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DIVERSITY OF FUNGI IN THE SEDIMENTS OF TIGER SHRIMP (*PENAEUS MONODON* FABRICIUS) CULTURE PONDS, MALAYSIA

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ABST1 RACT

The diversity of fungi in tiger shrimp *P. monodon* culture pond sediments was investigated. The culture pond types sampled were aged (>8 years), moderately aged (3 years) and new (<1 year); the former was situated in the state of Perak and the latter two in Malacca, Malaysia. Three sampling periods were selected, namely during stock (DS), middle of culture (MC) and after harvest (AH). Fungal propagules were found in sediment samples for all 3 sampling periods. Distribution of fungi was highest during MC for the aged pond but for the moderately aged and new ponds, the highest was at AH. The overall fungal distributions were found to increase with the increased culture period probably due to organic load during culture activities. The genus *Aspergillus* was the dominant group followed by *Penicillium* in shrimp pond sediments.

Key words: Fungi; sediment; shrimp pond; Malaysia

INTRODUCT1 ION

Fungi are cosmopolitan in distribution, occurring even in marine environments where the salinity is high (Abdullah and Hishamuddin, 2001). The fungi were first recorded from marine habitats were described in the middle of the nineteenth century (Durieu and Montagne, 1846-50 *in* Kohlmeyer, 1969), but the reports were not published. Cribb and Cribb (1955) were the first to report on fungi from the surface of mangrove roots. Since then, information on marine fungi from tropical and subtropical regions started to increase. About 50,000 fungal species are known from terrestrial habitats (Ainsworth, 1968) but in contrast, less than 500 have been described from marine habitats (Kohlmeyer and Kohlmeyer, 1979).

Besides bacteria, fungi also act as decomposers of organic materials in the aquatic system, especially where C:N ratios are high (Barlocher and Kendrick 1974; Suberkropp and Klug, 1976). Moriarty (1997) stated that microorganisms, most of which were bacteria and fungi, utilized the organic material as nutrients to construct microbial assemblages. Studies by Vala *et al*. (2000) stated that marine fungi, along with other microorganisms played an important role in the coastal productivity processes, particularly in the solubilisation of iron and making them available to phytoplankton. Some species of fungi like *Saprolegnia* sp., *Aphanomyces piscicida*, *Ichthyophonus hoferi* and *Fusarium solani* can be pathogenic, causing numerous infections to culture organisms (SEAFDEC, 1988; Hatai, 1998). Certain fungi together with bacteria are known to provoke white spots on the shrimp cuticle (Clifford and Cook, 2002).

In an aquaculture system, both biotic and abiotic factors play 1 major roles. The biotic component consists of organisms such as phytoplankton, zooplankton, bacteria and fungi, while the abiotic part encompasses pH, dissolved oxygen, mineral content, temperature, pressure and other related matters (Chanratchakool *et al*., 1995). Bacteria and fungi make up the most c ommon biodegrader populations in any aquatic ecosystem; they provide a natural way of eliminating pollution, create a beneficial microbial environment and at the same time inhibit the growth of harmful microbes (Keawchum, 1993). The role of fungi as biodegrading agents in the terrestrial ecosystem is well known, but its diversity and ecology in the sediments of brackish water culture ponds have never yet been examined, especially in Malaysia. Therefore, this study presents a preliminary survey of the distribution and diversity of fungi in pond sediments as well as examines the relationship of their distribution with culture duration in commercial aquaculture ponds. It is hoped that this first step towards documentation of fungi in shrimp pond sediments would contribute positively towards improved culture management in shrimp *P. monodon* ponds in the future.

MATERIALS AND METHODS

Pond location and sediment sampling

The study was conducted at Progressive Impact Aquaculture farm in Perak (5º 452 N and 101º 372E) and Lembaga Pertubuhan Peladang shrimp farm (2º 08´ 50º N latitude and 102º 24´ 00º E longitude) in Malacca,

Malaysia. The culture ponds were categorized into 3 types namely aged (>8 years; at Perak), moderately aged (3 years; at Malacca) and new (<1 year; at Malacca). Three periods of sediment sampling 1 were done i.e. during shrimp stocking (DS), middle of culture (MC) and after harvesting (AH) of the culture ponds. Sediment samples were collected from 4 point of shrimp pond using an Ekman grab sampler covering an area 225 cm2. Samples were collected in a sterilised bag and brought back to laboratory within 2 to 4 hrs after collection. In the laboratory, about 10 g of samples were transferred into sterilised glass vials. The vials were capped, labelled and kept at 10ºC-15ºC in a refrigerator before analysis.

Preparation of agar media

Rose bengal agar (RBA) was used for the direct isolation of fungal colonies from sediment samples, where each emerging colony was quantified as one colony forming unit (cfu). Seawater (salinity 21-22‰) was incorporated into the potato dextrose agar (PDA), which was used for the subculture and maintenance of pure fungal colonies as described by Manoch (1998).

Identification of fungi

Temporary slides were made by putting mycelial fragments from PDA culture onto clear glass slides and covered with cover slips. The slides were then examined under high power microscope (Zeiss, MC 100) for morphological characteristics. Identification of fungal genera or species was made based on these characteristics using taxonomic keys in standard reference manuals.

RESULTS AND DISCUSSI1 **ON**

This study found that the fungal propagules were present in sediment samples at all three sampling periods of DS, MC and AH (Table 1). The colony culture and photograph of microscopic characteristics of each species is presented in Fig. **1**. Based on colony forming units (cfu/g) sediment, fungal population were highest during middle of culture (MC) period (22 x 10^2 cfu/g) in the aged pond at Perak. In contrast, the highest counts were found at after harvest (AH) in both moderately aged (19 x 10^2cfu/g) and new culture pond (13 x 10^2cfu/g) at Malacca. In terms of fungal distribution, very low cfu was recorded during the earlier phase (DS) of culture in the ponds studied. A total of 10 species of fungi belonging to 4 genera were obtained from the all three sampling sites. The genus *Aspergillus* was the most dominant fungi in all culture ponds followed by *Penicillium*. One *Curvularia* sp. was found in the aged culture pond at Perak and one *Trichoderma* sp. in the moderately aged ponds at Malacca.

Except for the aged culture pond in Perak, the diversity of fungi was higher at the end of culture period in other ponds. The proliferation of fungi may be deduced to be in response to a various cultural activities such as presence of shrimp faeces, uneaten feeds, and shrimp metabolites during the progress of culture. This organically rich sediment provides a constant source of carbon and nitrogen, which are the major nutrients for fungal growth (Chanratchakool *et al*., 1995). However, some fungi are able to survive in low pH and poor nutrient conditions (Dix and Webster, 1995). The least variable of fungal species with low fungal counts were found from the new culture ponds at Malacca (Table 1). This was probably because the sediments had low organic content since they have been newly constructed for shrimp culture after clearing the mangrove forests. Exposure to sun after constructed especially in new pond may also kill fungi, which could not withstand under high temperatures (Chanratchakool *et al*., 1995).

The present study showed that the fungi found in shrimp pond sediments are similar to those found in soils on land. Based on the screening of fungi, the present study showed that there is fungal diversity in the shrimp pond sediments. This may be expected because fungi do not need sunlight and all accumulated organic substances in pond sediments offer sources of carbon and nitrogen for food. Therefore, they can proliferate. Their presence can also be manipulated by man towards their advantage. Shrimp pond water tended to be cloudy toward the culture because of the excess food and nitrogenous by products, which may cause low water quality. In this aspect, the selected and tested non-pathogenic fungi probably may introduce to utilize the high organic matter in the pond, which may improve water quality. There is no previous report on the composition and abundance of fungal flora in shrimp pond sediments. The study found that the fungal propagules could be found in various degrees in shrimp pond sediments. The fungal counts could be increased enormously due to culture activities that provided higher levels of nitrogenous and carboniferous materials.

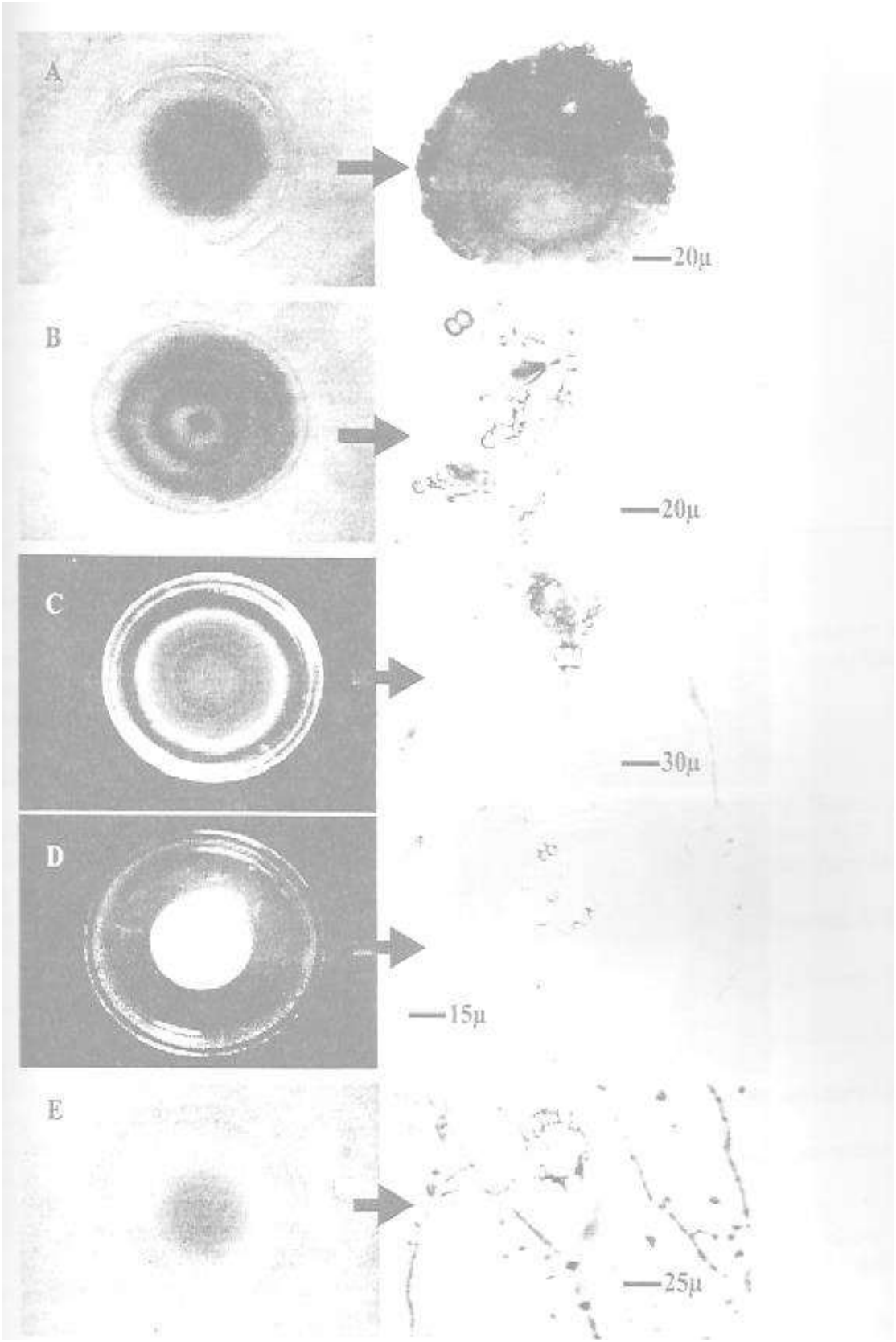

Fig.1. Mature surface and typical conidial head of isolated fungi from shrimp pond sediments [A] *Aspergillus niger* [B] *Trichoderma* sp. [C] *Penicollium decumben* series [D] *P. oxaliccum* series [E] *A. fumigatus* group, *Cont'd …*

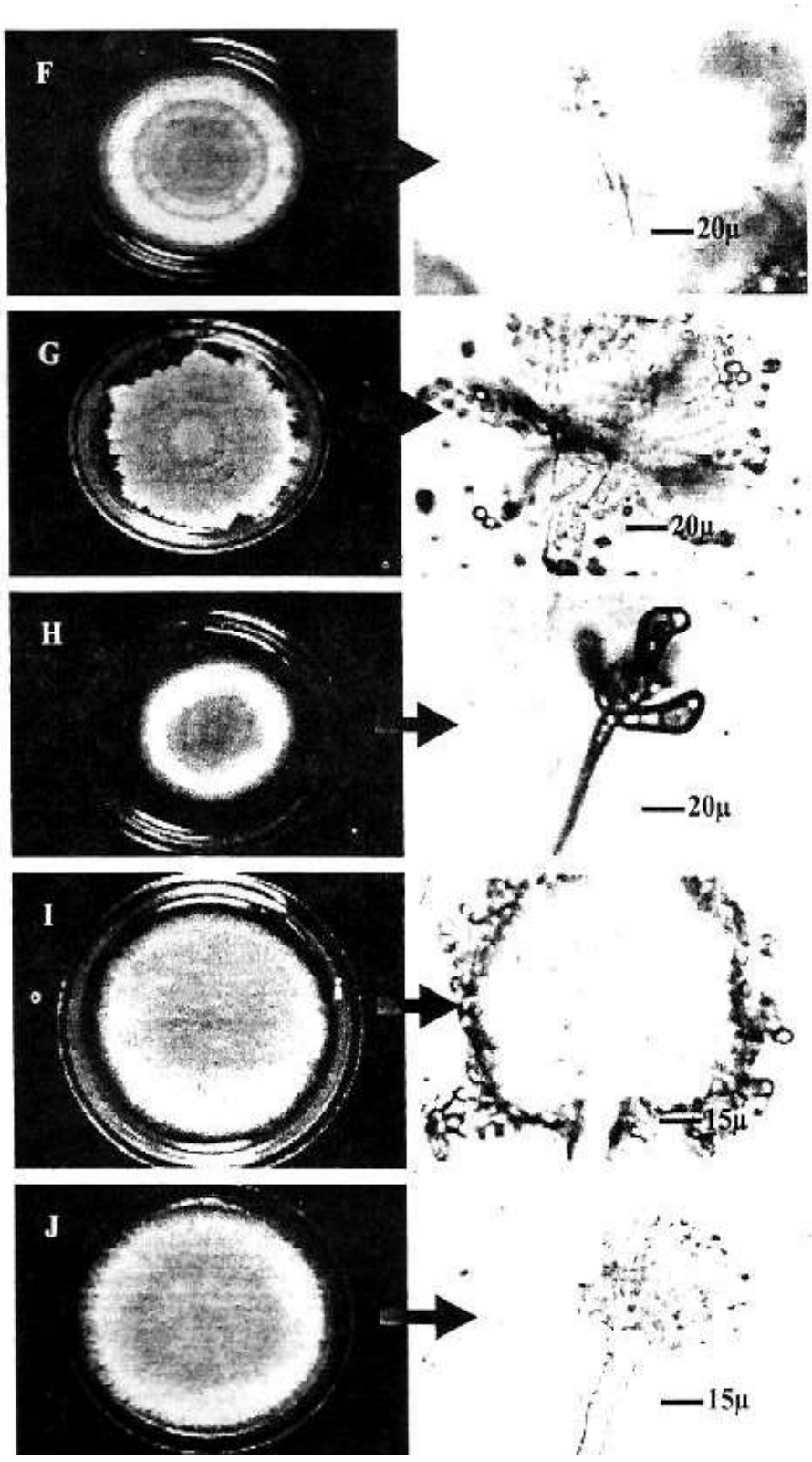

Fig.1 *cont'd.* [F] *P. chysogenum* series [G] *A. terreus* group [H] *Curvularia* sp. [I] *A. flavus*group [J] *A. flavipes* groups

Table 1. Soil fungi isolated from shrimp culture ponds during the culture period (cfu/g x10^2).

Fungi	During Stock (DS)	Middle of Culture (MC)	After Harvest (AH)
Aged pond Perak			
Curvularia sp.	2	9	-
Penicillium decumben series	6	4	2
Aspergillus fumigatus groups	-	3	-
A. flavipes groups	-	7	-
A. niger	-	-	1
Moderately aged ponds, Malacca			
Penicillium decumben series	9	2	6
Trichoderma sp.	-	-	1
A. flavus groups	-	-	2
Aspergillus sp.	-	-	2
A. oxalicum seris	-	-	8
New ponds, Malacca			
Penicillium decumben series	1	2	9
A. flavipes groups	-	-	3
P. chrysogenum series	-	-	1

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