has been suggested that the long hair may facilitate infection either by enabling the spores to attach to the fish more effeciently (Meir and Webster 1954; Beakes 1983) or by enabling the cysts to remain suspended in the water for longer periods than those of the saprophyte, thus improving the chance of encountering the host (Pickering *et al* 1979).

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