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Research Article

Studies on the Rhizosphere Mycoflora of Mangroves

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Abstract: Rhizosphere soil of mangrove plants (*Avicennia marina, Rhizophora mucronata, Aegiceras corniculatum*, and *Ceriops tagal*) was collected from coastal areas. Almost all samples showed a sandy to sandy loam texture. pH of the soil samples ranged from 7 to 10 and water content ranged from 8% to 9%. In all, 18 species of fungi belonging to 11 genera were isolated from the rhizosphere soil of all the mangrove species by direct plating method, whereas 20 fungal species belonging to 11 genera were isolated by serial dilution. Results showed that the greatest number of fungi was isolated by serial dilution. The maximum number of species was obtained from the rhizosphere soil of *A. marina*, whereas the lowest number of fungi was obtained from the rhizosphere soil of *A. corniculatum*.

Key Words: Rhizosphere soil, mycoflora, mangrove plants, coastal areas

Introduction

The coastline of Pakistan stretches for over 590 miles, 440 miles of which belong to Baluchistan on the western coast and 150 miles to the province of Sindh on the southern coast. The Pakistan coastline can be divided into 2 distinct types, the Sindh and the Indus delta (semi arid zone), and the Baluchistan/Makran (arid zone). The coastal soil is saline, in general, and unproductive. The decay of coastal halophytic flora forms the nutrients and organic matter; however, the saline soils have very little organic carbon that can serve as an energy source for soil mycoflora (Malik et al., 1980). The concept of the rhizosphere is expressed as the zone of increased microbial activity. Qualitative as well as quantitative distribution of fungi in the rhizosphere and non-rhizosphere soil has been discussed in detail (Harley & Waid, 1955; Parkinson & Waid, 1960; Burges & Raw, 1967). Information available on the rhizosphere mycoflora of mangroves is scanty (Lee & Baker, 1973). Fungi represent a very important component of the ecosystem, along with other microbes of the biomass (Jones et al., 1988; Hyde, 1990, 1992; Harrison et al., 1994). A survey of the literature showed that a number of fungi have been reported from salt marshes, mangrove mud, estuaries, and other coastal habitats. These fungi include *Absidia corymbifera*, *Alternaria* spp., *Aspergillus* spp., *Chaetomium indicum, Cladosporium oxyporum*, *Drechslera* spp., *Fusarium* spp., *Nigrospora sphaerica*, *Paecilomyces* spp., *Penicillium* spp., *Rhizoctonia solani*, *Rhizopus stolonifer*, *Syncephalastrum racemosum*, and *Trichoderma viride* (Domsch et al., 1980). Mehdi & Saifullah (1992a,b) reported that *Aspergillus* spp. were the most diverse genus isolated from a mud sample from Clifton and Korangi Creek. In general, Deuteromycota was the most dominant group, with the largest number of species. Among *Aspergillus* spp., *A. flavus* was the most abundant. Experiments were therefore carried out to study the rhizosphere mycoflora of mangroves.

Materials and Methods

Rhizosphere soil of mangrove plants (*Avicennia marina*, *Rhizophora mucronata*, *Aegiceras corniculatum*, and *Ceriops tagal*) was collected from different coastal areas and there were 3 replicates of each rhizosphere soil. Samples were kept in sterile polythene bags in a refrigerator.

The soil texture was determined by wet sieving technique (Barbour et al., 1980). Soil pH was determined by electrometric method, following Brady (1990).

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Moisture content of soil samples was calculated by oven drying the soil and determining the weight loss (Garrett, 1963).

With the direct plate method, soil samples (0.01g) were dispersed in 1 ml of sterile distilled water in a sterilized petri dish and then approximately 10 ml of molten, cooled sterile agar was added and mixed. The soil particles were distributed throughout the medium by rotating the petri dish. The petri dishes were incubated for 5-7 days at 25 ± 2 ºC (Warcup, 1950). For serial dilution, soil samples (2.0g) were suspended in 18 ml of sterilized distilled water, which gave a dilution of 1:10. Serial dilutions of 1:100, 1:1000, and 1:10,000 were prepared, and then a 1-ml aliquot from the 1:1000 dilution was added to a petri dish containing penicillin (20,000 units/l) and streptomycin (200 µg/l). Then, approximately 10 ml of sterile potato dextrose agar medium was added to each dish. Each dilution was replicated 3 times and the dishes were incubated for 5-7 days at 25 ± 2 ºC. The number of colonies produced by a fungus was multiplied by the dilution factor to obtain the total number of propagules/g of soil (Waksman & Fred, 1922). The fungi growing on plates were identified using the standard literature (Raper & Fennel, 1965; Ellis, 1971; Domsch et al., 1980; Nelson et al., 1983). The data were analyzed and subjected to analysis of variance (ANOVA), following Gomez & Gomez (1984).

Results

Physical and chemical properties

Physically, the texture of all rhizosphere soil samples was sandy loam, except that of *Aegiceras corniculatum*, which was sandy. The pH of the sites ranged from 7 to 10 and the water content of the soil samples ranged between 8% and 9% (Table 1). The ability of the mangrove rhizosphere soils to stimulate fungal activity, irrespective of the varying salinity levels, could be attributed to their pronounced rhizosphere effect (Mehdi & Saifullah, 1992a,b). Soil properties have a major impact on mangrove nutrition and growth. Some of the most important characteristics are siltiness, electrical conductivity, pH, and cation exchange capacity (Kusmana, 1990; Rao et al., 1992; Pezeshki et al., 1997). The present work also showed that the texture of the soil samples was sandy loam. Sandy soils have low porosity. Water moves into and drains out of sandy soil with much greater ease than with a fine textured soil, which means that sandy soils are more permeable than fine textured soils. Such soils are nutritionally rich, but can be agriculturally problematic due to low permeability and aeration (Barbour et al., 1980).

Isolation of fungi from rhizosphere soil of mangrove plants by different techniques

By direct plating, 18 species of fungi belonging to 11 genera were isolated from the rhizosphere soil of the 4 mangrove species (Table 2). *Avicennia marina* had 13 species and 6 genera, *Rhizophora mucronata* had 11 species and 7 genera, *Aegiceras corniculatum* had 5 species and 3 genera, and *Ceriops tagal* had 9 species and 6 genera, viz. **Absidia corymbifera* (Cohn) Sacc.& Trotter, **Alternaria alternata* (Fr.) Keissl., **Aspergillus candidus* Link, *A. flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., **A. parasiticus* Speare, **A. sulphureus* (Fresen.) Thom and Church, *A. terreus* Thom, **A. wentii* Wehmer, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, **Drechslera australiensis* (Bugnic.) Subram. & B.L. Jain, *Fusarium solani* (Mart.) Sacc., **Monilia* sp., *Mucor* sp., *Penicillium* sp., **Rhizopus stolonifer* (Ehrenb.) Vuill., and *Trichoderma viride* Pers. Species marked with an asterisk are new reports from Pakistan (Mehdi et al., 2000; Mehdi & Saifullah, 2000). The present work

Table 1. Physical and chemical properties of rhizosphere soil of sampled mangrove plants.

Mangrove name	Properties		
	pH	Water content (%)	Texture
A. marina	7.89-10.02	8.22-9.01	Sandy-sandy loam
R. mucronata	9.82	8.21	Sandy loam
C. tagal	7.89	9.11	Sandy loam
A. corniculatum	7.85	9.7	Sandy

Table 2. Direct plating of rhizosphere soil fungi from mangrove plants.

	Avicennia marina		*Rhizophora mucronata*		*Aegiceras corniculatum*		*Ceriops tagal*	
Name of Fungi	NSI	1% ± SD	NSI	1% ± SD	NSI	1% ± SD	NSI	1% ± SD
Absidia corymbifera	0	0	1	0.55 ± 0.00	1	0.33 ± 0.00	1	0.16 ± 0.00
Alternaria alternata	3	1.49 ± 6.35	2	0.33 ± 0.233	1	1.33±0.00	1	0.66 ± 0.00
Aspergillus candidus	2	0.3 ± 0.70	0	0	0	0	0	0
A. flavus	9	6.62 ± 7.50	3	2.21 ± 0.509	1	6.66 ± 0.00	2	2.16 ± 0.70
A. fumigatus	4	3.08 ± 7.74	2	1.99 ± 3.76	1	0.33 ± 0.00	0	0
A. niger	10	7.02 ± 15.04	3	3.77 ± 4.11	1	2.66 ± 0.00	2	1.16 ± 0.70
A. paraciticus	3	3.09 ± 15.04	2	1.66 ± 1.18	0	0	1	9.66 ± 0.00
A. sulphureus	2	0.26 ± 0.00	2	0.22 ± 0.00	0	0	1	0.16 ± 0.00
A. terreus	1	0.06 ± 0.00	0	0	0	0	0	0
A. wentii	4	0.73 ± 1.73	0	0	0	0	0	0
Cladosporium cladosporioides	0	0	1	0.88 ± 0.00	0	0	0	0
Dreshlera australiensis	5	0.99 ± 1.026	2	1.33 ± 0.94	0	0	0	0
F. solani	4	0.89 ± 2.29	1	0.11 ± 0.00	0	0	2	1.99 ± 0.47
Monilia sp.	0	0	0	0	0	0	2	1.99 ± 2.35
Mucor sp.	0	0	0	0	0	0	1	2.16 ± 0.00
Penicillium sp.	1	0.23 ± 0.00	0	0	0	0	0	0
Rhizopus stolonifer	2	0.26 ± 0.947	0	0	0	0	0	0
Trichoderma viride	0	0	1	0.55 ± 0.00	0	0	0	0

NSI: Number of samples infected.
SD: ± standard deviation.
1%: Percentage of infected seeds.

showed that 19 species were isolated from *A. marina* by direct plating and serial dilution techniques. Among these, *A. niger*, *A. flavus* ($P < 0.01$), and *A. alternata* were observed in all mangrove species. The present work showed that *Monilia* sp. and *Mucor* sp. ($P < 0.05$) were found only in the soil of *C. tagal*, whereas *A. candidus*, *A. terreus*, *A. wentii*, *Penicillium* sp., and *R. solani* were found only in the soil of *A. marina*. Most were representatives of Deuteromycota, although members of Zygomycota were also recorded. Similarly, Mehdi & Saifullah (2000) and Mehdi et al. (2000) observed that Deuteromycota was the most common and dominant group. Results of the present study showed that the high frequency of *Fusarium* spp., *Drechslera australiensis*, and *Aspergillus* spp. from the mangrove soil samples suggests that they are common components of the soil mycoflora. Similar results have been reported by Mehdi & Saifullah (2000); *Aspergillus* was the most diverse genus, followed by *Penicillium*. *A. marina* yielded the highest number of fungi, followed by *R. mucronata* and *C. tagal*, whereas the least number of fungi was observed in *A. corniculatum* ($P < 0.001$). *A. niger* and *A. flavus* occurred in 100% of *A. corniculatum* samples, whereas *A. candidus, A. terreus*, *Cladosporium cladosporioides*, *Monilia* sp., *Mucor* sp., *Rhizopus stolonifer*, *Penicillium sp.*, and *Trichoderma viride* occurred in mangrove rhizosphere soil with much lower frequency (Table 2). With the serial dilution method, 20 fungal species belonging to 11 genera were isolated (Table 3). Among these, *Absidia corymbifera, Alternaria alternate*, *Aspergillus candidus, A. flavus, A. fumigatus*, *A. niger, A. parasiticus, A. sulphureus, A. sydowii* (Bainier & Sartory) Thom & Church, *A. terreus, A. wentii, Chaetomium globosum* Kunze, *Cladosporium cladosporioides, Drechslera australiensis, Fusarium semitectum* Berk & Ravenel, *F. solani*, *Monilia* sp., *Penicillium* sp., *Rhizopus stolonifer,* and *Trichoderma viride* were observed (Table 3). *A. alternata*, *A. flavus*, *A. fumigatus*, *A. niger*, and *F. solani* were common in the rhizosphere soil of the 4 species of mangrove plants sampled. The present results

Table 3. Number of propagule/g of soil based on the serial dilution technique.

Name of Fungi	*Avicennia marina*	*Rhizophora mucronata*	*Aegiceras corniculatum*	*Ceriops tagal*
Absidia corymbifera	2300	0	0	0
Alternaria alternata	27,900	5500	13,300	8300
Aspergillus candidus	1900	0	0	1650
A. flavus	25,200	25,500	16,600	14,950
A. fumigatus	4900	17,700	13,300	0
A. niger	22,600	23,200	66,600	14,950
A. paraciticus	24,900	0	16,600	3300
A. sulphureus	6600	6600	3300	6650
A. sydowii	3300	0	0	0
A. terreus	3300	0	0	0
A. wentii	900	0	0	0
Chaetomium globossum	1300	0	0	0
Cladosporium cladosporioides	0	0	0	6650
Dreshelera australiensis	2300	0	0	8300
Fusarium semitectum	300	0	0	0
F. solani	5300	8800	16,600	9950
Monilia sp.	665	0	0	0
Penicillium sp.	300	0	0	1650
Rhizopus stolonifer	1100	0	0	0
Trichoderma viride	600	0	0	0

showed that the greatest number of fungi was isolated by serial dilution ($P < 0.001$). Manzoor et al. (2004) reported similar results with coastal soil. As fungi and other microbes play vital roles in the turnover of the biomass, they form a very important part of the ecosystem (Jones & Hyde, 1988). There is, therefore, a need for extensive study of fungi associated with mangroves in different areas of Pakistan, which could produce a better picture of the mycoflora associated with mangroves.

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