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# Mycobiota of Mangrove Forest Soil in Thailand

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**Summary**

To examine the distribution and ecology of the fungi inhabiting mangrove forest soil in a tropical region, 20 samples from mangrove sites and 3 from terrestrial sites were collected in Thailand in March 1999. Soil fungi were isolated by heat incubation at 45°C and the dilution plate method at 25°C. Comparison of the findings with those of an earlier survey of subtropical mangroves in Okinawa, Japan revealed the following. 1. The average numbers of colonies and species per sample from the Thai mangroves were smaller than in samples from Japanese mangroves. 2. *Talaromyces byssochlamydoides*, a thermophilic fungus, and *Aspergillus aculeatus* were frequently detected in the Thai samples but were not found in Japanese mangroves. 3. Species of the mitosporic genera *Coniothyrium, Phialophora*, and *Phoma* occurred less frequently in the Thai than the Japanese mangroves. 4. Species of the genus *Aspergillus* were abundantly detected in the Thai samples. The smaller population and lower ecological diversity of fungi in the Thai mangrove soil might be due to the lower nutrient content and anaerobic nature of the soil, which is mostly composed of clay particles. However, *Aspergillus* species, which adapt to high temperature, showed higher diversity in the Thai soil.

Keywords: diversity, fungal populations, mangrove forest, mycobiota, Thai soil.

Mangrove forest is distributed along estuaries and rivers in tropical and subtropical regions. As an ecosystem, it has unique characteristics and is one of the most diverse reserves of biological organisms. Recent attention has focused on the diversity of microorganisms and the important role that they play in the dynamics of the mangrove ecosystem. 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 (10). Mycobiota on mangrove and its mud have been investigated in the subtropical zone in Japan (4, 5), and tropical regions (7, 8, 9).

To examine the distribution and ecology of the fungi inhabiting mangrove forest soil in a tropical region, soil samples were collected from mangroves in Thailand. Fungi were

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isolated and identified, and several isolates were tested for optimum growth temperature and tolerance to sodium chloride. The results were compared with those of a study conducted in 1997 on the mycobiota of the mangrove rhizosphere in the subtropical region of Okinawa (4, 5).

A part of this study was carried out as a co-operative project of the IFO (Japan), Kasetsart University (Thailand), and National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand.

## Materials and Methods

*Sampling sites*. Twenty soil samples from the rhizosphere of eight mangrove species were collected from the Ranong Research Center of Kasetsart University and Phang-nga forest in southern Thailand, and three samples were collected from the campus of Kasetsart University (K.U.) in Bangkok on March 1999 as a terrestrial soil samples (Table 1). The mangrove collection sites were in the estuaries of two rivers debouching into the Andaman Sea. This region has a tropical monsoon climate, with annual rainfall of 2800 mm, an average annual temperature of 27°C, and a hot season (March) temperature of 32-36°C. Water temperature is 25-26°C, salinity is 5-30 ppt, and depth is 1-2 m. The mangrove species *Rhizophora mucronata* Lum., *R. apiculata* Bl., *Xylocarpus obovatus* A. Juss., *Avicennia alba* Bl., *Lumnitzera racemosa* Willd., *Nypa fruticans* Wurmb., *Bruguiera gymnorrhiza* (L.) Lam., *B. indica, Kandelia candel* Druce, and *Sonneratia alba* J.A. Sm. dominate the vegetation of these estuaries. Only two species of collected species, *Bruguiera gymnorrhiza* and *Sonneratia alba*, were common in Japan. Table 2 compares the mangrove forest environments of Thailand and Japan.

*Isolation methods*. The isolation methods adopted are the same as in the survey of Japanese mangrove forests reported previously (4, 5). The mud samples were suspended in physiological salt solution containing 0.85 % sodium chloride. Two isolation methods

Table 1. Soil samples of mangrove rhizosphere collected in Thailand.

| Sample No. | Date sampled | Locality | Vegetation of Mangrove tree |
|---|---|---|---|
| Th- 1 - Th- 2 | 18/3/'99 | Ranong | *Avicennia alba* |
| Th- 3 - Th- 4 | 18/3/'99 | Ranong | *Ceriops tagal* |
| Th- 5 - Th- 6 | 18/3/'99 | Ranong | *Rhizophora mucronata* |
| Th- 7 - Th- 8 | 18/3/'99 | Ranong | *Xylocarpus obovatus* |
| Th- 9 - Th-10 | 18/3/'99 | Ranong | *Bruguiera sexangula* |
| Th-11 - Th-12 | 18/3/'99 | Ranong | *Rhizophora apiculata* |
| Th-13 - Th-14 | 18/3/'99 | Ranong | *Sonneratia alba* |
| Th-15 - Th-16 | 19/3/'99 | Phang-nga | *Avicennia alba* |
| Th-17 - Th-18 | 19/3/'99 | Phang-nga | *Bruguiera sexangula* |
| Th-19 | 19/3/'99 | Phang-nga | *Sonneratia alba* |
| Th-20 | 19/3/'99 | Phang-nga | *Bruguiera gymnorrhiza* |
| Th-21 - Th-23 | 22/3/'99 | Kasetsart Univ. | terrestrial soil |

Table 2. Comparison of mangrove forest environments in Thailand and Japan.

| | Thailand | Japan |
|---|---|---|
| Locality | Rannong | Okinawa |
| | Phang-nga | Iriomote and Ishigaki Is. |
| | 99° E, 9° N | 127° E, 26° N |
| Climate | tropical | subtropical |
| | 27℃ (Mar.) | 17-19℃ (Mar.) |
| | 2800 mm | 2000 mm |
| Mangrove | *Avicennia alba* | *Avicennia marina* |
| | *Bruguiera gymnorrhiza* | *Bruguiera gymnorrhiza* |
| | *Bruguiera sexangula* | *Kandelia candel* |
| | *Ceriops tagal* | *Lumnitzera racemosa* |
| | *Rhizophora apiculata* | *Rhizophora stylosa* |
| | *Rhizophora mucronata* | *Sonneratia alba* |
| | *Sonneratia alba* | |
| | *Xylocarpus obovatus* | |
| Soil type | Clay | Sandy loam |
| Salinity | 15-30‰ | 15-25‰ |

were applied: incubation at 45°C and the standard dilution plate method with × 10 and × 100 dilution. Two plates were used for each sample and method. All fungi appearing from each sample were incubated at 45°C and 25°C for 2 and 4 days, respectively. Single colonies were picked up from the plates under a dissecting microscope and transferred to a half-strength malt extract agar slant.

*Isolation medium*. Malt extract-yeast extract-agar (per liter: 100 g glucose, 5 g peptone, 3 g malt extract, 3 g yeast extract and 20 g agar) containing 50 μg/ml of tetracycline antibiotic was used.

*Identification of isolates*. Isolates were inoculated on the plates of the appropriate medium for identification, such as oatmeal, potato carrot, cornmeal and potato sucrose agar, and incubated at 45°C and 25°C for appropriate periods. To identify the isolates, one representative strain of each species was used. Most were identified at K. U., and the remainder were identified at IFO. For species identification, some strains of IFO Culture Collection were used as reference.

## Results and Discussion

*Fungal population density*

The number of fungi found in each sample by the dilution plate method was counted

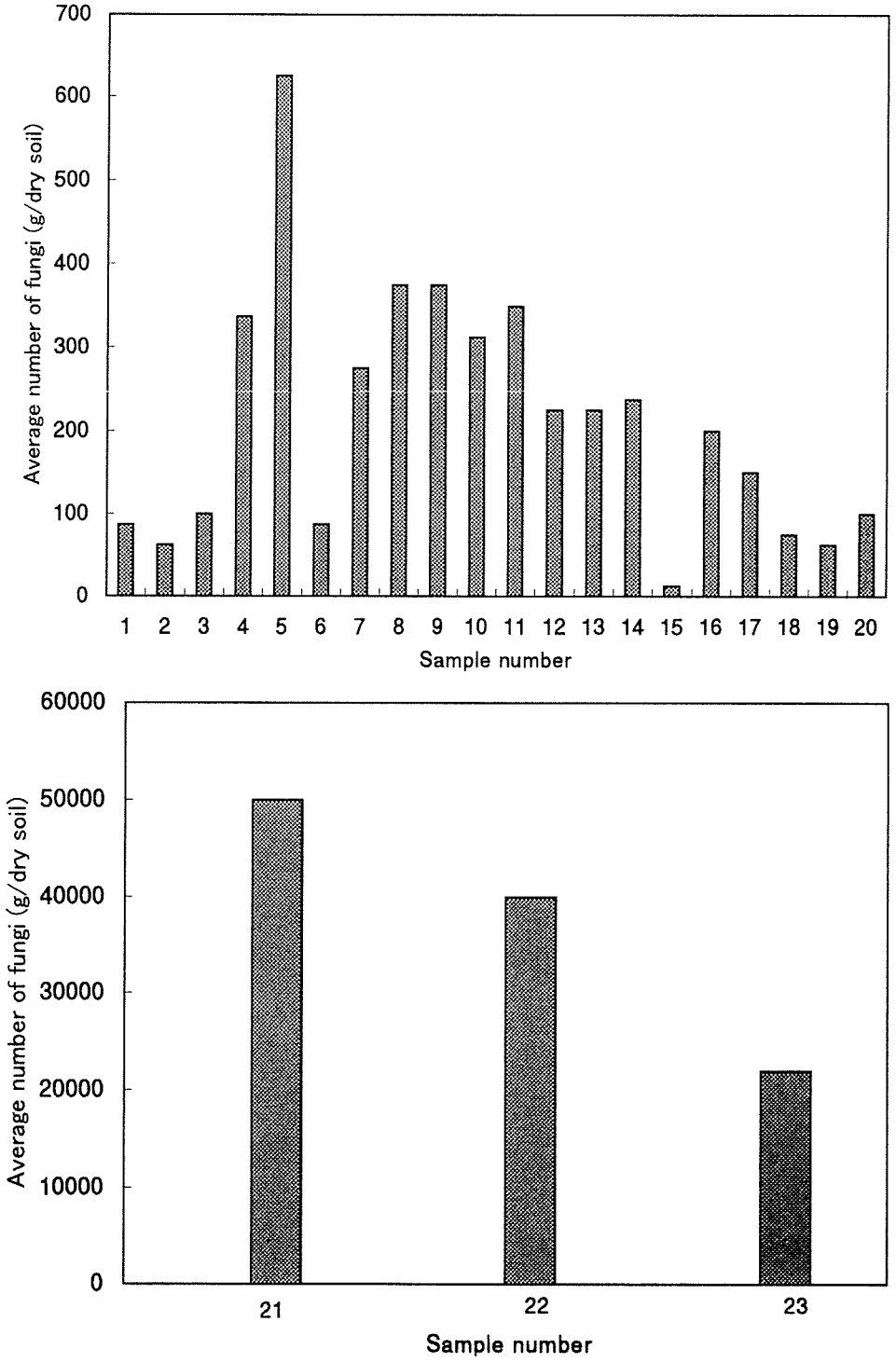


Fig. 1. Number of fungi isolated per sample by the dilution plate method.

under dissecting microscope, and the results are shown in Figure 1. The average number of fungi found for each mangrove species ranged from $2.0\times10^{1}$ to $6.2\times10^{2}$ cfu/g of dry

Table 3. Fungi isolated by two isolation methods from rhizosphere soil, by mangrove species.

| Fungus | Mangrove species | | | | | | | | Frequency |
|---|---|---|---|---|---|---|---|---|---|
| | *Avicennia alba* | *Ceriops tagal* | *Rhizophora mucronata* | *Xylocarpus obovatus* | *Bruguiera sexangula* | *Rhizophora apiculata* | *Sonneratia alba* | *Bruguiera gymnorrhiza* | (%)[a] |
| *Acremonium curvulum* W. Gams | | | | | | | 1 | | 5 |
| *Acremonium* spp. | | 1 | 1 | | 3 | | 1 | | 30 |
| *Aspergillus aculeatus* Iizuka | 1 | 1 | 1 | | 1 | | | 2 | 30 |
| *Aspergillus clavatus* Desmaz. | 2 | | | | | | | | 10 |
| *Aspergillus fumigatus* Fres. | | 1 | 1 | 1 | | | | | 15 |
| *Aspergillus niger* van Tiegh. | | | | 1 | 1 | | | | 10 |
| *Aspergillus niger* aggr. | 2 | 1 | | | | | 2 | | 25 |
| *Aspergillus* spp. | | 2 | | | 1 | 1 | | | 20 |
| *Aspergillus terreus* Thom | | 2 | 1 | | | | 1 | | 20 |
| *Cladosporium cladosporioides* (Fres.) de Vries | | 1 | 1 | 1 | | 1 | | | 20 |
| *Coniothyrium* sp. | | | 1 | | | | | | 5 |
| *Cylindrocladium parvum* Anders. | | | 1 | | | | | | 5 |
| *Eupenicillium javanicum* (van Veyma) Stolk & Scott | 1 | 1 | 1 | 1 | 1 | | | | 25 |
| *Eupenicillium* sp. | 1 | | | | | | | 1 | 10 |
| *Fusarium oxysporum* Schlecht. emend. Sny. & Hans. | 1 | | | | | | | | 10 |
| *Fusarium solani* (Mart.) Appel & Woll. emend. Sny. & Hans. | 1 | 1 | | | | | | | 10 |
| *Fusarium* sp. | | 1 | | | | | | | 5 |
| *Geotrichum candidum* Link: Pers. emend. Carmich. | | | | | 1 | | | | 5 |
| *Gongronella butleri* (Lend.) Peyr. & Dal Vesco | | 1 | | | 1 | | | | 10 |
| *Metarhizium anisopliae* (Metschnik.) Sorok. | 1 | | 1 | | | | | 1 | 15 |
| *Microascus cinereus* (Emil.-Weil & Gand.) Curzi | | | 1 | | | | | | 5 |

a: Number of positive samples/total number of samples x 100.

(Continued).

| Fungus | Mangrove species | | | | | | | | Frequency |
|---|---|---|---|---|---|---|---|---|---|
| | ***Avicennia alba*** | ***Ceriops tagal*** | ***Rhizophora mucronata*** | ***Xylocarpus obovatus*** | ***Bruguiera sexangula*** | ***Rhizophora apiculata*** | ***Sonneratia alba*** | ***Bruguiera gymnorrhiza*** | (%)[a] |
| *Mucor* sp. | | | 1 | | | 1 | | | 10 |
| *Neosartorya fischeri* (Fenn. & Raper) Mall. & Cain var. *glabra* (Wehm.) Mall. & Cain | | | | | | | 1 | | 5 |
| *Paecilomyces variotii* Bain. | | 1 | 2 | | | | 1 | | 20 |
| *Paecilomyces* sp. | | 1 | | | | | | | 5 |
| *Penicillium citrinum* Thom | 1 | | | | | | 1 | | 10 |
| *Penicillium funiculosum* Thom | | | | 1 | | | | | 5 |
| *Penicillium purpurogenum* Stoll | | | | | | | 1 | | 5 |
| *Penicillium verruculosum* Peyr. | | 1 | 1 | 1 | | | | | 15 |
| *Penicillium* sp. -1 | 1 | 1 | | | 1 | | | | 10 |
| *Penicillium* sp. -2 | 1 | | | | 1 | 1 | | | 15 |
| *Penicillium* spp. | | 1 | 2 | 2 | 3 | | 1 | 1 | 50 |
| *Pestalotiopsis* sp. | | | 1 | | | | | | 5 |
| *Phoma* sp. | | 1 | | | | | | | 5 |
| *Talaromyces byssochlamydoides* Stolk & Samson | 1 | | 2 | | | 1 | 1 | | 25 |
| *Talaromyces flavus* (Klock.) Stolk & Samson var. *flavus* | | 1 | | | 1 | | | | 10 |
| *Talaromyces wortmannii* C.R. Benj. in Stolk & Samson | 1 | | | | | | | | 5 |
| *Trichoderma harzianum* Rifai | 2 | 2 | | 2 | | 1 | 1 | | 40 |
| *Trichoderma koningii* Oudem. | | 1 | | | | | | | 5 |
| *Trichoderma pseudokoningii* Rifai | 1 | 1 | 1 | | | 1 | | | 15 |
| *Trichoderma* spp. | | 2 | | | 1 | 1 | | 1 | 25 |
| Unidentified strains | | | 2 | 2 | | | 2 | | 30 |
| Total number of strains | 18 | 26 | 22 | 12 | 16 | 8 | 14 | 6 | |
| Number of samples | 4 | 2 | 2 | 2 | 4 | 2 | 3 | 1 | 20 |

a: Number of positive sample/total number of samples x 100.

Table 4. Fungi isolated from soils of campus of Kasetsart univesity.

| | |
|---|---|
| *Acremonium* sp. | 1[a] |
| *Aspergillus awamori* Nakazawa | 1 |
| *Aspergillus candidus* Link | 1 |
| *Aspergillus fumigatus* Fresen. | 1 |
| *Aspergillus niger* aggr. | 3 |
| *Aspergillus* sp. | 3 |
| *Aspergillus tamarii* Kita | 1 |
| *Aspergillus terreus* Thom | 3 |
| *Cladosporium cladosporioides* (Fres.) de Vries | 3 |
| Coelomycetes | 1 |
| *Curvularia lunata* (Wakker) Boed. | 1 |
| *Emericella nidulans* (Eidam) Vuill. | 1 |
| *Eupenicillium* sp. | 1 |
| *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. | 1 |
| *Fusarium solani* (Mart.) Appel & Woll. emend. Snyd. & Hans. | 1 |
| *Fusarium* sp. | 1 |
| *Gliocladium roseum* (Link) Bain. | 1 |
| *Mucor* spp. | 3 |
| *Myceliophthora thermophila* (Apinis) van Oorsch. | 1 |
| *Myrothecium cinctum* (Corda) Sacc. | 1 |
| *Neurospora crassa* Shear & Dodge | 1 |
| *Nigrospora sphaerica* (Sacc.) Mason | 1 |
| *Penicillium citrinum* Thom | 1 |
| *Penicillium funiculosum* Thom | 3 |
| *Penicillium* spp. | 3 |
| *Pithomyces graminicola* R.Y. Roy & Rai | 1 |
| *Rhizomucor* sp. | 1 |
| *Scolecobasidium* sp. | 1 |

a: Number of positive samples (3 samples tested).

soil, being lower than that in the subtropical zone of Okinawa ($2.3\times10^3$ cfu/g of dry soil). On the other hand, campus soil samples from K. U. gave counts ranging from $2.5\times10^5$ to $5.0\times10^5$ cfu/g of dry soil, similar to those of agricultural soil in subtropical Japan ($2.1\times10^5$ cfu/g of dry soil) (4). These findings indicate a poor distribution of fungal propagules in the tropical mangrove mud. The reason may be the semi-anaerobic condition of the mud, which is more clayey than mangrove soil in Japan.

*Mycobiota of mangrove rhizosphere soil*

The fungi isolated from mangrove soil in Thailand were compared with those isolated in Japan. Eighty percentage of the Thai isolates were also found in Japan, including less dominant species (4), and almost all of them are known as typical soil-borne fungi (1, 2, 3, 6). Table 3 lists all the species of fungi isolated from mangrove soil samples by the two isolation methods and their frequency. The number of species found per sample was

Table 5. Fungi isolated from soil of washed mangrove roots by the dilution plate method in Japan.

| Species | No. of positive samples | Frequency (%)[a] |
|---|---|---|
| *Acremonium* spp. | 11 | 50.0 |
| *Cladosporium cladosporioides* (Fres.) deVries | 6 | 27.3 |
| *Coniothyrium* spp. | 12 | 54.5 |
| *Exophiala* sp. | 5 | 22.7 |
| *Fusarium* spp. | 8 | 36.4 |
| *Gliocladium roseum* (Link) Bain. | 3 | 13.6 |
| *Metarhizium anisopliae* (Metschn.) Sorok. | 3 | 13.6 |
| *Nodulisporium* sp. | 3 | 13.6 |
| *Paecilomyces lilacinus* (Thom) Samson | 8 | 36.4 |
| *Paecilomyces* spp. | 5 | 22.7 |
| *Penicillium citrinum* Thom | 6 | 27.3 |
| *P. crustosum* Thom | 3 | 13.6 |
| *P. janthinellum* Biour. | 4 | 18.2 |
| *P. purpurogenum* Stoll | 8 | 36.4 |
| *Penicillium* spp. | 3 | 13.6 |
| *Phialophora fastigiata* (Langerb. & Melin) Conant | 5 | 22.7 |
| *Phialophora* spp. | 6 | 27.3 |
| *Phoma* spp. | 13 | 59.1 |
| *Scopulariopsis* spp. | 5 | 22.7 |
| *Trichoderma harzianum* Rifai | 10 | 45.5 |
| *T. koningii* Oudemans | 3 | 13.6 |
| *Trichoderma* spp. | 3 | 13.6 |
| Sterile mycelium | 16 | 72.7 |

a: Number of positive samples/total number of samples x 100 (22 samples).
Mangrove species: *Avicennia marina, Rhizophora stylosa, Sonneratia alba, Bruguiera gymnorrhiza, Kandelia candel, Lumnitzera racemosa.*

fewer than in the Japanese mangrove (2.1 species/sample). Isolates mainly belong to the mitosporic fungi. Fungi isolated by the heat incubation method were mainly *Aspergillus fumigatus* Fresenius, *A. terreus* Thom, *A. aculeatus* Iizuka, *Paecilomyces variotii* Bainier and *Talaromyces byssochlamydoides* Stolk & Samson. The latter three of these species were dominant in the Thai samples but were not detected in the subtropical zone of Japan. Conversely, *Acremonium alabamense* Morgan-Jones, which was dominant in Japan (4), was not detected in the Thai samples. This may reflect a geological difference in fungal diversity between tropical and subtropical zones.

Species of the genera *Penicillium* and *Trichoderma* were dominantly detected by the dilution plate method. These species, which decompose plant debris, are commonly isolated from subtropical soil. Species of the mitosporic genera *Coniothyrium, Phialophora*, and *Phoma* occurred less frequently than in the subtropical mangroves (4). The reason is suggested to be differences in the mangrove environment. The fungi detected in this survey are typical terrestrial soil fungi that are considered to have been carried in from

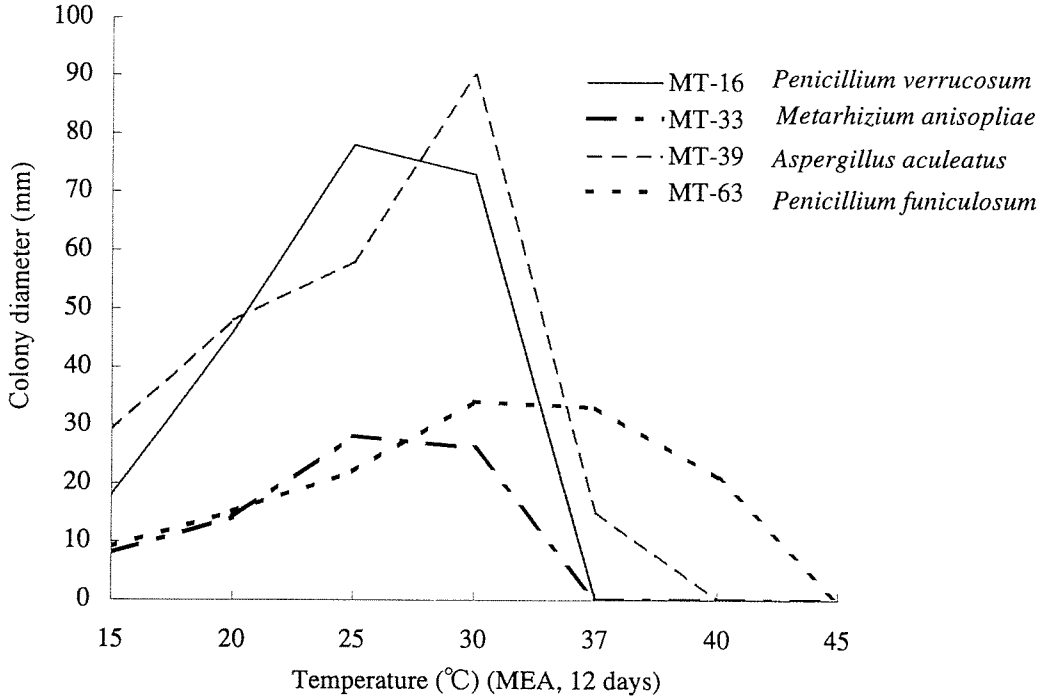


Fig. 2. Hyphal growth of four strains

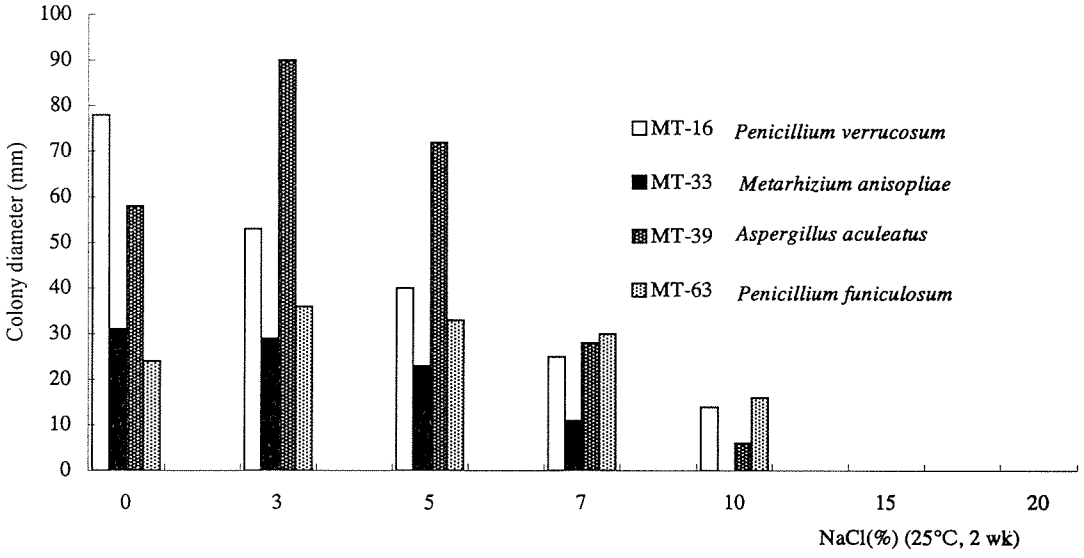


Fig. 3. Salinity range for growth of four strains.

surrounding substrates and adapted to the mangrove environment.

*Aspergillus* species were dominantly isolated from campus soil of K.U.: *A. terreus, A. tamarii* Kita, *A. awamori* Nakazawa, *A. candidus* Link, and *A. fumigatus* (Table 4).

Except for *A. terreus* and *A. fumigatus* (4), these species are less dominant in subtropical zone. This difference represents a difference in the fungal diversity in terrestrial soil between tropical and subtropical zones. *Penicillium funiculosum* was characteristically isolated from soil of campus of K.U., but it was not predominantly isolated in the subtropical zone. To confirm why do such differences occur, research into mangrove rhizosphere mycobiota should continue.

Table 5 shows the fungi dominantly detected in Japanese mangrove forest soils by the dilution plate method (5).

*Optimum growth temperature*

The effect of temperature on hyphal growth was investigated for four of the isolated fungi: *A. aculeatus, Metarhizium anisopliae* (Metschnikoff) Sorokin, *Penicillium verruculosum* Peyronel, and *P. funiculosum*. Figure 2 shows the results. The optimum temperature for *A. aculeatus, M. anisopliae* and *P. verruculosum* was 25°C to 30°C, and that for *P. funiculosum* was 30°C to 35°C. These fungi may be adapted to the conditions of the mangrove rhizosphere.

*Tolerance to sodium chloride*

Tolerance to sodium chloride was tested for the same four strains. Except for *M. anisopliae*, the fungi grew at NaCl concentrations of up to 10 % (Fig. 3). These fungi have been isolated from natural substrates and soil (1). Their salt tolerance means that these fungi can withstand the high osmotic pressure if the soil in which mangrove is growing dries up or if they are carried into salt water by the tide. That is, they are probably able to adapt to the mangrove environment.

The smaller population and lower diversity of fungi in the Thai mangrove soil might be due to the lower nutrient content of soil, which is mostly composed of clay particles. However, the *Aspergillus* species, which adapt to high temperature, showed higher diversity in the Thai soil.

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