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Abundance and Diversity of Mould Inhabiting Muara Layang Estuary Sediment, Bangka Belitung Islands

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ABSTRACT

Estuary basin is a fertile area which has important role for sustaining many organisms from estuary and sea. Mould and other saprobic microorganisms have important role to decomposing organic material in estuary water. A study on diversity and abundance of mould inhabiting Muara Layang estuary sediment, Bangka Belitung Islands has not been conducted before. The objective of this study is to investigate the abundance and diversity of mould inhabiting Muara Layang estuary sediment, Bangka Belitung Islands. The mould isolation was based on sample dilution method with Rose Bengal Chloramphenicol Agar mould isolation media. The abundance of mould was counted by measuring the average Colony Forming Unit (CFU)/ml of all mould colonies which grown on isolation media by Total Plate Count (TPC) method. The diversity of isolated mould was identified based on phenotypic characters by observing both of macroscopic and microscopic mould morphology. The result showed that the growth average of mould colonies is about (5-27.5) $x10^2$ CFU/mL. The result of mould identification showed that eight mould genera, *Aspergillus* (6 species), *Chaetomium*, *Eupenicillium* (3 species), *Gliocladium*, *Paecilomyces*, *Penicillium* (3 species), *Scopulariopsis*, *Trichoderma* (3 species), one group identified in class level (Coelomycetes), and three groups of unidentified sterile mould isolates were isolated.

Key words: estuary, mould, sediment, abundance, diversity.

INTRODUCTION

Estuary zone is an area with high nutrition and has an important role in transporting the nutrient into the sea (Mahasneh, 2001). Due to its fertility, estuary is used by fish and other aquatic biota as feeding, spawning and nursery ground. Microorganisms, such as bacteria and mould, play part in nutrient degradation and regeneration in estuarine. Degradation of leaves litter by microorganisms produce detritus which will be utilized through food chain by fish, shrimp, mollusk, benthic animal, and other aquatic biota (Chandramohan, 1984; Rao and Nair, 1986). Moulds have hypha with filament structures that can penetrate to the substrates. Their enzymatic ability is higher comparing to bacteria, especially in decomposing organic compounds like lignin and cellulose (Cromack and Caldwell, 1992; Mille-Lindblom, 2005). Moreover, moulds also produce extra cellular enzyme which can provide nitrogen source, vitamin and amino acid (Berg and Matzner, 1997).

Varieties of moulds have been isolated from estuarine environment. *Alternaria maritima*, *Aspergillus flavus*, *Aspergillus niger*, *Aureobasidium pullulans*, *Cladosporium* sp., *Humicola* sp., *Mucor* sp., *Penicillium* sp., and *Rhizopus* sp. have been isolated from Korangi and Clifton bay, Pakistan (Mehdi and Saifullah, 1992). *Acremonium alabamense, Aspergillus terreus, Penicillium crustosum, Penicillium purpurogenum, Phialophora fastigiata, Talaromyces flavus,* and *Trichoderma harzianum* have been isolated from mangrove mud in Okinawa island (Ito and Nakagiri, 1997). Endophytic microfungi from Coelomycetes, Hyphomycetes, and Xylariales taxa are also isolated from vegetation in estuarine and mangrove area (Okane et al., 2001a, b). Nives-Rivera (2005) found number of moulds which are classified most in Ascomycetes dan Deuteromycetes taxa from estuarine in Puerto Rico.

Muara Layang estuary in Bangka Belitung islands is an area which has low impact from tin mining, so it is planned to be the fishery conservation area. Research in physicochemical and biological have been conducted but the roles of microbes especially moulds in this area have not been conducted. Therefore, the aim of this research is to investigate the abundance and diversity of moulds inhabiting

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Muara Layang estuary sediment in Bangka Belitung islands which is related to the physical and chemical factors in water of Muara Layang estuary.

MATERIALS AND METHODS

Sampling location

Sample of sediment was taken from Muara Layang estuary in Bangka Belitung islands in May 2007 (Table 1). Ekman dredge was used for taking sediment sample in each of sampling stations. Sediment was put in polybag or sterile bottle and either plated immediately or stored in a 4°C refrigerator. Measurement of water quality such as pH, salinity, temperature, turbidity, and dissolved oxygen were done during the sampling by using Water Quality Checker DKK TOA (Table 2).

Table 1. Description of sampling sites in Muara Layang Estuary, Bangka Belitung Islands

Station	Sub area	Coordinate	Sediment types
I	Jelutung river estuary	S: 1°46'8.4" E:105°47'31.2"	Soft texture
II	Kelam river estuary	S: 1°47'15.72" E:105°48'42.12"	Soft texture
III	Lumut river estuary	S: 1°47'39.12" E:105°49'10.56"	Sandy texture
IV	Maras river estuary	S: 1°48'25.92" E:105°48'44.64"	Coarse and sandy texture
V	Manjang river estuary	S: 1°46'19.8" E:105°49'57"	Coarse and sandy texture
VI	Melandur river estuary	S: 1°46'53.16" E:105°52'11.64"	Soft texture

Table 2. Data of physical and chemical quality in water of Muara Layang Estuary, Bangka Belitung Islands in May, 24, 2007

Station	pH	Turbidity NTU	Dissolved Oxygen (mg/L)	Temperature (°C)	Salinity (‰)
I	7.08	26.0	5.84	29.9	20.0
II	7.21	28.3	5.52	29.8	14.1
III	6.95	15.3	5.29	29.6	12.2
IV	6.99	16.2	5.76	30.3	12.2
V	6.85	26.6	5.27	29.4	7.9
VI	6.57	117.3	5.78	29.5	2.5

Isolation and enumeration of moulds

Isolation was conducted in the laboratory with dilution method and the procedure will be done as followed (Ando et al., 2003; Ilyas et al., 2006): 10 g of sediment sample was weighed and put in test tube with 90 mL sterile aquadest (10^{-1} dilution) then homogenized (preparation I). One mL of preparation I was inoculated into 9 mL sterile aquadest (10^{-2} dilution) and homogenized (preparation II). The same procedure was used to make 10^{-3} dilution (preparation III). 0.2 mL preparation I, II, and III was poured and spread on the Rose Bengal Chloramphenicol Agar. Each was plated in triplicate. Culture was incubated for 3-7 days in room temperature. Mould colony was counted in colony forming unit (CFU)/mL and chosen for isolation then transferred to Potato Dextrose Agar (PDA).

Purification of mould colonies

Mould cultures that grow during the isolation were purified with colony propagation by cutting and transferring aseptically part of mould mycelium to fresh culture media (Alexopoulos et al., 1996). The Isolates that grow on Rose Bengal was chosen and transferred to PDA chloramphenicol in a 6 cm diameter petri dish. Colonies was incubated for 3-7 days in 27ºC. The isolates were identified microscopically and macroscopically. The purified colonies were chosen and reculture in slant tube (PDA). The process was done in duplicate.

Mould identification

The isolated and purified moulds were identified base on phenotypic characters using guide books from Barnett (1955), Ellis (1971), Domsch et al. (1980), Sutton (1980), Webster (1980), Samson et al. (1995), Barnett and Hunter (1998), and Gandjar et al. (1999). Characteristic and morphology were identified both macroscopically and microscopically. The macroscopic characters consist of color and colony's surface (granular, powder, elevate, smooth), texture, zonation, growing zone, radial and concentric line, reverse color, and exudates drop. Microscopic observation includes existence of hypha, hypha pigmentation, existence of clamp connection on hypha, form and ornamentation of spore and sporangiophore.

RESULT AND DISCUSSION

The result demonstrated that the average of mould colonies from sediment sample of Layang estuary was (5-27.5) $x10^2$ CFU/mL. The sequence of mould colonies number from the lowest to the highest was stated as follows. The lowest average of mould colonies was 5.0 $x10^2$ CFU/mL obtained from station V in sub estuary of Manjang river. It was followed by station III in sub estuary of Lumut river which was 6.5 $x10^2$ CFU/mL. Station IV was in the third position in terms of the average number which has 7.0 $x10^2$ CFU/mL, followed by station I and II in sub area of Jelutung and Kelam river which has 18.5 $x10^2$ CFU/mL. The highest average of mould colonies (27.5 $x10^2$ CFU/mL) was in station VI in sub area of Melandur river. Data of the abundance of mould colonies in each of the sampling site can be seen in Table 3.

Table 3. The average of mould colonies from sediment of Muara Layang Estuary, Bangka Belitung Islands, May 24, 2007

Station	Average of mould colonies ($x10^2$ CFU/ mL)
I	18.5
II	18.5
III	6.5
IV	7.0
V	5.0
VI	27.5

In general, the average of mould colonies in 6 sampling sites were low because they all have average CFU ≤ 10^4 per gram or milliliter sample. Mould abundance was affected by environmental factors such as humidity, temperature, pH, nutrient and other chemical compound (Alexopoulos et al., 1996; Gandjar et al., 2006). One of the limiting factors for mould growth in 6 sampling sites was pH. Measurement of pH from 6 sampling sites showed that the pH tended to be neutral or base with its range about 6.57-7.21 (Table 2). Commonly, mould reaches its optimum growth in acid environment (Gandjar et al., 2006). Large numbers of the mould colonies have optimum growth in pH range between 5 and 6. Only a few can grow in a wide range of pH, for instances *Aspergillus niger* grows in pH between 2-8, *Penicillium italicum* grows in pH 1.9-9.3, and *Fusarium oxysporum* grows in pH 1.8-11.1 (Barnett and Hunter, 1998).

Other factor that makes the abundance of mould colonies in 6 sampling sites was low in average CFU/mL was salinity. Osmotic pressure and (water activity/a_w) will be affected by the salinity. As the salinity increases, the osmotic pressure will increase and water activity/a_w will decrease. The low level of water activity has caused the mould difficult to take water from the environment for their metabolism activity. The result of salinity in 6 sampling sites showed that the salinity was between 2.5-20‰ (Table 3). According to Välikangas (1933), brackish water has salinity between 5-17‰ and sea water has 17‰ salinity. Generally, mould cannot be grown in high salinity. Only a few taxa can grow optimum in high salinity. Nives-Rivera (2005) found *Chaetomium* in hypersaline environment. Mould that can be grown in high salinity known as halophilic mould and can be isolated from mangrove, sea water, as well as food product such as salted fish (Gandjar et al., 2006). Even though halophilic mould that grows optimum in salinity between 5-20‰ is also found in brackish water, halophilic mould grows optimum in salinity between 25-40‰ (Rheinheimer, 1980).

Mould is heterotrophic microorganism and generally grows as saprophyte. Organic compounds are needed as a nutrient source for their growth. The accumulation of organic compound in sediment tends to be higher compared to water body; therefore it can generate mould growth in sediment. Sediment texture in 6 sampling sites was different. Based on the texture, station III, IV, and V have sand texture and station I, II, and VI have soft texture (Table 2). The average CFU showed that sediment texture also affected the number of mould colonies. The highest average of mould colonies was found in soft sediment texture. In general, sediment with soft texture contains higher organic matter than those that are in sandy texture. The presence of sufficient organic matter as a nutrition for mould growth will support the life sustainability of mould.

Phenotypic identification showed that there are 23 different taxa which different morphologically (Table 4). The result of mould identification showed that eight mould genera, *Aspergillus* (6 species), *Chaetomium*, *Eupenicillium* (3 species), *Gliocladium*, *Paecilomyces*, *Penicillium* (3 species), *Scopulariopsis*, *Trichoderma* (3 species), whereas one group is identified in class level (Coelomycetes). Three groups of unidentified sterile mould isolates were isolated. They were sterile and did not form sexual reproduction structure during the incubation. Most of isolated mould colonies from 6 sampling sites were cosmopolite and saprophytic microorganisms.

The most diverse of mould was from station I in sub area of Jelutung river. Station I had the highest variety in species, which was 12 different taxa. The isolates were *Aspergillus* spp.1, *Aspergillus* spp.3, *Aspergillus* spp.4, *Eupenicillium* sp.1, *Eupenicillium* sp.2, *Gliocladium*, *Penicillium* spp.2, *Penicillium* spp.3, *Trichoderma* spp.1, *Trichoderma* spp.2, and two groups of unidentified sterile mould.

Isolation and identification result showed that mould from genera *Aspergillus* and *Penicillium* found in most of the sampling sites. *Aspergillus* and *Penicillium* are saprophytic mould that grows in variety of habitats (Samson et al., 1995). In estuarine area, these moulds live in sandy sediment, leaf litter and decay trunk (Nives-Rivera, 2005). Anand dan Sridhar (2004) reported that *Penicillium* and *Aspergillus* are terrestrial moulds which are commonly found around estuary. Numbers of mould isolating from estuary sediment such as *Penicillium* and *Aspergillus* were originally terrestrial mould. Their spores washed and diluted into river and then adapted and lived in estuarine (Jones and Hyde, 1988).

Other saprophytic moulds which were found in sampling sites were mould from genera *Chaetomium*, *Gliocladium*, *Paecilomyces*, *Scopulariopsis*, and *Trichoderma*. *Chaetomium* was isolated from station II, *Gliocladium* was found in station I, V, dan VI, *Paecilomyces* was found in station III dan V, *Scopulariopsis* was found in station V, while *Trichoderma* was isolated from all of the sampling sites (Table 4). *Chaetomium*, *Gliocladium*, *Paecilomyces*, and *Scopulariopsis* can be found and isolated from estuarine environment (Nives-Rivera, 2005). While *Trichoderma harzianum* and *Trichoderma* sp. was isolated from rhizosphere and

Table 4. Mould generas and species that had been isolated from Muara Layang Estuary Sediment, Bangka Belitung Islands, May 24, 2007.

Generas	Species/explanation	Station					
		I	II	III	IV	V	VI
Aspergillus	*niger*	-	-	+	-	-	+
Aspergillus	spp.1, dark green colony	+	-	-	+	+	-
Aspergillus	spp.2, bluish green colony	-	+	+	-	+	-
Aspergillus	spp.3, brown colony	+	+	-	-	-	-
Aspergillus	spp.4, dark brown colony	+	-	-	+	-	+
Aspergillus	spp.5, brownish grey colony	-	+	-	-	-	-
Chaetomium	sp.	-	+	-	-	-	-
Eupenicillium	sp.1, brown colony	+	-	-	-	-	-
Eupenicillium	sp.2, greenish yellow colony	+	+	-	-	-	-
Eupenicillium	sp.3, orange colony	-	+	-	-	-	-
Gliocladium	sp.	+	-	-	-	+	+
Paecilomyces	sp.	-	-	+	-	+	-
Penicillium	spp.1, brown colony	-	-	-	+	-	-
Penicillium	spp.2, grey colony	+	-	+	-	-	-
Penicillium	spp.3, greenish blue colony	+	+	+	+	+	+
Scopulariopsis	sp.	-	-	-	-	+	-
Trichoderma	*harzianum*	-	-	-	-	+	+
Trichoderma	sp.1, blackish green colony	+	-	+	+	-	+
Trichoderma	sp.2, greenish yellow colony	+	+	-	+	+	+
Coelomycetes	sp.	-	-	+	+	-	-
Unidentified	Sterile, cottony mycelia in yellowish white	+	+	+	+	-	+
Unidentified	Sterile, cotton mycelia in dark grayish	-	-	+	-	+	-
Unidentified	Sterile, cottony mycellia in black	+	-	-	+	-	+

Note: (+) = found, (—) = not found. Morphology Identification was done in mould isolates that grow on PDA media, incubated in 27º C for 7-14 days.

mangrove mud (Ito and Nakagiri, 1997; Ito et al., 1999).

Coelomycetes was isolated from station III and IV. These moulds known have strong association with plants. Coelomycetes is endophytic mould which has parasitic and mutualistic association with plants which become its host. That kind of relationship enables them to transfer their genetic matter (Tanaka et al., 1999). Coelomycetes is typically found in leaf litter. However, most of Coelomycetes was not sporulated while culturing in the laboratory (Itazaki et al., 1992). Number of those moulds can only be identified based on morphology reproduction structure in their host. Large numbers of Coelomycetes produce both sexual (teleomorph phase) and asexual (anamorph phase) spore in their natural habitat or hosts. Nevertheless, these moulds are sterile and tend to fail sporulating in culture media (Paulus et al., 2003). Some of Coelomycetes, such as *Pestalotiopsis* and *Phoma*, was isolated from mud and rhizosphere of mangrove plants (Ito et al., 1999).

CONCLUSION

The average of mould colonies of Muara Layang estuary sediment was between (5-27.5)x10^2 CFU/mL. The highest average abundance was in station VI in sub area of Melandur river. The result of mould identification showed that eight mould genera, *Aspergillus* (6 species), *Chaetomium*, *Eupenicillium* (3 species), *Gliocladium*, *Paecilomyces*, *Penicillium* (3 species), *Scopulariopsis*, *Trichoderma* (3 species). In addition, one group was identified in class level (Coelomycetes), and three groups of unidentified sterile mould isolates were isolated. The highest diversity was found in station I in sub area of Jelutung river with 12 different species. Generally, result showed that the abundance of mould from 6 sampling sites was low and the diversity was dominated by cosmopolite and saprophytic moulds.

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