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Mycoflora of the Rhizospheres of Mangrove Trees

Tadayoshi ITO and Akira NAKAGIRI

Summary

Mycoflora of the rhizospheres of mangrove trees collected from Okinawa Pref. in Japan was investigated. After washing the collected mangrove roots and surrounding soil samples, their root- and soil-inhabiting fungi were isolated by washing and dilution plate methods, respectively. *Pestalotiopsis* sp.-1 and *Trichoderma harzianum* were dominant species on the rhizoplane of mangrove trees, and these two species were considered to be the main components of mangrove rhizoplane mycoflora. The genera *Acremonium, Coniothyrium, Penicillium, Phoma* and *Trichoderma*, known terrestrial fungi, were isolated from the washed root soils. Thus, the mycoflora of the mangrove rhizoplane differed from that of its surrounding soil.

Keywords: Mycoflora, mangrove rhizosphere, terrestrial fungi, root- and soil -inhabiting fungi

The microorganisms in the rhizosphere of higher plants are known to grow by using dead plant debris or secretions from plant root tissues (8). They are also reported to form a kind of micro-community in the rhizosphere (8). The soils of mangrove forests in tropical and subtropical regions are reported to be semi-aerobic, low in nutrients, and to have higher concentrations of heavy metals and higher salinity than terrestrial soils (9). There have been few reports on the mycofloras in such adverse conditions in the mangrove rhizosphere. The present investigation treats mangrove rhizosphere samples collected from estuaries in the South-West Islands of Okinawa prefecture.

Materials and Methods

Sampling sites. Twenty-two healthy root samples of *Avicennia marina* Vierh., *Rhizophora stylosa* Griff., *Sonneratia alba* J.A. Sm., *Bruguiera gymnorrhiza* Lamk., *Kandelia candel* Druce and *Lumnitzera racemosa* Willd. were collected from estuaries of the Maira, Shiira and Urauchi rivers of Iriomote Is. and the Nagura and Fukidou rivers of Ishigaki Is., Okinawa prefecture, Japan in October 1995 (Table 1).

Table 1. List of mangrove rhizosphere samples collected in Okinawa.

Sample No.	Date sampled	Locality	Collected Samples
H7-18-23	24/10/'95	Maira river Iriomote Is.	*Avicennia marina* *Rhizophora stylosa* *Sonneratia alba*
H7-24-31	24/10/'95	Shiira river Iriomote Is.	*Bruguiera gymnorrhiza* *Avicennia marina* *Sonneratia alba*
H7-32-35	25/10/'95	Urauchi river Iriomote Is.	*Kandelia candel*
H7-36-37	26/10/'95	Nagura river Ishigaki Is.	*Lumnitzera racemosa*
H7-38-39	26/10/'95	Fukido river Ishigaki Is.	*Rhizophora stylosa*

Isolation methods. Slender root samples were cut into sections of 5 cm in length and washed with physiological saline solution. Five sections of each sample were put on each of two plates containing cornmeal agar and incubated at 15°C for 4 wk.

All fungi appearing during the incubation were isolated at 2, 3 and 4 wk under the dissecting microscope. Soil fungi were isolated by the dilution plate method from the soil suspension obtained in the first washing. Isolates were then transferred to half-concentration malt extract agar slant.

Isolation medium. Cornmeal agar containing 50 μg/ml of the tetracycline antibiotics was used for the slender mangrove root samples. For soil samples, malt-yeast extract agar containing the same sort and concentration of antibiotic was used.

Identification of isolates. Isolates were inoculated on plates of potato-carrot, malt extract, potato-sucrose and oatmeal agars and incubated at 24°C for appropriate periods. To identify the isolates, one representative strain of each species was used.

Results and discussion

Table 2 lists the fungi isolated from mangrove root samples and their frequency of isolation. Isolated species mainly belong to the taxon Deuteromycotina.

The sample of *Rhizophora stylosa* yielded the largest number of isolates, 18 strains of 18 species; and *Kandelia candel* yielded the fewest, 6 strains of 2 species.

Trichoderma harzianum Rifai was detected in the highest frequency from the 22 samples used (50.0% frequency) and from all six species of mangrove trees. This fungus is well known from plant debris and soil worldwide. It has also been isolated from the rhizospheres of poplar, pine, tobacco, beet and wheat, and is reported to decompose cellulose and starch (1). Therefore, this fungus is considered to have a high affinity for mangrove roots. The second dominant species was *Pestalotiopsis* sp-1. (27.3% frequency). Species of this genus are known as parasites which causes the *Pestalotia* disease of apple, chestnut, persimmon, loquat and coconut palm trees and the leaf spot disease of

Table 2. Fungi isolated from roots of mangrove trees and their frequency of isolation.

Species	Host						Frequency (%)[a]
	So. alba	Rh. sty.	Br. gym.	Av. mar.	Ka. can.	Lu. rac.	
Acremonium sp.[b]	1[c]	1				1	13.6
Alternaria alternata	1	1					9.1
Aspergillus aculeatus				1			4.5
Cladosporium cladosporioides		1					4.5
Cylindrocarpon destractans	1	1				1	13.6
Coelomycetes		3					13.6
Engyodontium album				1			4.5
Eupenicillium sp.			1				4.5
Fusarium moniliforme	1		1				9.1
Fusarium sp. 1		1					4.5
Gliocladium virens						1	4.5
Gliomastix murorum				1			4.5
Myrothecium sp.		1					4.5
Pestalotiopsis sp.-1	1	1	1		2	1	27.3
Penicillium citrinum		1	2				13.6
Pencillium sp. 1	1	1	1				13.6
Pencillium sp. 2			1	1			9.1
Pencillium sp. 3			1	1			9.1
Pencillium sp. 4			1	1			9.1
Phoma sp. 1		1					4.5
Phoma sp. 2		1					4.5
Scolecobasidium humicola				1			4.5
Trichoderma aureoviride			1				4.5
Trichoderma harzianum	3	1	1	1	4	1	50.0
Trichoderma sp.	1						4.5
Virgaria nigra				1			4.5
Volutina concentrica		1					4.5
Westerdykella dispersa		1					4.5
Unidentified strains	2	1	1	1		1	27.3
Total number of strains	12	18	12	10	6	6	
Number of samples	4	4	4	4	4	2	22

a: Total number of positive samples / total number of samples.

b: Bold type shows species detected in the previous investigation (1994).

c: Number of positive samples in each mangrove tree.

maidenhair and Japanese cedar (5, 6). This is the first time it has been detected from mangrove roots.

The species isolated in this investigation agree closely with those of an earlier investigation in October 1994 (unpublished). Therefore, it is considered that the dominant species of the mangrove rhizosphere are *Trichoderma harzianum* and *Pestalotiopsis*

Table 3. Fungi isolated from soil of washed mangrove roots by dilution plate method.

Species	No. of positive samples	Frequency (%)[a]
DEUTEROMYCOTINA		
Acremonium spp.	11	50.0
Albophoma yamanashiensis	2	9.1
Arthrinium phaeospermum	1	4.5
Aspergillus clavatus	2	9.1
Aspergillus niger	1	4.5
Cladosporium cladosporioides	6	27.3
Coniothyrium spp.	12	54.5
Exophiala sp.	5	22.7
Fusarium spp.	8	36.4
Gliocladium roseum	3	13.6
Gliocladium virens	2	9.1
Gliocladium sp.	1	4.5
Gliomastix murorum	1	4.5
Metarhizium anisopliae	3	13.6
Myrothecium sp.	1	4.5
Nodulisporium sp.	3	13.6
Paecilomyces lilacinus	8	36.4
Paecilomyces spp.	5	22.7
Penicillium citrinum	6	27.3
P. corylophilum	2	9.1
P. crustosum	3	13.6
P. funiculosum	2	9.1
P. janthinellum	4	18.2
P. purpurogenum	8	36.4
Penicillium spp.	3	13.6
Pestalotiopsis sp.	1	4.5
Phialophora fastigiata	5	22.7
Phialophora spp.	6	27.3
Phoma spp.	13	59.1
Phomopsis spp.	2	9.1
Scopulariopsis spp.	5	22.7
Stilbum sp.	2	9.1
Trichoderma aureoviride	2	9.1
T. harzianum	10	45.5
T. koningii	3	13.6
T. pseudokoningii	2	9.1
Trichoderma spp.	3	13.6
ASCOMYCOTINA		
Achaetomium macrosporum	1	4.5
Talaromyces flavus var. *flavus*	1	4.5
ZYGOMYCOTINA		
Cunnighamella sp.	1	4.5
Mortierella sp.	1	4.5
Mucor sp.	1	4.5
Sterile mycelium	16	72.7

a: Number of positive samples / a total number of samples.

sp.-1. To investigate the correlation between fungi and mangrove roots, these two strains were inoculated on the sterilized root of *Rhizophora stylosa*. It is confirmed that the strain of *Trichoderma harzianum* invaded into the intercellular space and sporulated in the air space, and *Pestalotiopsis* sp.-1 strain exfoliated the cortical tissue and formed pycnidia in the mangrove root tissue. *Penicillium citrinum* Thom, a terrestrial fungus, was used as a control. It does not invade the root tissues and formed colonies only on the root surface. It is suggested that the former two species are the main components of the mangrove rhizoplane.

Table 3 shows the species detected and their frequency from 22 samples of soil surrounding roots.

Species of *Acremonium* Link, *Coniothyrium* Corda, *Fusarium* Link, *Paecilomyces* Bainnier, *Penicillium* Link, *Phoma* Sacc. and *Trichoderma* Pers. were the dominant species detected from these samples. These results agreed well with those of mud samples taken from mangrove forest in January 1994 and examined by the dilution plate method (4) and with those of mangrove mud in India (7). No difference was found between the mycoflora of the mangrove root soil and the soils of paddy, pineapple, plant garden and pasture concurrently collected from Iriomote Is. of Okinawa (4) or the soils of paddy and vegetable fields in Osaka prefecture (2, 3). Therefore, the fungi detected in mangrove root soil are considered to have been carried there from land by river.

It was confirmed that mycoflora on the mangrove rhizoplane differs from that of the surrounding root soil. In particular, *Trichoderma harzianum* and *Pestalotiopsis* sp.-1 are suggested an affinity for mangrove roots. Further experiments are needed to confirm the ecological distribution of these fungi.

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