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Biodiversity of Fungi in Seawater and Sediment from Mangrove Forest at Andaman Coastal Research Station for Development, Ranong Province

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Abstract

Fungal diversity in the seawater and sediment in the mangrove forest at Andaman Coastal Research Station for Development, Ranong Province was studied. Seawater and sediment were sampled in 5 sites during the rainy and dry season. The fungi were cultivated in Potato Dextrose Agar (PDA) supplemented with Streptomycin and Chloramphenical and incubated at room temperature for 7 days. Eighty nine and 99 fungal isolates were found in the sediment and seawater samples, respectively. Fungal identification was done using their molecular study. Forty-five fungal species could be identified using molecular study and morphological features including : *Acremonium furcatum, A. nepalense, Aspergillus aculeatus, A. flavus, A. fumigatus, A. niger, A. nomius, A. nutans, A. oryzae, A. tubingensis, Aspergillus sp., Ceratocystis paradoxa, Cladosporium cladosporioides, C. oxysporum, Colletotrichum gloeosporioides, Diaporthe helianthi, Didymellaceae sp., Dothideomycetes sp., Eupenicillium shearii, Fusarium equiseti, F. moniliformis, F. nelsonii, F. oxysporum, F. proliferatum, F. solani, F. subglutinans, Fusarium sp., Ganoderma cupreum, Gliocephalotrichum simplex, Gongronella butleri, Hypocrea jecorina, Lasiodiplodia theobromae, Neosartorya fischeri, Penicillium citrinum, P. funiculosum, P. griseofulvum, P. janthinellum, P. lilacinum, P. oxalicum, P. rademirici, P. simplicissimum, P. stecki, P. vasconiae, Penicillium sp., Pestalotiopsis mangiferae, Pleurotus pulmonarius, Rigidoporus vinctus, Sordaria sp., Talaromyces assiutensis, Trichaptum laricinum, Trichoderma asperellum, Trichoderma sp.* and *Xylaria apoda.* The common fungal species found in this study were *Penicillium, Aspergillus* and *Fusarium.*

Keywords : *fungi, mangrove forest, seawater, sediment*

1. Introduction

Mangrove forests are open ecosystems that straddle the land and the sea, from freshwater to seawater areas. The brackish water plant communities cover the world in a total area of approximately 18.1 million hectares (1). They distribute along 3 major low-lying tropical and subtropical coastlines of the world: 1) Tropical Asia, Australia and Oceania, with 46.5% of the world's mangroves; 2) Tropical America, with 34.9%; 3) Tropical Africa, with 18.7% (2). The majority of global mangrove forests are found mainly in the tropical Indo-Pacific region. At present, Thailand mangrove forests cover a total area of, about 0.17 million hectares (1). Most of the remaining mangrove forests in Thailand can be found on the Andaman coastline. The Andaman coastline runs from Ranong Province to Satun Province, with mangrove forests found in 85% of the total area (3). Mangroves have been found to be in decline over 50% of the overall areas of the country since 1960 (1).

Mangroves are a very important ecosystem with high productivity and valuable coastal resources. They play a very important role for human life as a source of fuel, food, ecologically protected areas along the coastlines and the economy of the country. They have long been recognized as a very important part in supporting marine life such as shrimps, crabs, shellfish, fish and other benthic fauna in the estuarine and coastal waters. Mangrove forests also protect soil erosion and windbreak along the coastline caused by severe storms and strong currents.

The fungi kingdom is estimated to contain about 1.5 million species (4). Fungi are important actors in the ecological processes occurring in mangroves and are thought to play a role in organic matter decomposition pathways. Fungi are a very important part of providing nutrients in the mangrove system by decomposing leaves. Fungi are ubiquitous and grow in a wide range of habitats such as soil, water and in association with other organisms (4).

Leightley and Eaton(5) determined the ability to degrade wood cell wall components in several marine fungi belonging to the genera *Cirrenalia, Culcitalna, Holosphaeria, Humicola, Nia* and *Zalerion*. Pisano et al., 1964 (6) reported that 13 marine fungi could produce gelatinase activity, with Halosphaeria mediosetigera producing the highest activity. Peters et al.(7) reported the compounds commonly found in higher marine fungi including alanine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, tryptophan, threonine, tyrosine and valine. The conclusion is that higher marine fungi could be an important source of amino acids. Triglyceride fatty acids, oleic, palmitic and linoleic acids were isolated from *Corollospora maritime* and *Zalerion maritimum(8)*. Choline sulfate (ester) was found to be the principal amine produced by 10 marine Ascomycetes and Deuteromycetes(9).

In this study, the diversity of fungi in the mangrove forest was examined. The fungi in the sediment and seawater of the Andaman Coastal Research Station for Development, Ranong Province, Thailand were isolated and identified using their morphological features and molecular method.

2. Materials and Methods

2.1 Study site

The study area was located in a mangrove forest of the Andaman Coastal Research Station for Development (under Kasetsart University) Laemson National Park, Ranong Province. Five plots were selected as illustrated in Figure 1. (plot A: N 09° 22' 14.9", E 098° 24' 38.2"; plot B: N 09° 21' 48.8", E 098° 24' 19.6"; plot C N 09° 21' 53.6", E 098° 24' 26.7"; plot D: N 09° 22' 50.1", E 098° 24' 08.1"; plot E: N 09° 22' 16.2", E 098° 23' 59.9") The salinity of the seawater from all sampling sites varied from about 0-26 psu. in the rainy season and 8-32 psu. in the dry season. The soil texture was mostly sandy and the vegetation was mangrove plantation.

Figure 1. Five sampling plots in mangrove forest at the Andaman Coastal Research Station for Development, Laemson National Park, Ranong Province.

2.2 Samples collection

Seawater and sediment were randomly sampled in 5 plots during the rainy season (May and August, 2012) and dry season (October, 2012 and January, 2013). The sediment samples were collected at a 5 cm. depth from the surface and kept in plastic bags. Seawater samples were collected at 2 different levels, lower level (about 1 meter from the water surface) and upper level (about 5 cm. from the water surface), and kept in 600 ml. plastic bottles. All samples were incubated in cold temperature (10-20ºC) and examined at a laboratory in Bangkok.

2.3 Fungal isolation

Five grams of each sediment sample were diluted in 45 ml. of sterile distilled water (1:10 w/v). One ml. of this suspension was then added to 9 ml. of sterile distilled water (1:100 v/v) and 1 ml. of this final volume being spread on a potato dextrose agar (PDA) supplemented with antibiotics (0.2 g/l of chloramphenicol and streptomycin) to prevent the growth of

bacteria. Each sample was done in triplicate. Each seawater sample was filtrated through 0.45 µm. filter paper with the filter paper plated on the PDA plate supplemented with antibiotics (0.2 g/l of chloramphenicol and streptomycin). The filter paper was rinsed using 1 ml of sterile distilled water. The petri dishes were incubated at room temperature for 3-7 days. After 7 days, the fungal colonies grown on PDA were counted and recorded. The fungal colonies were transferred to new PDA plates to get pure cultures. The isolated strains were investigated for their morphology and confirmed by molecular techniques.

2.4 Identification of the isolated fungi

The fungal isolates were cultured on Potato Dextrose Agar (PDA) to study their morphological characteristics and molecular methods. The morphological characteristics of the isolated strains which similar to *Aspergillus* and *Penicillium* were studied by cultivation on 3 selective media (Czapeck agar, Malt extract agar and Yeast extract sucrose agar) and their morphological characteristics were illustrated on each medium (colors, appearances and colony diameter)(1). Ribosomal DNA from the fungal isolates were extracted from 10 mg of fresh mycelium weight using the CTAB (N-cetyl-N,N,N- trimethyl –ammonium bromide) method and amplified the ITS gene by PCR using the primers ITS1 and ITS4 (10). The Internal Transcribed Spacer (ITS) region was chosen because it shows high variation between the most species of fungi in both length and sequence. It is commonly used in molecular studies of fungi. The 50 µl of PCR mix used for each sample contained fungal DNA template 50-100 ng/ ml, 10X ExTaq buffer 5 µl, dNTP 5 µl, ExTaq 0.5 µl, ITS1 (10 pmol) 1 µl, ITS4 (10 pmol) 1 µl and sterile de-ionized water. The PCR cycle began with an initial denaturation at 95ºC for 1 min, followed by 30 cycles of denaturation at 95ºC for 2 min, annealing at 50ºC for 1 min, extension at 72ºC for 1 min, and final extension at 72ºC for 7 min. The PCR products were run on 1% agarose gel to separate the DNA bands, with individual bands cut out from the gel and purified using gel extraction kit (fermentas, Canada) following the manufacturer's instructions. The purified PCR products were sequenced using ITS1 and ITS4 primers. The PCR products were sequenced by Macrogen Inc. in Korea with the same primer as in the PCR amplification. The sequence results were checked manually and made an alignment using ClustalW. A blast search was done (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to check sequence similarity with fungal sequences in the database (data not shown).

3. Results and Discussion

Salinity is a major factor affecting the diversity of marine fungi. Physiological studies of marine fungi concentrate on their salinity requirements in the belief that there is a requirement for sodium chloride at concentrations found in seawater. Salinity in each season is different. In the rainy season (1st and 2nd sampling), salinity is less than in the dry season (3rd and 4th sampling). Plot A has the lowest salinity because of its proximity near fresh water. Plot D has the highest salinity because of its proximity near the sea. The salinity of the water at the upper level and lower level are not different (0-32 psu.). The salinity of the soil in each season and each plot are not different (0-0.5psu.).

Acidity-alkalinity (pH) of the seawater and sediment of the plot in each sampling time was about 7.0-9.0 and 3.0-9.0, respectively. Marine fungi grow and sporulate at around pH 7.5 depending on the habitat. They are able to tolerate the concentration of ions present in seawater and prefer the alkaline pH. Acidity–alkalinity affects production of some substances.

The temperature is an important factor in the geographical distribution of marine fungi. In the dry season, humidity is low and the fungi can survive in a dormant state such as spores, sclerotium and chlamydospores. In the dry season, the water temperature at both the upper and lower level is highest. In the rainy season, the water temperatures are the lowest (25 ºC).The maximum temperature was found in plot D (34 ºC) and mangrove trees were rare because of this site's proximity near the sea. The temperatures of the soil and water were similar (25-34 ºC).

Fungal diversity is greatly affected by the nature of the substratum. The nature of the substratum can have a major effect on the fungi colonizing it, even from one timber species to the next. Competition between fungi can markedly affect fungal diversity, and species composition.

A total of 188 fungal isolates were obtained from both sediment and seawater samples. From these 188 fungal isolates, 89 fungal isolates (44%) were isolated from sediment samples. and 99 fungal isolates (56%) were isolated from seawater samples. In the case of seawater samples, 51 and 48 fungal isolates were obtained from the upper level and lower level samples, respectively, with 188 fungal isolates able to be identified into 45 species. Most of the identified species were isolated from the seawater samples.

Table 1. Type of the fungi isolated from sediment and seawater in each sampling time

Name	Sediment				Seawater			
	Sampling time				Sampling time			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Ascomycetes								
Acremonium nepalense					+			
Acremonium sp.					+			
Aspergillus aculeatus	+							+
Aspergillus flavus								+
Aspergillus fumigatus			+					
Aspergillus niger					+			

Name	Sediment				Seawater			
	Sampling time				Sampling time			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Aspergillus nomius		+						
Aspergillus nutans							+	
Aspergillus oryzae								+
Aspergillus sp.		+	+	+				
Aspergillus tubingensis		+			+			
Blastobotrys malaysiensis			+					
Ceratocystis paradoxa					+	+		
Cladosporium cladosporioides								+
Cladosporium oxysporum							+	
Colletotrichum gloeosporioides								+
Fusarium proliferatum						+		
Fusarium solani		+	+		+	+		
Fusarium sp.					+			
Fusarium subglutinans					+			
Hypocrea jecorina								+
Neosartorya fischeri							+	
Penicillium citrinum	+	+		+				+
Penicillium funiculosum						+		
Penicillium griseofulvum								+
Penicillium janthinellum		+			+			
Penicillium oxalicum								+
Penicillium purpurogenum		+						
Penicillium sp.	+	+	+	+	+			

Name	Sediment				Seawater			
	Sampling time				Sampling time			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Pestalotiopsis adusta		+						
Pestalotiopsis mangiferae					+			
Phoma pereupyrena	+							
Scedosporium apiospermum	+	+						
Scopulariopsis sp.					+			
Talaromyces assiutensis					+			
Trichoderma asperellum	+						+	
Trichoderma harzianum	+	+	+	+				
Trichoderma koningiopsis	+							
Trichoderma sp.	+	+	+	+				
Trichoderma virens		+						
Xylaria apoda					+			
Basidiomycetes								
Ganoderma cupreum							+	
Pleurotus pulmonarius					+			
Zygomycetes								
Gongronella butleri	+						+	
Rhizomucor variabilis		+						

The results show that the fungal biodiversity in the rainy season is more varied and diverse than in the dry season. Ten fungal species were found in both seasons, including: *Aspergillus aculeatus, Aspergillu sp., Fusarium solani, Penicillium citrinum, Penicillium janthinellum, Penicillium sp. Trichoderma asperellum, Trichoderma harzianum, Trichoderma sp.* and *Gongronella butleri.* Nineteen fungal species found only in the rainy season, are *Acremonium nepalense, Acremonium sp., Aspergillus nomius, Aspergillus tubingensis, Aspergillus niger, Ceratocystis*

paradoxa, Fusarium proliferatum, Penicillium funiculosum, Penicillium janthinellum, Penicillium purpurogenum, Pestalotiopsis adusta, Pestalotiopsis mangiferae, Phoma pereupyrena, Scedosporium apiospermum, Scopulariopsis sp., Talaromyces assiutensis, Trichoderma virens, Pleurotus pulmonarius and *Rhizomucor variabilis*. The 13 fungal species found only in the dry season included: *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nutans, Aspergillus oryzae, Blastobotrys malaysiensis, Cladosporium cladosporioides, Cladosporium oxysporum, Colletotrichum gloeosporioides, Hypocrea jecorina, Neosartorya fischeri, Penicillium griseofulvum, Penicillium oxalicum* and *Ganoderma cupreum*. The fungi obtained from seawater samples are more diverse than sediment samples shown by the number of fungal species isolated from seawater and sediment samples at 32 and 21 species, respectively. Of the fungi isolated from the five plots, plot A showed the highest fungal diversity, while plots D and E showed the lowest fungal diversity. Temperature plays a major role in the geographical distribution of marine fungi with species that are typically tropical. Salinity is also important in affecting species composition. Many fungi occur primarily in fully saline waters, while terrestrial and freshwater species may be able to grow at lower salinities. In mangroves, many fungal species can tolerate great variation in the salinity of the water. Biodiversity of the fungi found in this study revealed that the rainy season had more diversity than in the dry season. Rainfall also represents an important environmental variable in addition to salinity.

4. Conclusion

In the study of 45 fungal species were identified decomposing in the sediment and seawater of the Andaman Coastal Research Station for Development, Ranong Province. The dominant fungi were *Aspergillus, Fusarium, Trichoderma, Acremonium, Scopulariopsis, Colletotrichum, Rhizopus* and *Penicillium*. It is generally accepted that many species of these genera can survive in dry environments. The fungi obtained from the seawater and sediment samples included 32 species and 21 species, respectively. Most of the fungal species identified in this study were isolated from the seawater.

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