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Mycoflora of the rhizosphere of *Salicornia europaea* L., a halophytic plant

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Summary

To compare the mycoflora of its rhizosphere with that of mangrove, *Salicornia europaea* L., a halophytic plant like mangrove, was collected from Hokkaido, Japan. After washing the collected *S. europaea* roots and surrounding soil samples, their root- and soil-inhabiting fungi were isolated by washing and dilution plate methods, respectively. Population density of the rhizosphere soil showed 7.8 x 10^3 average colony-forming units (cfu)/g of dry weight. *Acremonium strictum* W. Gams, *Alternaria alternata* (Fr.) Keissler, and *Cladosporium cladosporioides* (Fres.) de Vries were dominant species on the rhizoplane of *S. europaea*, and these species were considered to be the main components of the rhizoplane mycoflora. The genera *Acremonium* Link, *Cladosporium* Link, *Penicillium* Link, *Phoma* Sacc., and *Trichoderma* Pers., were predominantly isolated from the washed root soils. Consequently, the mycoflora of the *S. europaea* rhizoplane differed from that of its surrounding soil. The mycoflora of the *S. europaea* rhizoplane also differed from that of mangrove, but the soil mycoflora of its rhizosphere was almost the same as that of mangrove. Tolerance to sodium chloride was tested for some isolates.

Keywords: Mycoflora, *Salicornia europaea* rhizosphere, halophytic plant, root-and soil inhabiting fungi.

Terrestrial microorganisms in the rhizoplane of higher plants are known to grow by using dead root tissue or secretions of root tissue, and to establish a micro-community in the roots and surrounding soil (1, 14, 15). The mycoflora of rice fields is affected by inundation with water (9, 14) and that of the mangrove rhizosphere by semi-anaerobic conditions and a high content of heavy metals (7, 8). *S. europaea* plant are also adapted to these conditions as a halophytic plant. To compare the mycoflora of *S. europaea* rhizosphere with that of mangrove, *S. europaea* samples were collected and surveyed. This is a preliminary study as we are studying for clarification of ecological and species diversity of endophytic fungi.

S. europaea is a halophytic plant that is distributed in the temperate region of the Northern Hemisphere. In Japan, it is found mainly in Hokkaido, growing in soils that

are semi-aerobic, low in nutrient, and have higher salinity than terrestrial soils, like soils of the mangrove habitat. The mycoflora of the *S. europaea* rhizosphere where such adverse conditions prevail has not been studied.

The present study compares the mycoflora of the *S. europaea* rhizosphere with the results of mangrove rhizosphere obtained in 1996 (7, 8).

Materials and Methods

Sampling sites. Fifteen healthy roots of *S. europaea* were collected from the side of Lake Notoro (4 samples) and Lake Saroma (11 samples), Abashiri and Monbetu-gun, Hokkaido, Japan on 1 and 2 August 1997. Five samples were collected from pasture, vegetable, and wheat fields near the lakes as root-free soil. This region has a so-called Okhotsk-type climate, with annual rainfall of 845 mm and average annual temperature of 5.9°C. The locality is at longitude 139°40'E and latitude 37°40'N. Soil type is sandy loam and soil pH is 7.2. Content of NaCl is 3.0%.

Isolation method. The same isolation method was adopted as in the previous report (8). Eight out of 15 root samples collected were used. Slender root were cut into sections of ca 5 cm in length and washed with physiological water (0.85%). Five sections of each sample were incubated on two plates at 15°C for one month. All fungi appearing during the incubation were isolated at 2, 3 and 4 wk under the dissecting microscope. Soil fungi were isolated from 15 samples after the first washing by x 50 and x 100 dilution plate methods.

Isolation medium. Cornmeal agar containing 50 μg/ml of tetracycline antibiotics was used for the slender root samples. Malt-yeast extract agar containing the same concentration and type of antibiotics was used.

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

For species identification the following references and strains of IFO Culture Collection were consulted: *Acremonium* (6), *Penicillium* (10), *Fusarium* Link (2), Dematiaceous Hyphomycetes (4, 5), general taxonomy (3).

Results and Discussion

Fungal population density

The number of fungi present in each sample by the dilution plate method was counted under the dissecting microscope.

The average number of fungi for each site of *S. europaea* was 7.8 x 10^3 cfu/g of dry soil, whereas that of root-free soils of *S.europaea*, namely, pasture, vegetable, and wheat field soils, was 9.4 x 10^3 cfu/g. No significant differences were found in the average number of fungi between the *S. europaea* rhizosphere and root-free sample. The average number of fungi in the *S. europaea* rhizosphere was higher than that of mangrove soil

Table 1. Fungi isolated from *S. europaea* and mangrove roots, and their frequency of occurrence.

Species name	*S. europaea* frequency[a]	Species name	Mangrove frequency[b]
Acremonium strictum W.Gams	37.5	*Acremonium* sp.	3.6
Acremonium sp.	12.5	*Cylindrocarpon destractans*	
Alternaria alternata (Fr.) Keissler		(Zins.) Sholten	13.6
Chrysosporium sp.	75.0	Coelomycetes	13.6
Cladosporium cladosporioides	12.5	*Pestalotiopsis* sp.−1	27.3
(Fres.) de Vries	50.0	*Penicillium citrinum* Thom	13.6
Cladosporium herbarum (Per.)	12.5	*Penicillium* sp.−1	13.6
Link ex S.F.Gray		*Trichoderma harzianum* Rifai	50.0
Sterile mycelium	12.5		
Number of samples	8		22

[a]: Number of positive samples / total number of samples x 100 (%).

[b]: Data are cited from the IFO Res. Commun. 18: 40-44 (1997).

(1.2 x 10^3 cfu/g) (7). The reason is unclear, but the strongly anaerobic state and high content of heavy metals of the mangrove soil may be involved.

Mycoflora of rhizoplane of S. europaea

Table 1 shows the frequency of detection of fungi isolated from *S. europaea* root samples. Only six species were detected. *Alternaria alternata* (75.0% frequency), *Cladosporium cladosporioides* (50.0%), and *Acremonium strictum* (37.5%) were dominantly detected. The dominant species in roots of mangrove trees were *Trichoderma harzianum* Rifai (50.0%) and *Pestalotiopsis* sp.-1 (27.3%) (8). This difference is considered to be due to the host specificity.

Mycoflora of rhizosphere soil

Table 2 lists all the species of fungi isolated from 15 rhizospere and 5 root-free samples.

Isolated species mainly belong to the taxon Deuteromycotina. The dominantly detected fungi from soil of *S. europaea* rhizosphere were *Acremonium* sp.-1 (66.7%), *Penicillium* spp. (33.3%), *Phoma* spp. (46.7%), *Trichoderma harzianum* (33.3%), and *T. koningii* Oudemans (33.3%), followed by *Acremonium strictum* (26.7%), *Alternaria alternata* (26.7%), *Cladosporium herbarum* (26.7%), and *Mucor hiemalis* Wehmer (26.7%). In root-free soil of *S. europaea*, *Apiospora montagnei* Sacc. (60.0%), *Mortierella nana* Linnemann (60.0%), *Mucor* spp. (80.0%) and *Phoma* spp. (60.0%) were detected. These fungi are typical soil fungi worldwide (3). It is considered that some of these fungi are psychrophilic or are adapted to low temperature.

Most of the fungi detected in the *S. europaea* rhizosphere resembled the mangrove rhizosphere fungi (7, 8, 11, 12, 13). Notable exceptions not found in the mangrove

Table 2. Fungi detected in *S. europaea* rhizosphere and root-free soil samples by the dilution plate method.

Rhizosphere soil Species name	Frequency[a]	Root-free soil Species name	Frequency
Acremonium murorum (Corda) W.Gams	13.3	*Acremonium strictum* W.Gams	20.0
Acremonium strictum W.Gams	26.7	*Acremonium* spp.	40.0
Acremonium sp.1	66.7	*Apiospora montagnei* Sacc.	60.0
Acremonium spp.	20.0	*Chrysosporium merdarium* (Link:Fr.)	
Alternaria alternata (Fr.:Fr.) Keissler	26.7	Carmichael	20.0
Apiospora montagnei Sacc.	6.7	*Cladosporium cladosporioides* (Fres.) de Vries	40.0
Arthrinium phaeospermum (Corda) M.B.Ellis	6.7	*Cladosporium herbarum* (Pers.:Fr.) Link	20.0
Aureobasidium pullulans (de Bary) Arnaud	20.0	*Cladosporium* sp.	20.0
Beauveria bassiana (Balsamo) Vuill.	6.7	*Coniothyrium* sp.	20.0
Beauveria brongniartii (Sacc.) Petch	6.7	*Fusarium* spp.	40.0
Bipolaris sp.	6.7	*Gliocladium virens* Miller et al.	20.0
Chrysosporium sp.	6.7	*Gliocladium* sp.	20.0
Cladosporium cladosporioides (Fres.) de Vries	20.0	*Gongronella butleri* (Lendner) Peyronel	
Cladospotium herbarum (Pers.:Fr.) Link	26.7	& Dal Vesco	20.0
Cladosporium sp.	6.7	*Mortierella alpina* Peyronel	20.0
Coniothyrium sp.	6.7	*Mortierella minutissima* van Tieghem	40.0
Curvularia lunata (Wakker) Boedijn	6.7	*Mortierella nana* Linnemann	60.0
Cylindrocarpon sp.	6.7	*Mortierella ramanniana* (Moller)	
Eurotium amstelodami Mangin	6.7	Linnemann var. *angulispora*	
Eurotiales	6.7	(Naumov) Linnemann	20.0
Fusarium graminearum Schwabe	20.0	*Mortierella* sp.	20.0
Fusarium oxysporum Schlecht.:Fr.	6.7	*Mucor hiemalis* Wehmer	40.0
Fusarium spp.	13.3	*Mucor racemosus* Fres.	20.0
Metarhizium anisopliae (Metschn.) Sorokin	6.7	*Mucor* spp.	80.0
Mortierella alpina Linnemann	6.7	*Penicillium islandicum* Sopp	20.0
Mortierella minutissima van Tieghem	6.7	*Penicillium spinulosum* Thom	20.0

Table 2. (continued)

Rhizosphere soil Species name	Frequency[a]	Root-free soil Species name	Frequency
Mortierella spp.	13.3	*Penicillium viridicatum* Westling (?)	20.0
Mucor hiemalis Wehmer	26.7	*Penicillium* spp.	40.0
Mucor sp.	6.7	*Phialophora cyclaminis* van Beyma	20.0
Paecilomyces marquandi (Massee) Hughes	13.3	*Phoma* spp.	60.0
Paecilomyces sp.	6.7	*Rhizopus* sp.	20.0
Penicillium citrinum Thom	20.0	*Scopulariopsis* sp.	20.0
Penicillium aurantiogriseum Dierckx	13.3	*Trichoderma aureoviride* Rifai	20.0
Penicillium chrysogenum Thom	6.7	*Trichoderma harzianum* Rifai	20.0
Penicillium roquefortii Thom	6.7	*Trichoderma polysporum* (Link:Fr.) Rifai	20.0
Penicillium waksmanii Zaleski	6.7	*Trichoderma viride* Per.:Fr.	20.0
Penicillium spp.	33.3	*Trichoderma* sp.	20.0
Phialophora sp.	6.7	Unidentified strains	40.0
Phoma spp.	46.7	Sterile mycelium	80.0
Rhinocladiella sp.	6.7		
Scopulariopsis spp.	20.0		
Talaromyces helicus C.R.Benjamin var. *major* Stolk & Samson	6.7		
*Trichoderma harzianum*Rifai	33.3		
Trichoderma koningii Oudem.	33.3		
Verticillium fungicola (Preuss) Hassebr.	6.7		
Unidentified strain	6.7		
Sterile mycelium	6.7		
Total number of samples	15		5

[a]: Number of positive samples / total number of samples x100 (%).

rhizosphere were *Alternaria alternata*, *Cladosporium herbarum* (Per.: Fr.) Link, and *Fusarium graminearum* Schwabe. The optimal growth temperature of these fungi are at 15-28°C and the minimum temperature are in the range 5-8°C. It is assumed that they grow in low temperature.

The genera *Mortierella* Coemans and *Mucor* Fresenius, which belong to the taxon Zygomycotina, were often detected in root-free soils. These fungi are known to be found dominantly in tomato field and forest soil (1, 3), but they were scarcely detected in mangrove root-free samples in Okinawa (7). It is considered that these fungi like to cold temperature and the optimum temperature is low. Consequently, low temperature is assumed to be one of the factors determining their geographical distribution.

Tolerance to sodium chloride

Tolerance to sodium chloride was tested for some isolates, namely, *Acremonium* sp.-1 (H9-6-7), *Mortierella ramanniana* (Möler) Linnemann var. *angulispora* (Naumov) Linnemann (H9-18-2), *Mucor hiemalis* (H9-18-1), *Penicillium aurantiogriseum* (H9-4-3), *Phoma* sp. (H9-3-3), and *Trichoderma harzianum* (H9-16Na-2). The fungi other than *M. ramanniana* var. *angulispora* grew at the concentration of NaCl from 7.0% to 15.0%. These fungi have been isolated from natural substrates and sugar, salted food products, and concentrated fruit juice (3). The results show that these fungi can withstand the high osmotic pressure if the soil in which *S. europaea* is standing dries up at high temperature or if they are carried into the salty water of a lake. *M. ramanniana* var. *angulispora* tolerated NaCl only up to the concentration of 2.0%. This means that this species will not survive in lake water of 3.0% NaCl when it is carried into the lake.

It was considered that the mycoflora of the *S. europaea* rhizosphere differs from that of the mangrove rhizosphere, but soil mycofloras of the two habitats are almost the same in spite of the differences in host plants and geographical distribution. Further investigations on ecology and species diversity are necessary.

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