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A Mycofloral Study on Mangrove Mud in Okinawa, Japan

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Summary

The fungal flora of mangrove mud in Okinawa, Japan was investigated by four isolation methods. From 36 mud samples, 36 genera of fungi representing 11 Ascomycotina, 21 Deuteromycotina, 2 Zygomycotina, and 2 unidentified Basidiomycotina were detected. The most dominant species were, in order, *Penicillium purpurogenum, Aspergillus terreus, Trichoderma harzianum, Penicillium crustosum, Acremonium alabamense, Talaromyces flavus* var. *flavus* and *Phialophora fastigiata.*

No significant differences were found in the numbers of species and isolates detected in the estuaries of six rivers. Fewer fungal populations were detected in mangrove mud than in agricultural soils. Tolerance to sodium chloride was tested for some isolates.

Keywords: fungal flora, mangrove mud, number of fungi.

Mangrove forest in Japan is mainly distributed in Okinawa prefecture, which lies in the subtropical zone. The salinity and pH of the mud in mangrove forest in Japan were reported to be 2.6-5.1% and 6.1-8.1 at 20 cm depth, respectively. Mangrove mud is also reported to be deficient in dissolve oxygen and to show accumulation of heavy metals (8). Mangrove trees are known to adapt to these conditions (8). The fungal flora in mud of mangrove forest, however, has not been surveyed in Japan, and only a few surveys have been reported from other countries (5, 6, 7).

Materials and Methods

Thirty-six samples of mangrove mud were collected from Okinawa prefecture on 25-27 January 1994 (Fig. 1). The collection sites are estuaries in three areas of Okinawa prefecture: Urauchi, Maira and Shiira rivers in Iriomote Is.; Nagura river, Ishigaki Is.; and Kesaji and Ooura rivers, Okinawa Is. The vegetation of these estuaries is dominated by plants such as *Rhizophora stylosa* Griff, *Avicennia marina* Vierh., *Bruguiera gymnorrhiza* Lamk., *Kandelia candel* Druce and *Sonneratia alba* J. E. Sm.

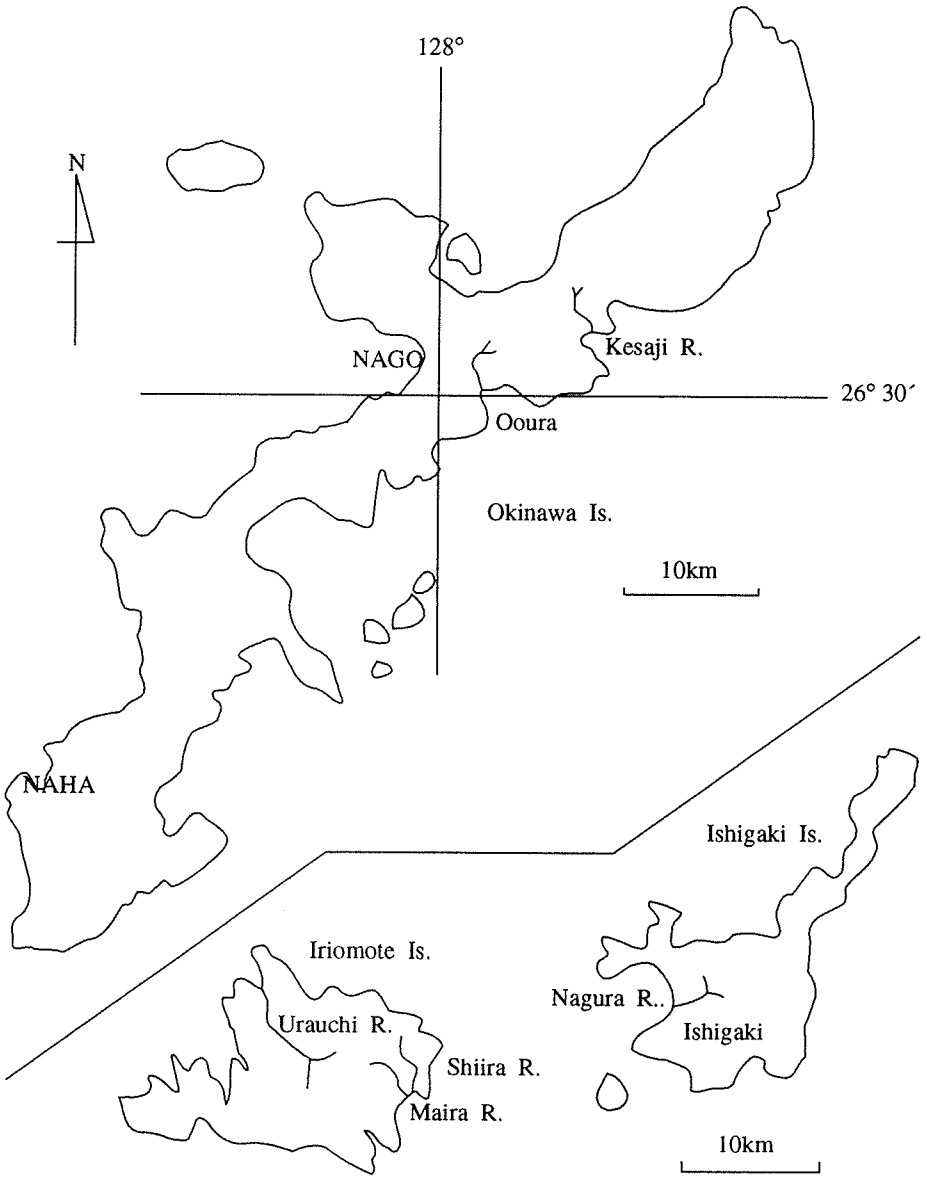

Fig. 1. Map of Okinawa prefecture showing the study sites.

The mud samples were collected from about 5 cm depth at each site, and the total of 36 samples obtained are listed in Table 1. To compare the number of fungal propagules in agricultural soil, three soil samples of pineapple, banana and paddy fields were also collected from Iriomote Is. The samples were suspended in physiological salt solution containing 0.85% sodium chloride. Four isolation methods were applied: incubation at 45°C, treatment with 50% ethanol for 15 min., heat treatment at 70°C for 15 min., and the standard dilution plate method. Plates for heat incubation and for other methods were incubated at 45°C and 24°C for three to four days, respectively. Single colonies were picked up from the plates under a dissecting microscope and transferred to malt agar slants. The isolation medium was malt extract-yeast extract-agar in which distilled water

Table 1. List of mangrove mud samples collected in Okinawa, and the number of species isolated from each sample.

Sample No.	Date sampled	Locality	Predominant vegetation	Number of species isolated
1	25/1/'94	Urauchi river Iriomote Is.	*Rhizophora stylosa* *Kandelia candel*	3
2	〃	〃	〃	2
3	〃	〃	〃	3
4	〃	〃	〃	4
5	〃	〃	〃	2
6	〃	〃	〃	0
7	〃	〃	〃	3
8	〃	〃	〃	3
9	26/1/'94	Shiira river Iriomote Is.	*Avicennia marina* *Rhizophora stylosa* *Sonneratia alba*	8
10	〃	〃	〃	2
11	〃	〃	〃	7
12	〃	〃	〃	13
13	〃	〃	〃	3
14	26/1/'94	Maira river Iriomote Is.	*Avicennia marina* *Rhizophora stylosa* *Sonneratia alba*	3
15	〃	〃	〃	6
16	〃	〃	〃	3
17	〃	〃	〃	4
18	〃	〃	〃	9
19	〃	〃	〃	1
20	〃	〃	〃	4
21	〃	〃	〃	7
22	〃	〃	〃	11
23	〃	〃	〃	0
24	27/1/'94	Nagura river Ishigaki Is.	*Kandelia candel* *Rhizophora stylosa*	11
25	〃	〃	〃	19
26	〃	〃	〃	15
27	〃	〃	〃	15
28	28/1/'94	Oora river Okinawa Is.	*Kandelia candel* *Bruguiera gymnorrhiza*	11
29	〃		〃	4
30	〃	〃	〃	7
31	〃	〃	〃	2
32	〃	〃	〃	5
33	28/1/'94	Kesaji river Okinawa Is.	*Rhizophora stylosa*	0
34	〃	〃	〃	0
35	〃	〃	〃	5
36	〃	〃	〃	2

was replaced by 2.0% artificial sea water (Jamarin S; Jamarin Laboratory, Osaka) containing tetracycline antibiotics (50 μg/ml) to inhibit bacterial growth, as previously reported (3, 4, 9). To isolate fungi decomposing chitin, trap method using a crab shell was tried. Tolerance to sodium chloride was tested for some isolates.

Results and Discussion

Fig. 2 shows the number of fungi in each sample by the dilution plate method.

The average number of fungi for each river site ranged from 4.5×10^2 to 2.3×10^3 per gram of dry soil, whereas agricultural soil samples of pineapple, banana and paddy fields ranged from 7.5×10^4 to 2.1×10^5. Four mud samples collected from the Urauchi, Maira and Kesaji rivers contained no viable fungal propagules (Table 1) even though four isolation methods were tried. This was also confirmed by the direct soil plate method and trap method using the crab shell. It indicates a poor distribution of fungal propagules in the mangrove mud. The reason is considered to be the semi-anaerobic condition and the high content of heavy metals in the mud.

Table 2 lists all the species of fungi isolated from the 36 samples. Forty-three species in 36 genera were identified and classified into 16 species in 11 genera of Ascomycotina, 25 species in 21 genera of Deuteromycotina, 2 species in 2 genera of Zygomycotina, and 2 unidentified species of Basidiomycotina.

Almost all the species identified were common, typical soil fungi which have been recorded worldwide (1, 2, 3, 4, 9). Both the total numbers of fungus species detected (Table 2) and the number of species in each sample were low in all 36 samples.

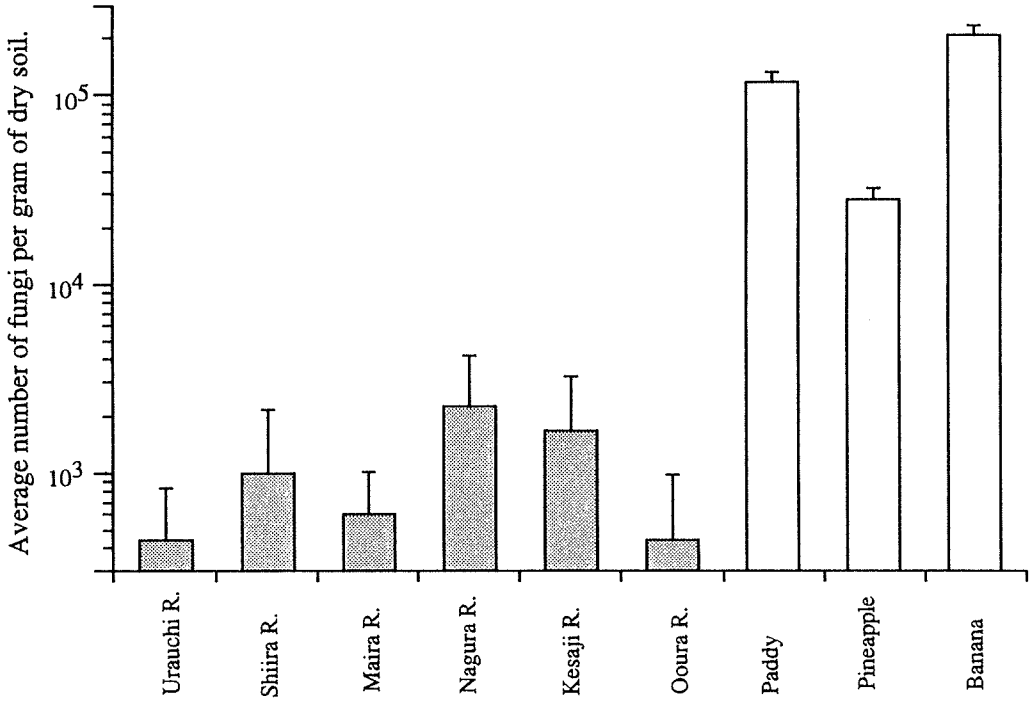

Fig. 2. Number of fungi isolated each site by the dilution plate method.

Eleven genera of Ascomycotina were encountered in this survey. *Talaromyces flavus* (Klocker) Stolk & Samson var. *flavus* (22.2%), *T. stipitatus* C. R. Benjamin apud Stolk & Samson (11.1%) and *T. wortmannii* C. R. Benjamin apud Stolk & Samson (11.1%) occurred frequently. It is considered that these species are isolated in higher frequency by the ethanol and heat treatments of soil samples, although they have often been detected from soil worldwide (1, 2, 3, 4, 9).

Table 2. Fungi isolated from mangrove muds by four isolation methods.

Species detected	Sample No.	Method[a]	Frequency[b] (%)
ASCOMYCOTINA			
Achaetomium macrosporum Rai et al	11	D	2.8
Chaetomium aureum Chivers	29	D	2.8
Emericella nidulans (Eidam) Vuillemin var. *nidulans*	1	H	2.8
Eupenicillium parvum (Raper & Fennell) Stolk & Scott	9, 12	T, E	5.6
Eurotium rubrum Konig et al.	27	E	2.8
Microascus cinereus (Emile-Weil & Gaudin) Curzi	20	D	2.8
Neosartorya fischeri (Wehmer) Malloch & Cain var. *glabra* (Fennell & Raper) Malloch & Cain	24, 25, 27	H, T	8.3
N. quadricincta (Yuill) Malloch & Cain	13, 27, 28	H, T	8.3
Penicilliopsis clavariaefomis Solms-Laubach	12	E	2.8
Talaromyces flavus (Klocker) Stolk & Samson var. *flavus*	9, 10, 12, 24, 25, 27, 35	H, T, E, D	22.2
T. ohiensis Pitt	25	T	2.8
T. helicus C.R. Benjamin apud Stolk & Samson var. *helicus*	24, 25	T, E	5.6
T. stipitatus C.R. Benjamin apud Stolk & Samson	24, 25, 26, 27	T, E, D	11.1
T. wortmannii C.R. Benjamin apud Stolk & Samson	11, 18, 21, 24	D	11.1
Thermoascus aurantiacus Miehe	27, 30	H	5.6
Thielavia terricola (Gilman & Abott) Emmons	24	H	2.8
Westerdykella multispora (Saito & Minoura) Cejp & Milko	25	D	2.8
DEUTEROMYCOTINA			
Acremonium albamense Morgan-Jones	18, 24, 25, 26, 27, 36	H	16.7
A. terricola (Miller et al.) W. Gams	27	D	2.8
Acremonium spp.	(18)[c]	D	50.0
Arthrinium phaeospermum (Corda) E.B. Ellis	25	E	2.8
Aspergillus clavatus Desmazieres	12, 17	T, D	5.6
A. fumigatus Fresenius	30	H	2.8
A. terreus Thom	14, 17, 18, 22, 24, 25, 26, 27	H, D	22.2
Chalara sp.	12	D	2.8
Cladosporium cladosporioides (Fresenius) de Vries	11, 28	D	5.6
Coniothyrium spp.	4, 7, 8, 11, 18, 24, 26, 29, 35	D	25.0
Exophiala sp.	32	D	2.8

Table 2. (Continued).

Species detected	Sample No.	Method[a]	Frequency[b] (%)
Fusarium sp.	35	D	2.8
Gliocladium virens Miller et al.	25	D	2.8
Gliocladium sp.	9	D	2.8
Metarhizium anisopliae (Metschnikoff) Sorokin	12, 26	D	5.6
Nodulisporium sp.	3	E	2.8
Paecilomyces lilacinus (Thom) Samson	12, 22, 25	D	8.3
Paecilomyces spp.	12, 22, 35	D	8.3
Penicillium citrinum Thom	26	D	2.8
P. corylophilum Dierckx	18	D	2.8
P. crustosum Thom	9, 12, 25, 27, 28, 32	D	16.7
P. janthinellum Biourge	21, 25	D	5.6
P. purpurogenum Stoll	9, 11, 12, 15, 17, 18, 19, 20, 22, 26, 28	D	30.6
P. rugulosum Thom	16	D	2.8
Penicillium spp.	9, 18	D	5.6
Pestalotiopsis sp.	25	D	2.8
Phialophora fastigiata (Lagerberg & Melin) Conant	1, 9, 21, 27	D	11.1
Phialophora spp.	3, 4, 11	D	8.3
Phoma herbarum Westend	13, 24	D	5.6
Phoma spp.	(19)	D	52.8
Phomopsis spp.	13, 21, 30, 32	D	11.1
Scopulariopsis brumptii Salvanet-Duval	26, 29	D	5.6
Scopulariopsis spp.	16, 22	D	5.6
Thermophymatospora fibrigera Udagawa et al.	27	H	2.8
Trichoderma aureoviride Rifai	12, 22, 25	D	8.3
T. harzianum Rifai	17, 18, 20, 25, 26, 27, 30	D	19.4
T. koningii Oudemans	36	D	2.8
T. pseudokoningii Rifai	10	D	2.8
Trichoderma spp.	24, 25	D	5.6
Virgaria nigra (Link) Nees ex S.F. Gray	20	D	2.8
ZYGOMYCOTINA			
Mucor hiemalis Wehmer f. *hiemalis*	25	D	2.8
Rhizomucor pusillus (Lindt) Schipper	26	H	2.8
BASIDIOMYCOTINA			
Unidentified species	1, 22	E, D	5.6
Sterile mycelium	(26)	E, D	69.4

a: H, heat incubation; E, ethanol treatment; T, heat treatment; D, dilution plate. b: Number of positive samples/total number of samples. c: Total number of samples in which the fungi were detected.

Forty-five genera of Deuteromycotina were detected. *Acremonium alabamense* Morgan-Jones, *Aspergillus terreus* Thom, *Penicillium crustosum* Thom, *P. purpurogenum* Stoll, *Trichoderma harzianum* Rifai were frequently isolated from 16.7% to 30.6% of all samples collected. These species are commonly isolated from various soils and materials in many parts of the world (1, 2, 3, 4, 9). Many strains of *Phoma* spp. were isolated. Almost all of these strains are considered to adapt to the conditions of mangrove mud by the formation of enduring tissues such as pycnidia.

Only two species of Zygomycotina were detected, although these species are dominant in cold areas and forest soil (1, 3, 4, 9). The scarce distribution of Zygomycotina is probably due to the deficiency of dissolved oxygen in mangrove mud.

Two unidentified species of Aphylophorales in Basidiomycotina were isolated. These species had typical clamp connections, but primordia or fruit bodies were not formed during cultivation on sawdust plus rice bran. Some fungi which occur in low frequency in mud were also detected in this investigation. A strain of *Penicilliopsis clavariaeformis* Solms-Laubach belonging to Ascomycotina was isolated from a sample from the Shiira river in Iriomote Is. This species has only been isolated from Iriomote Is. in Japan with its anamorphic state. *Thermophymatospora fibrigera* Udagawa et al. and *Virgaria nigra* (Link) Nees ex S. F. Gray belonging to Deuteromycotina were detected from Nagura river in Ishigaki Is. and Maira river in Iriomote Is., respectively. The former species was originally isolated from soil of a date palm plantation in Iraq as a thermotolerant fungus of basidiomycetous Hyphomycetes. The latter is often reported worldwide from various kinds of wood and leaves, but it is rare in soil.

Using the trap method, strains of *Acremonium strictum* W. Gams, *Aspergillus niger* v. Tieghem, *A. terreus* Thom, *Fusarium* sp. *Paecilomyces lilacinus* (Thom) Samson, *Scopulariopsis brumptii* Solvanet-Duval, and *Talaromyces stipitatus* C. R. Benjamin apud Stolk & Samson were isolated. Other than *T. stipitatus*, these fungi are known to decompose chitin and have been isolated from saltmarshes by Domsh et al. (1980).

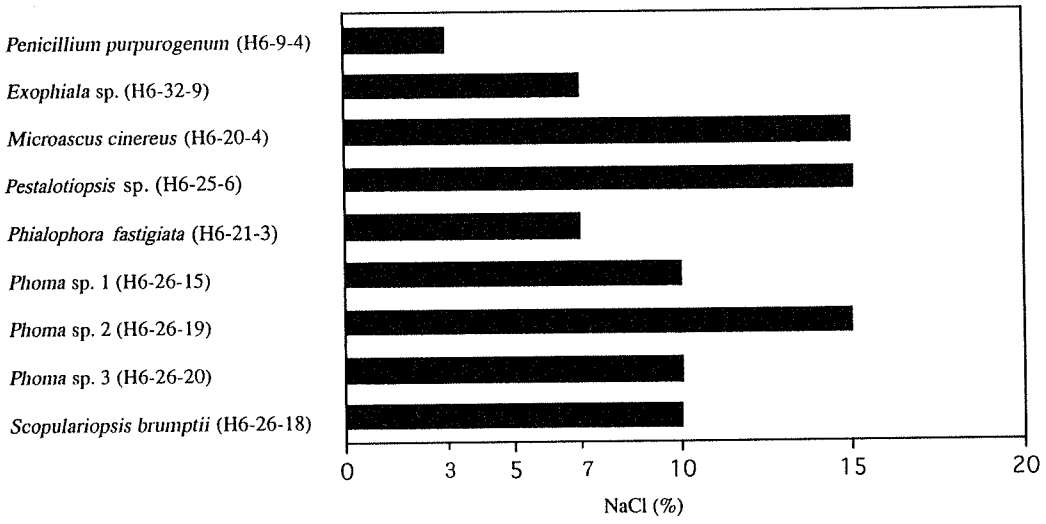

Fig. 3. Salinity range for growth of some isolates. (Growth on malt extract agar containing NaCl after incubation for three weeks at 24 °C.)

Figure 3 shows the results of the test for sodium chloride tolerance. The fungi tested grew up to the concentration of 7.0-15.0% NaCl. These fungi, which are termed as osmophiles, have been isolated and reported most frequently from sugar, salted food products and concentrated fruit juices (1). They are probably able to adapt to the high osmotic pressure that results when mangrove mud dries up at high temperature.

The fungal flora of mangrove mud in Okinawa, which is semi-anaerobic and has a high content of heavy metals, was characterized by fewer species and fewer propagules than those of agricultural soil samples.

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