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Effect of sea water concentration on hyphal growth and antimicrobial metabolite production in marine fungi

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We studied the effect of sea water concentration in a culture medium on fungal growth and the production of antimicrobial metabolites. Most of the marine fungal isolates were identified as members of the same genera as terrestrial isolates, such as *Aspergillus* and *Trichoderma*. Many of the marine fungi isolated grew more abundantly as the sea water concentration increased. The production of antimicrobial materials was improved as the sea water concentration increased. Even though the marine fungi were considered to be similar to fungi from terrestrial environments, from a mycological perspective, the two types have different physiological characteristics. The fungi from marine samples are useful microbial resources in the search for new bioactive compounds.

Key Words——antimicrobial metabolite; hyphal growth; marine fungi; salinity; sea water.

Fungi exist not only on land but also in marine environments, which account for 70% of the earth's surface. In the oceans, fungi live as saprophytes, parasites, and symbionts on various matrices such as sea sand, logs, water, soil, bubbles, as well as algae and other marine organisms. Various fungi have been isolated from samples obtained from marine environments in recent years. These fungi are useful resources in screening for bioac-

Table 1. Strains used in the study.

Strain[a]	Source	Sample collected at	Genus	Strain[a]	Source	Sample collected at	Genus
FT-0011	marine sponge	Pohnpei	*Aspergillus*	FT-0449	seaweed	Palau	*Aspergillus*
0012	marine sponge	Pohnpei	*Cladosporium*	0468	fallen leaf	Palau	*Aspergillus*
0034	sea sediment	Pohnpei	unidentified	0500	marine sponge	Palau	unidentified
0104	marine sponge	Pohnpei	*Aspergillus*	0507	marine sponge	Palau	*Penicillium*
0108	marine sponge	Pohnpei	unidentified	0554	marine sponge	Palau	*Aspergillus*
0111	fallen twig	Pohnpei	*Trichoderma*	0555	marine sponge	Palau	*Trichoderma*
0138	mangrove fruits	Pohnpei	unidentified	0566	seaweed	Palau	*Pestalotiopsis*
0180	fallen leaf	Pohnpei	*Phomopsis*	FO-7474	soil	Okayama	*Penicillium*
0205	sea water	Pohnpei	unidentified	7486	soil	Hokkaido	unidentified
0222	sea water	Pohnpei	*Cladosporium*	7584	soil	Tokyo	*Penicillium*
0294	sea sediment	Pohnpei	*Aspergillus*	7663	soil	Tokyo	*Cladosporium*
0302	sea water	Pohnpei	*Gliomastix*	7930	soil	Saitama	*Aspergillus*
0317	marine sponge	Pohnpei	*Aspergillus*	8269	plant leaf	Tokyo	*Fusarium*
0331	sea squirt	Pohnpei	*Trichoderma*	8287	plant leaf	Tokyo	*Aureobasidium*
0358	marine sponge	Pohnpei	*Aspergillus*	8343	plant leaf	Tokyo	*Chaetomium*
0407	fallen leaf	Palau	unidentified	8348	plant leaf	Tokyo	*Fusarium*
0445	seaweed	Palau	*Trichoderma*				

[a] FT: Marine isolate, FO: terrestrial isolate.

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Table 2. Effect of sea water concentration on mycelial growth of fungi isolated from marine and terrestrial samples.

Type	Mycelial growth in 0, 50 and 100% sea water[a)]	Marine isolate	Terrestrial isolate
A	0<50<100	FT-0011, 0012, 0034, 0104, 0180, 0222, 0294, 0302, 0317, 0358, 0449, 0468, 0554, 0566	FO-7584, 7663, 8269
B	0=50=100	FT-0108, 0111, 0205, 0507	FO-7486, 7930, 8343, 8348
C	100<50<0	FT-0138, 0331, 0407, 0445, 0500, 0555	FO-7474, 8287

[a)] Observed after incubation at 25°C for 7 d.

tive compounds (Pietra, 1997; Renner et. al., 1998). They are also utilized in studies of fungal physiology and ecology. The effect of sea water concentration on hyphal growth and germination of conidia has been studied (Byrne et al., 1975; Nakagiri, 1990).

The present authors have isolated various fungi from marine and terrestrial samples, and have subjected these samples to a variety of screening systems in the search for bioactive metabolites. We have discovered several new bioactive compounds such as pyripyropenes (Ōmura et al., 1993), terpendoles (Huang et al., 1995), arisugacins (Ōmura et al., 1995) and macrosphelides (Hayashi et al., 1995).

To optimize marine fungal cultures supplied for screening, we studied the effects of sea water concentration in a culture medium on fungal growth and production of antimicrobial metabolites.

Materials and Methods

Sample and fungal culture isolation At the site of culture isolation, samples were obtained from marine mud, water, algae and logs collected on the coasts of Pohnpei and Palau Islands, Micronesia in June–July, 1995. Fungi were isolated from the samples mainly by the agar dilution method. The isolation media used were potato-dextrose agar (Difco, PDA) prepared in 50% natural sea water (salt concentration 3.4%) and soluble starch-yeast extract agar, supplemented with 100 μg/ml of an antimicrobial antibiotic, chloramphenicol (sterilized at 121°C, for 15 min).

Strains used Of the fungi isolated from the marine samples by the above procedure, 24 strains (given FT No.) were selected randomly as test organisms. Nine terrestrial fungi (given FO No.) were also used for comparison. The strain numbers and their genera are listed in Table 1.

Effect of sea water concentration amd sodium chloride in medium on fungal growth Test organisms were inoculated on a PDA prepared with 50% and 100% natural sea water and cultivated for 7 d at 25°C. The diameters of colonies formed were measured. To examine the effect of sodium chloride, the test organisms were inoculated

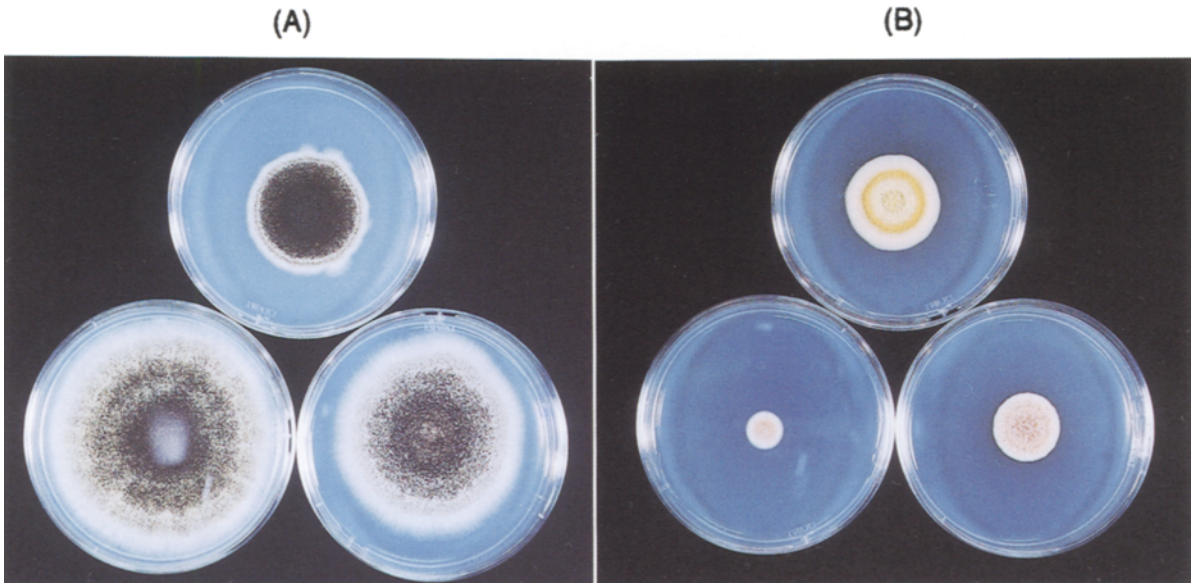

Fig. 1. Colonies of Types A and C fungi on different sea water concentrations. (A), *Aspergillus* sp. FT-0554; (B), *Aureobasidium* sp. FO-8287. Top, PDA medium (control); Right, PDA medium prepared in 50% concentration of natural sea water; Left, PDA medium prepared in 100% concentration of natural sea water.

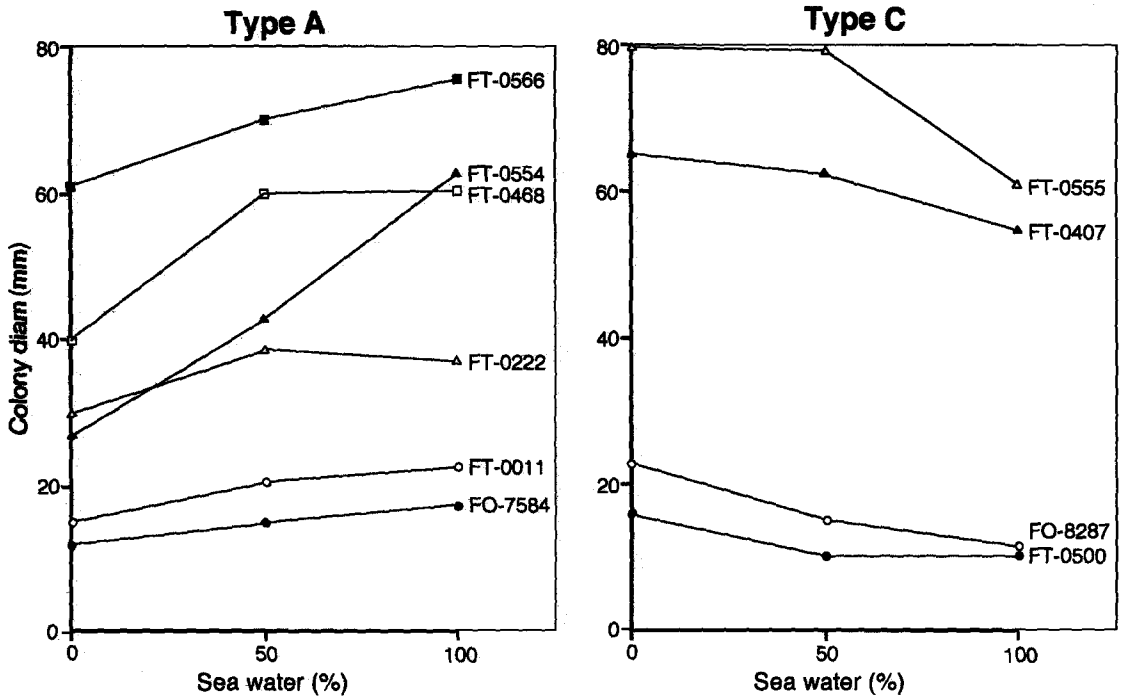

Fig. 2. Colony sizes of Types A and C fungi on different sea water concentrations after incubation at 25°C for 7 d.

on PDA medium containing 4% sodium chloride. The organisms were cultivated for 7 d at 25°C, and the growth of test fungi was compared with that on control media with no addition.

Effect of sea water concentration on production of antimicrobial compounds Test fungi were cultivated in a production medium prepared with 50% and 100% natural sea water for 7 d at 27°C with shaking culture. The antimicrobial activity of the culture fluid was then measured by the paper disk method (8 mm thick) using *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 KB211 (ATCC 6633) and *Mucor racemosus* Fresenius KF223 (IFO 4581) as test organisms.

Results

Effect of sea water concentration on mycelial growth Mycelial growth of the fungi (Table 1) in different sea water concentrations (0, 50 and 100%) was compared after incubation at 25°C for 7 days, and the results are summarized in Table 2. The fungi were classified into three types from the growth. In Type A, the colony size increased with increasing sea water concentration. In Type B, the growth did not change with changes in sea water concentration. In Type C, the size decreased with increasing sea water concentration. Figure 1 shows typical profiles of a colony of Type A strain (FT-0554) and Type C strain (FO-8287) in different sea water concentrations. The colony sizes in different sea water concentrations were measured for 6 strains of Type A and 4 strains of Type C (Fig. 2). Of the 24 marine fungi tested, 14 isolates including FT-0222 and FT-0554 were classified as Type A. The growth of some of the type C fungi was strongly suppressed at the sea water concentration of 100%. Characteristic growth responses to sea water concentration were observed among terrestrial fungi.

Effect of sodium chloride on mycelial growth Table 3 shows the effect of sodium chloride on fungal growth. Strains FT-0011, FT-0222, FT-0468, FT-0554, and FT-0556 of Type A were found to grow abundantly on the medium containing 4% sodium chloride. Growth of three strains of Type C was inhibited by sodium chloride. These results correlated well with those of growth in sea water.

Several fungi of marine or terrestrial origin (e.g., FT-

Table 3. Effect of sodium chloride on mycelial growth.

Type	Strain	Growth in the presence of NaCl[a]	
		None	4%
A	FT-0011	+[b]	+++
	0222	+	+++
	0468	+	+++
	0554	+	+++
	0566	+	+++
C	0407	+++	+
	0500	+++	+
	0555	+++	+
A	FO-7663	+++	+
	8269	+++	+
C	7474	+++	+

[a] Potato dextrose agar containing 4% sodium chloride.
[b] + growth, +++ abundant growth.
Observed after incubation at 25°C for 7 d.

Table 4. Effect of sea water concentration on production of antimicrobial substances.

Type	Strain	Test organism[a]	Inhibition zone (mm)			Type	Strain	Test organism[a]	Inhibition zone (mm)		
			Sea water						Sea water		
			None	50%	100%				None	50%	100%
A	FT-0034	B	13	12	14	A	FO-7584	B	30	18	20
	FT-0104	B	14	18	20	B	FO-7930	M	23	16	14
	FT-0294	B	24	24	23	C	FO-7474	B	12	14	—
	FT-0317	B	21	23	25		FO-8287	B	19	20	14
	FT-0449	B	12	24	24						
	FT-0468	B	22	23	23						
	FT-0566	B	13	12	12						
C	FT-0108	B	29	23	22						
	FT-0500	M	17	18	16						

[a] B: *Bacillus subtilis* KB211 (ATCC 6633), M: *Mucor racemosus* KF223 (IFO 4581)

0566, FT-0407, FT-0555, FO-8269, and FO-7474) showed tolerance to a wide range of metal ions (data not shown).

Effect of sea water concentration on production of antimicrobial substances Among the fungi listed in Table 1, the culture broths of nine FT strains and four FO strains were found to show antimicrobial activity against *Bacillus subtilis* and/or *Mucor racemosus* (Table 4). The anti-*B. subtilis* activity of the culture broths of FT-0104, FT-0449, and FT-0317 strains increased with sea water concentration. These three strains all belong to Type A, suggesting that they are more adaptable to the marine environment. The antimicrobial activities of the four terrestrial strains (FO) were suppressed by sea water. The production of substances showing antimicrobial activity in some fungi belonging to Type A were not affected by sea water concentration (Table 4). FT-0554, a producer of new bioactive compound named nafuredin (Ōmura et al., 2001; Ui et al., 2001), and other strains required an optimal sea water concentration (25–50%) for the maximal production of bioactive compounds (data not shown).

Discussion

The effect of sea water concentration in a culture medium on fungal growth and the production of antimicrobial metabolites was studied. Of the 24 fungi isolated in media containing sea water, 14 strains grew more abundantly as the sea water concentaration increased, while growth of 6 strains was suppressed (Table 2). It was found that the marine strains that grew in high sea water concentration grew rapidly in the medium containing 4% sodium chloride. They are probably able to adapt to the marine environment. However, the growth of terrestrial strains was suppressed by 4% sodium chloride.

The relationship between growth and conidiogenesis was examined. On a culture medium without sea water, which was a poor growth medium, the type A strain FT-0554 produced dense conidia. However, on the medium prepared with full-strength sea water, which was the best for growth (i.e., giving the maximum colony diam), it produced only faint and sparse conidia. This suggests that the optimum concentrations of sea water for growth of FT-0554 and for its conidiogenesis are different. A similar phenomenon was observed for other strains of type A (data not shown). Further study of this phenomenon should provide an insight into the physiology of conidiogenesis in marine fungi.

A tendency for sea water to improve the production of antimicrobial materials was found only among fungi isolated from marine environments. The production of antimicrobial materials by fungi isolated from terrestrial environments was inhibited by sea water (Table 4). This suggests that even though the marine fungi were considered to belong to the same genera as those from terrestrial environments, some isolates appear to have evolved a mechanism to produce a greater abundance of antimicrobial substances in marine environments. This interesting ability has not yet been fully exploited.

We have recently discovered novel bioactive compounds such as nafuredin, phenochalasin (Tomoda et al., 1999) and roselipin (Ōmura et al., 1999), all of which were produced by fungi isolated from marine environments. This demonstrates clearly that fungi from marine samples are useful microbial resources in the search for new bioactive compounds.

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Literature cited

Byrne, P. J. and Jones, E. G. 1975. Effect of salinity on the reproduction of terrestrial and marine fungi. Trans. Br. Mycol. Soc. **65**: 185–200.

Hayashi, M., Kim. Y. P., Hiraoka, H., Natori, M., Takamatsu, S., Kawakubo, T., Masuma, R., Komiyama, K. and Ōmura, S. 1995. Macrospelide, a novel inhibitor of cell-cell adhesion molecule. I. Taxonomy, fermentaion, isolation and biological activities. J. Antibiotics **48**: 1435–1439.

Huang, X.-H., Tomoda, H., Nishida, H., Masuma, R. and Ōmura,

S. 1995. Terpendoles, novel ACAT inhibitors produced by *Albophoma yamanashiensis*. I. Production, isolation and biological properties. J. Antibiotics **48**: 1–4.

Nakagiri, A. 1990. Marine fungi. Lifescience & Biotechnology **6**: 67–71. (In Japanese.)

Ōmura, S., Kuno, F., Otoguro, K., Sunazuka, T., Shiomi, K., Masuma, R. and Iwai, Y. 1995. Arisugacin, a novel and selective inhibitor or acetylcholinesterase from *Penicillium* sp. FO-4259. J. Antibiotics **48**: 745–746.

Ōmura, S., Miyadera, H., Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T., Takano, D., Sunazuka, T., Harder, A., Kölbel, H., Namikoshi, M., Miyoshi, H., Sakamoto, K. and Kita, K. 2001. An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria. Proc. Natl. Acad. Sci. USA **98**: 60–62.

Ōmura, S., Tomoda, H., Kim, Y. K. and Nishida, H. 1993. Pyripyropenes, high potent inhibitors of acyl-CoA cholesterol acyltransferase produced by *Aspergillus fumigatus*. J. Antibiotics **46**: 1168–1169.

Ōmura, S., Tomoda, H., Tabata, N., Ohyama, Y., Abe, T. and Namikoshi, M. 1999. Roselipins, novel fungal metabolites having a highly methylated fatty acid modified with a mannose and an arabinitol. J. Antibiotics **52**: 586–589.

Pietra, F. 1997. Secondary metabolites from marine microorganisms: bacteria, protozoa, algae and fungi. Achivements and prospects. Nat. Prod. Rep. **14**: 453–464.

Renner, M. K., Jensen, P. R. and Fenial, W. 1998. Neomangicols: Structures and absolute streochemistries of unprecedented halogenated sesterterpenes from a marine fungus of the genus *Fusarium*. J. Org. Chem. **63**: 8346–8354.

Tomoda, H., Namatame, I., Si, S. H., Kawaguchi, K., Masuma, R., Namikoshi, M. and Omura, S. 1999. Phenochalasins, inhibitors of lipid droplet formation in mouse macrophages, produced by *Phomopsis* sp. FT-0211. J. Antibiotics **52**: 851–856.

Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T., Takano, D., Sunazuka, T., Namikoshi, M. and Ōmura, S. 2001. Nafuredin, a novel inhibitor of NADH-fumarate reductase, produced by *Aspergillus niger* FT-0554. J. Antibiotics **54**: 234–238.