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パラオとインドネシアブナケン島のサンゴ礁における海綿と共存する海洋糸状菌の分布

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Distribution of Marine Filamentous Fungi Associated with Marine Sponges in Coral Reefs of Palau and Bunaken Island, Indonesia

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Abstract: Recent investigations indicate the tremendous potential of marine filamentous fungi as a source of new bioactive natural products. In the course of our study on bioactive metabolites from marine fungi, we isolated filamentous fungi associated with marine sponges at Palau and at Bunaken Island in North Sulawesi, Indonesia, during the voyage of the training vessel Shinyo-maru. Filamentous fungi were obtained from 82.7% and 98% of sponges at Palau and Bunaken Is., respectively. These rates were remarkably higher than those of other tropical areas. Distribution of marine filamentous fungi in the coral reef of Bunaken Is. at 6 sampling points was equally high (90-100%), while the 8 sampling points at Palau slightly varied in distribution.

Key words: *Marine filamentous fungi, Marine sponges, Distribution, Coral reef, Palau, Bunaken Island, North Sulawesi*

Introduction

Filamentous fungi are a prolific source of biologically active secondary metabolites. The first antibiotic and still the important antiinfectious agent, penicillin, was isolated from the fungus *Penicillium notatum*. A number of antibiotics have been obtained from the culture broths of filamentous fungi to date. Recent investigations on marine filamentous fungi as a bioresource for searching biologically active secondary metabolites indicate the tremendous potential of them as a source of new medicines[1-4)].

Marine sponges are benthic animals found in the wide range of marine environments. The species diversity of sponges is superior in the tropical coral reef environments. While, the sponges would be one of the most to-be-studied groups among reef fauna. Indeed, the sponges are often ignored by divers and naturalists, who appreciate encountering mobile animals, such as fish, turtles, mammals, rays, and even sharks, and looking at colorful corals, but sponges are quite interesting animals to be questioned to them on the origins of their diversity in species, colors, and morphologies and on the ecological significance as the members of tropical coral reef habitats. The sponges are also very important resources for searching the biologically active substances, which are useful to develop pharmaceuticals, agrochemicals and biochemical reagents and their lead compounds. The origins of these biologically active substances are recently thought to be the metabolites produced by the microorganisms associated with the sponges. Sponges are good homes not only for macroorganisms, such as worms, brittlestars, shrimp, crabs, etc., but also for a variety of microorganisms, such as bacteria, fungi, and microalgae, which live in the canals, between cells, and even inside the cell.

In the course of our systematic study on marine filamentous fungi as a useful and sustainable source of bioactive natural products[5-9)], we investigated the diversity and distribution of sponges and their associated filamentous fungi in the coral reefs of Palau and Bunaken Island in North Sulawesi, Indonesia. The expeditions were conducted on the occasions when the training vessel Shinyo-maru was anchored at Marakal in Palau between February 4 and 8, 2000 and at Bitung in North Sulawesi between March 3 and 7, 2001.

Marine filamentous fungi were obtained in high yield from marine sponges at both sites. Distribution of filamentous fungi at Bunaken Is. was equally high, and a slight difference in distribution was observed at Palau.

This report describes the results of the expeditions and the distribution of marine filamentous fungi isolated from

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marine sponges collected in the coral reefs of Palau and Bunaken Island.

Materials and Methods

Marine Sponges

Marine sponges were collected by scuba diving at 8 sampling points in Palau between February 4 and 7 in 2000 and at 6 sampling points in Bunaken Is. between March 4 and 6 in 2001. Photographs of the sponges were taken in the water by a camera (Nikonos V) equipped with an electronic flash. Each sponge was sealed in a sterile plastic bag in the water and stored in a cooler box with coolant for isolation of filamentous fungi.

Isolation of Filamentous Fungi

Treatment of marine sponges was done within 3 h in a laboratory of the research vessel Shinyo-maru. The sponges were cut into small pieces, and three to five pieces of each sponge were placed in a sterile mortar with 1-2 mL of sterile seawater and homogenized with a pestle. One- to two-hundred microliters of the liquid portion was placed on an agar plate (0.02% yeast extract (Difco), 0.1% soluble starch, 2% agar (Difco), and 200 ppm chloramphenicol in 90% natural seawater). The organism remaining in the mortar was pressed by pestle to remove liquid, and two to three pieces were applied on an agar plate. The plates were placed in the research room (25-26°C) of the ship and then incubated at 20°C after returning to the university.

The mycelia grown on an agar plate or on a substrate were inoculated on a slant in a culture tube (1/10 YSA: 0.02% yeast extract, 0.1% soluble starch, and 2% agar in 90% natural seawater).

Results and Discussion

Study Sites

Palau is located at latitude about 7°N and surrounded by the deep sea (Pacific Ocean) (Figure 1). The country is consisted of many islands, and coral reefs are well grown (Figure 2). Bunaken Island, located at little north of latitude 1°N, is a small island in North Sulawesi, Indonesia (Figures 1 and 3). The island is surrounded by a coral reef (Figure 4), which gives a steep drop off to 200-300 m depth. Several islands including Bunaken Is. are preserved their natural environments as the National Marine Park.

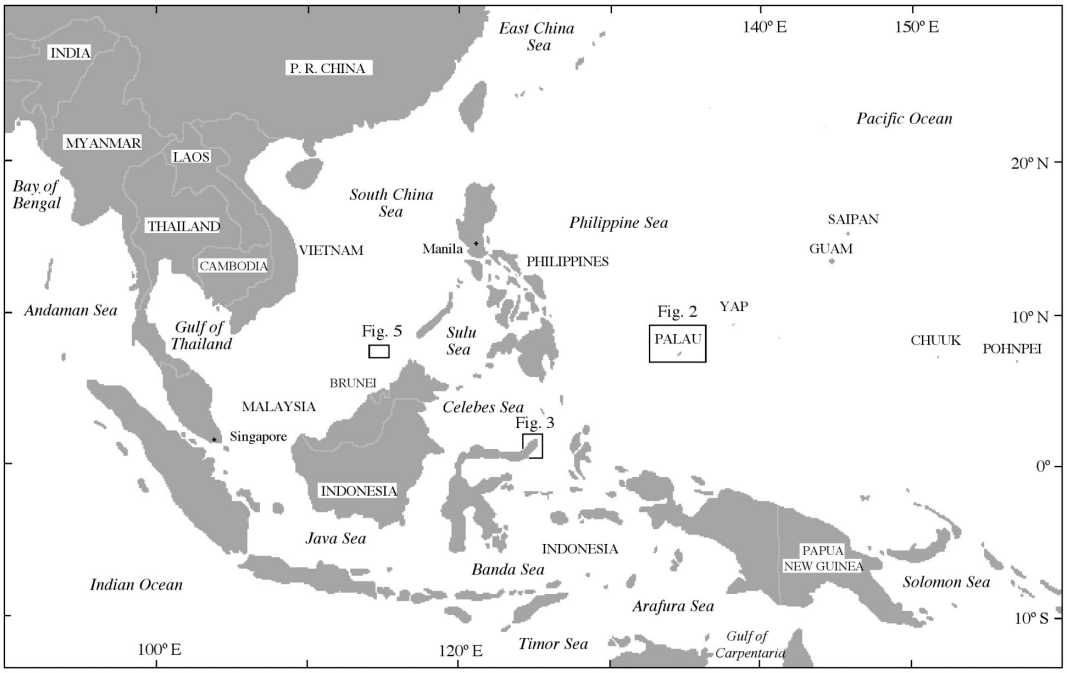

Figure 1. Map of Indopacific area.

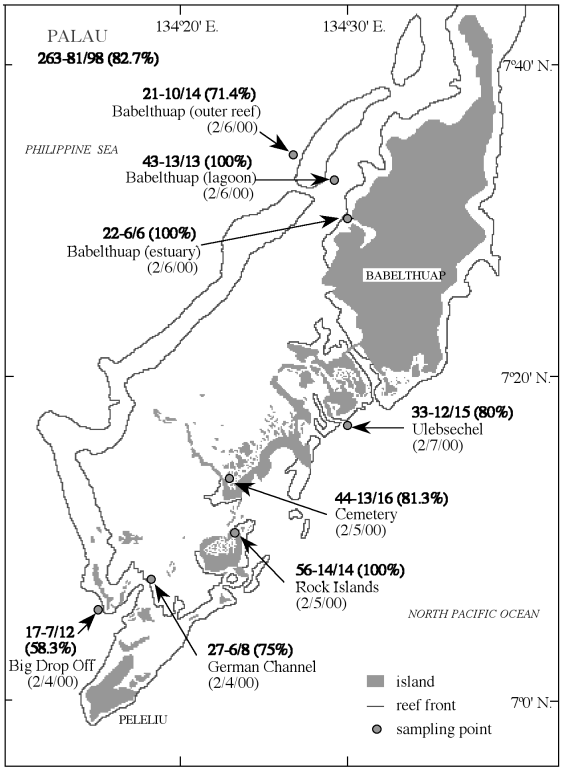

Figure 2. Sampling points and results of collection at Palau.
Total fungi-Sponges yielded fungi/Total sponges (Rate)

Figure 3. Map of North Sulawesi in Indonesia.

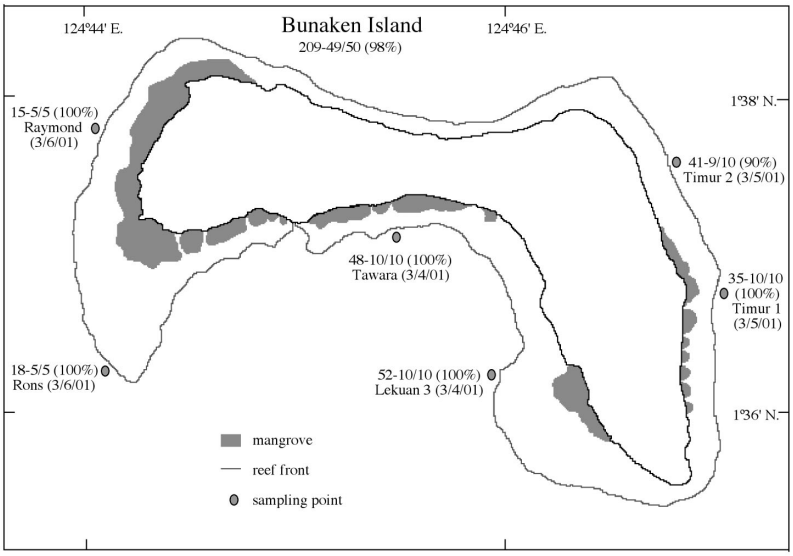

Figure 4. Sampling points and results of collection at Bunaken Island.
Total fungi-Sponges yielded fungi/Total sponges (Rate)

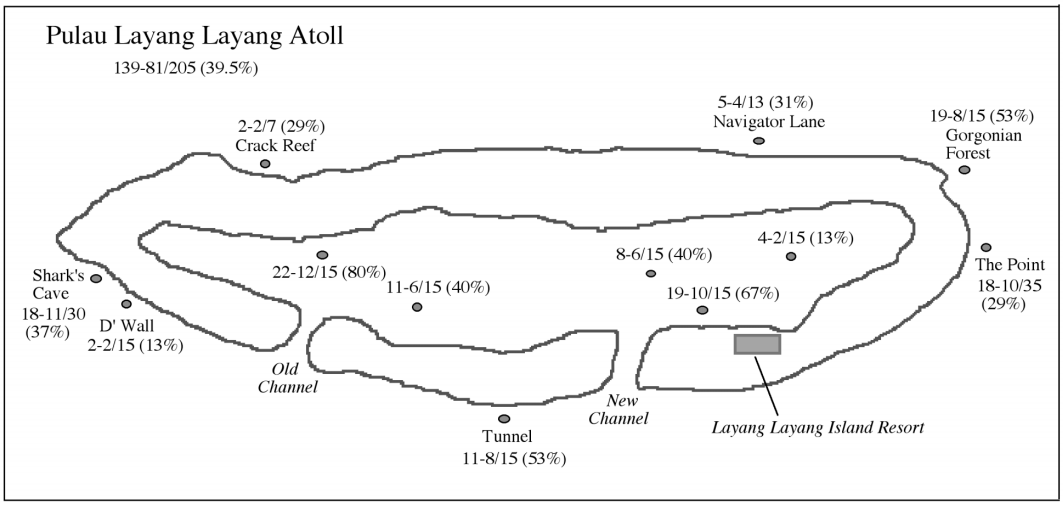

Figure 5. Sampling points and results of collection at Layang-Layang Island.
Total fungi-Sponges yielded fungi/Total sponges (Rate)

Marine Sponges

Diverse marine sponges were photographed and collected by scuba diving. A small portion of each sponge was sealed in a sterile plastic bag in the water and stored in a cooler box until the sponge was treated for isolating filamentous fungi.

The sum of 98 sponges were collected at 8 sampling points in Palau (Figure 2 and Table 1) and 50 sponges at 6 sampling points in Bunaken Is, (Figure 4 and Table 2). Different species of sponges were collected at a sampling point, and overlap of species was also avoided at resembled sampling points.

Table 1. Summary of results obtained from the Palau expedition

Date	Sampling Point	Number of Sponges				Number of Fungi Isolated		
		Col.*1	Liq.*2	Org.*3	Total*4	Liq.*5	Org.*6	Total
Feb. 4, 2000	German Channel	8	6 (75%)	3 (37.5%)	6 (75%)	20 [3.3]	7 [2.3]	27 [4.5]
	Big Drop Off	12	6 (50%)	5 (41.6%)	7 (58.3%)	9 [1.5]	8 [1.6]	17 [2.4]
Feb. 5, 2000	Rock Islands	14	11 (78.6%)	12 (85.7%)	14 (100%)	22 [2.0]	34 [2.8]	56 [4.0]
	Cemetery	16	10 (62.5%)	10 (62.5%)	13 (81.3%)	23 [2.3]	21 [2.1]	44 [3.4]
Feb. 6, 2000	Babelthuap estuary	6	5 (83.3%)	6 (100%)	6 (100%)	11 [2.2]	11 [1.8]	22 [3.7]
	Babelthuap outer reef	14	4 (28.6%)	7 (50%)	10 (71.4%)	10 [2.5]	11 [1.6]	21 [2.1]
	Babelthuap lagoon	13	10 (76.9%)	10 (76.9%)	13 (100%)	21 [2.1]	22 [2.2]	43 [3.3]
Feb. 7, 2000	Ulebsechel	15	8 (53.3%)	12 (80%)	12 (80%)	14 [1.8]	19 [1.6]	33 [2.8]
	Total	98	60 (61.2%)	65 (66.3%)	81 (82.7%)	130 [2.2]	133 [2.0]	263 [3.2]

*1 Sum of sponges collected.
*2 The number of sponges yielded at least one fungus from the liquid portion (the rate in parenthesis).
*3 The number of sponges yielded at least one fungus from the residual organism (the rate in parenthesis).
*4 Sum of sponges yielded at least one fungus (the rate in parenthesis).
*5 The number of fungi isolated from the liquid portion [the average in bracket].
*6 The number of fungi isolated from the residual organism [the average in bracket].

Table 2. Summary of results obtained from the North Sulawesi expedition

Date	Sampling Point	Numbers of Sponges				Numbers of Fungi Isolated		
		Col.*1	Liq.*2	Org.*3	Total*4	Liq.*5	Org.*6	Total
Mar. 4, 2001	Lekuan 3	10	10 (100%)	10 (100%)	10 (100%)	30 [3.0]	22 [2.2]	52 [5.2]
	Tawara	10	10 (100%)	9 (90%)	10 (100%)	26 [2.6]	22 [2.4]	48 [4.8]
Mar. 5, 2001	Timur 2	10	9 (90%)	8 (80%)	9 (90%)	21 [2.3]	20 [2.5]	41 [4.6]
	Timur 1	10	8 (80%)	10 (100%)	10 (100%)	15 [1.9]	20 [2.0]	35 [3.5]
Mar. 6, 2001	Raymond	5	5 (100%)	5 (100%)	5 (100%)	6 [1.2]	9 [1.8]	15 [3.0]
	Rons	5	5 (100%)	5 (100%)	5 (100%)	9 [1.8]	9 [1.8]	18 [3.6]
	Total	50	47 (94%)	47 (94%)	49 (98%)	107 [2.3]	102 [2.2]	209 [4.3]

*1 Sum of sponges collected.
*2 The number of sponges yielded at least one fungus from the liquid portion (the rate in parenthesis).
*3 The number of sponges yielded at least one fungus from the residual organism (the rate in parenthesis).
*4 Sum of sponges yielded at least one fungus (the rate in parenthesis).
*5 The number of fungi isolated from the liquid portion [the average in bracket].
*6 The number of fungi isolated from the residual organism [the average in bracket].

Filamentous Fungi Associated with Marine Sponges

Filamentous fungi were isolated from the liquid portion and remaining organism obtained by the homogenization of sponges. The shape and color of colonies, mycelia, and spores (if formed) were observed under a microscope, and different species were isolated and inoculated on slants separately. The results are listed in Tables 1 and 2. It is interesting that the most of fungi isolated from both liquid portion and organism of the same sponge were different species. Fungi obtained from organism may be associated strongly with the sponge.

The average of about two fungi was isolated respectively from the liquid portion and organism at both sites, although the numbers were varied among sampling points (Tables 1 and 2). There were variations in the rate of sponges yielded at least one fungus collected in Palau (Table 1). The sponges collected at Big Drop Off in Palau showed the minimum rate (58.3%). The sponges collected from the outside of the reef (Big Drop Off and Babelthaup) yielded lower rates (Table 1 and Figure 2). The average of 3.2 fungi was isolated at Palau, while the sponges at Bunaken Is. gave 4.3 fungi. Only one sponge did not yield a fungus among 50 sponges at Bunaken Is. (Table 2), and high rates (90-100%) were obtained at all sampling points (Table 2 and Figure 4). The sponges in the coral reef of Bunaken Is. and probably in North Sulawesi are little better providers of filamentous fungi than those of Palau.

We have done the similar study in Malaysia, Pohnpei, and Okinawa (Aka, Ishigaki, and Iriomote islands). The rates obtained in this study were remarkably higher than those obtained at the above sites (unpublished data). For example, the results obtained from two expeditions held at Layang-Layang Island, a small atoll located at about 300 km off the cost of Sabah State of East Malaysia (Figure 1), was shown in Figure 5. The sponges in Layang-Layang Atoll gave low rates at both inside and outside of the reef. Although it is not clear why the sponges in Palau and Bunaken Is. hold more filamentous fungi, it is certain that Palau and North Sulawesi are good study sites on these marine organisms.

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パラオとインドネシアブナケン島のサンゴ礁における海綿と共存する海洋糸状菌の分布

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海洋糸状菌は生物活性物質探索資源として重要であることが、最近の研究で明らかになってきている。我々は海洋糸状菌由来の生物活性物質探索の一環として、練習船神鷹丸の長期航海の際にパラオ共和国とインドネシア北スラウェシ地方のブナケン島で海綿と共存する糸状菌の分離を行った。パラオとブナケン島ではそれぞれ82.7%と98%の海綿から糸状菌が得られた。この率は他の熱帯地域の値よりも明らかに高かった。ブナケン島での採集地点6カ所における糸状菌の分布はほぼ同じ程度に高い値（90-100%）であったが、パラオの8カ所の分布には多少のばらつきがみられた。

キーワード: 海洋糸状菌, 海綿, 分布, サンゴ礁, パラオ, ブナケン島, 北スラウェシ