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Pathogenicity of fungi isolated from the larvae of the mangrove crab, *Scylla serrata*, in Indonesia

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Heavy mortality reaching almost 100% occurred in the larvae of the mangrove crab, *Scylla serrata*, from July to December 1997 at the hatchery of Gondol Research Station for Coastal Fisheries in Bali, Indonesia. Mortality was observed in larvae after hatching from the eggs. The affected larvae were whitish and filled with numerous aseptate hyphae. Three fungi belonging to the order Lagenidiales, *Lagenidium callinectes*, *Haliphthoros milfordensis*, and *Halocrusticida* sp., were isolated from the infected larvae. Pathogenicity tests of the infected fungi against the larvae of mangrove crab demonstrated that all isolates were pathogenic.

Key Words——fungal infection; Lagenidiales; pathogenicity; *Scylla serrata*.

Three species of mangrove crabs, *Scylla oceanica* Dana, *S. tranquebarica* Fabricius, and *S. serrata* Forskal, are found in Indonesia. Of these, *S. serrata* is the most adaptable species to seed production and has recently gained popularity as an export commodity. Seed production of the mangrove crab *S. serrata* has been successfully carried out in Gondol Research Station for Coastal Fisheries, Bali, Indonesia since 1992. However, it has faced the problem of high mortality of the larvae due to fungal infection (Rusdi et al., 1993; Roza et al., 1993; Roza and Johnny, 1998). Fungal diseases in mangrove crabs or swimming crabs are also serious problems in other countries. The causative agents of these diseases are fungi of the order Lagenidiales. *Lagenidium callinectes* Couch (Nakamura and Hatai, 1995), *L. scyllae* Bian et al. (Bian et al., 1979), and *Halocrusticida hamanaensis* Bian & Egusa (Bian and Egusa, 1980) have been reported from ova or larvae of *S. serrata*. Unidentified species belonging to the genera *Lagenidium*, *Halocrusticida*, and *Haliphthoros* have also been reported from the mangrove crab (Kaji et al., 1991; Hamasaki and Hatai, 1993; Zafran et al., 1993). *Haliphthoros milfordensis* Vishniac (Lightner, 1981; Alderman, 1982; Hatai, 1989) is also known as an important fungal pathogen of some crustaceans.

In 1997, fungal diseases occurred in the eggs and zoeae of the mangrove crab *S. serrata* at Gondol Research Station. Mortality reached almost 100% in seed production attempted from July to December. The infected larvae were whitish and filled with numerous aseptate hyphae.

In this paper, we compare the pathogenicities to the larvae of mangrove crab of the fungi isolated from the infected larvae of the mangrove crab.

Materials and Methods

Isolation and identification Zoeae of mangrove crab, *S. serrata*, which were produced at the hatchery of Gondol Research Station for Coastal Fisheries, Bali, Indonesia, were reared at 25–27°C in a 200-l tank and fed with rotifer, *Brachionus plicatilis* Müller, and brine shrimp, *Artemia salina* Linné. Fungal infection occurred in the zoeae in July 1997 (Fig. 1). Infecting fungi were isolated by using PYGS agar (1.25 g of Bacto peptone, 1.25 g of Bacto yeast extract, 3 g of glucose, 12 g of Bacto agar, and 1000 ml of seawater) containing 500 μg/ml each of streptomycin sulphate and ampicillin to retard bacterial contamination. The agar plates were incubated at 25°C for 3–5 d. Each fungal colony developed on the agar plates was transferred onto fresh PYGS agar to make a pure culture.

For morphological observation, the fungi were inoculated into PYGS broth and incubated at 25°C for 3–5 d. The small colonies in PYGS broth were transferred into 30 ml of sterilized artificial seawater (Aqua-Ocean®, Japan Pet Drugs Co.) and incubated at 25°C to induce zoospore production. The fungal isolates were classified according to Couch (1942), Bian and Egusa (1980), Hatai et al. (1992), Nakamura and Hatai (1995), and Vishniac (1958).

Artificial infection Larvae of mangrove crab, *S. serrata*, from zoea 1 to zoea 4, which were produced at the hatchery of Gondol Research Station, Bali, Indonesia, were reared in 200-l polycarbonate tanks with aeration and fed with rotifer, *B. plicatilis*, and brine shrimp, *A. salina*, until the artificial infection.

Haliphthoros milfordensis GSM 9701, *Halocrusticida* sp. GSM 9703, and *L. callinectes* GSM 9706 were tested

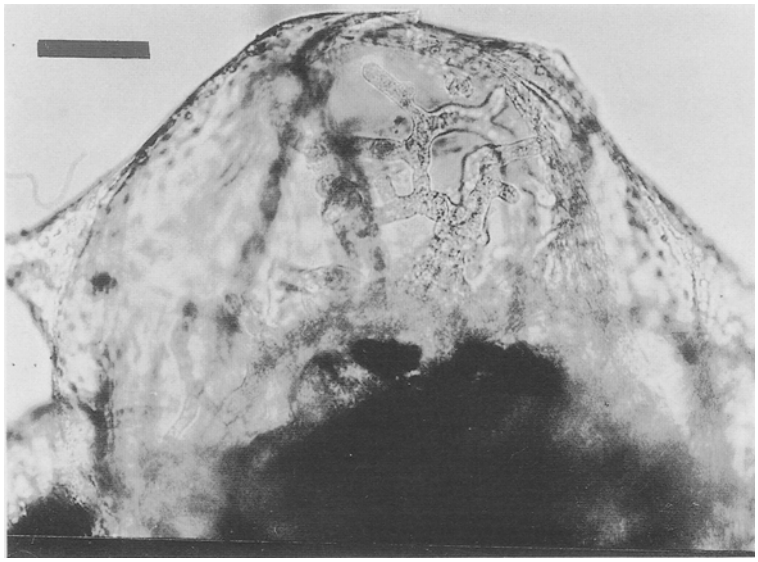

Fig. 1. Larva (zoea 1) of *Scylla serrata* naturally infected with fungus. Scale bar = 40 μm.

for pathogenicity to each zoea.

The larvae (zoea 1, 2, 3, and 4) were dipped in sterilized seawater containing 20 μg/ml of oxytetracycline for 1 h to reduce bacterial contamination before the artificial infection. Then, 45 ml of seawater with 30 larvae and 5 ml of zoospore suspensions were poured into a 100 ml beaker. The number of zoospores per ml of the suspension was adjusted to 10^2, 10^3 and 10^4. As a control, 50 ml of seawater with 30 zoeae in a 100-ml beaker was used. Each experiment was performed in triplicate. During the experiment, zoeae were not fed, but weak aeration was provided. After exposure to zoospores, the number of dead larvae was counted at 24 and 48 h. The larvae were then checked under microscope, and an attempt was made to reisolate the infecting organism from infected larvae using PYGS agar.

Results

Isolation and identification Three strains belonging to the order Lagenidiales were isolated from infected zoeae of the mangrove crab, *Scylla serrata* (Fig. 1). Three fungi with different characteristics (isolates GSM 9701, GSM 9703, and GSM 9706) were randomly selected from all of the fungal colonies. All isolates were maintained at 25°C and subcultured onto PYGS agar once a month.

Isolate GSM 9701 was classified as a member of the genus *Haliphthoros* from the characteristics of fragment formation and the mode of the zoospore production. It was identified as *H. milfordensis* from other characteristics of the asexual reproduction. Isolate GSM 9703 was found to be an unidentified species of *Halocrusticida* from the characteristics of the size and shape of zoosporangium and the production of one to several discharge tubes from each sporangium. Isolate GSM 9706 was identified as *Lagenidium callinectes* from the characteristics of the vesicle formation and other features.

The detailed and formal description of the isolates will be presented elsewhere.

Artificial infection Affected zoeae showing white coloration were found at the bottom of the beaker. They were often occupied by fungal hyphae (Fig. 2). The causative agents used in each experiment could be reisolated from the dying or dead larvae. In conrtol beaker, no fungal infection was observed during the experiments.

Table 1 shows the pathogenicity of the isolates toward the zoea 1 stage of mangrove crab. For isolate GSM 9701, the cumulative mortality after 24 h in zoeae challenged with 10^2, 10^3, and 10^4 zoospores/ml was 29.6, 37.7 and 53.6%, respectively. For isolate GSM 9703, the corresponding figures were 31.1, 42.1, and 60.5%; and for isolate GSM 9706, 28.8, 37.2, and 51.9%. In all cases, the cumulative mortality increased further between 24 h and 48 h. The mortality of the control group was less than 6.0%.

Table 2 shows the pathogenicity of the isolates toward the zoea 2 stage of mangrove crab. For isolate GSM 9701, the cumulative mortality after 24 h in zoeae challenged with 10^2, 10^3, and 10^4 zoospores/ml was 28.0, 36.6, and 53.3%, respectively. For isolate GSM 9703, the corresponding figures were 30.5, 41.0, and 58.5%; and for isolate GSM 9706, 21.9, 35.4, and 46.4%. In all cases, the cumulative mortality increased further between 24 h and 48 h. The mortality of the control group was less than 5.0%.

Table 3 shows the pathogenicity of the isolates toward the zoea 3 stage of mangrove crab. For isolate GSM 9701, the cumulative mortality after 24 h in zoeae challenged with 10^2, 10^3, and 10^4 zoospores/ml was 22.9, 34.3, and 49.1%, respectively. For isolate GSM 9703, the corresponding figures were 30.0, 40.0, and 52.9%; and for isolate GSM 9706, 17.8, 32.2, and

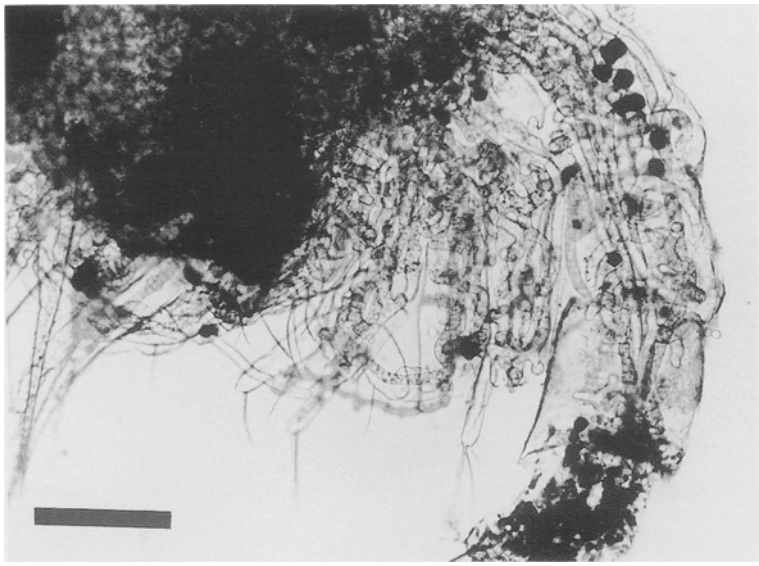

Fig. 2. Larva (zoea 2) of *Scylla serrata* artificially infected with *Lagenidium callinectes* GSM 9706. Scale bar = 100 μm.

Table 1. Mortality of zoea 1 larvae of mangrove crab, *Scylla serrata*, challenged with different numbers of zoospores of the fungi isolated from *S. serrata*.

Isolate	Mortality (%) of zoea 1 larvae[a] of mangrove crab after							
	24 h				48 h			
	10^2	10^3	10^4	Control	10^2	10^3	10^4	Control
GSM-9701	29.6	37.7	53.6	3.3	49.9	60.9	77.0	4.5
GSM-9703	31.1	42.1	60.5	2.0	65.5	69.9	79.2	3.8
GSM-9706	28.8	37.2	51.9	3.7	43.7	58.8	68.4	5.1

a) Thirty larvae were used in each test.

Table 2. Mortality of zoea 2 larvae of mangrove crab, *Scylla serrata*, challenged with different numbers of zoospores of the fungi isolated from *S. serrata*.

Isolate	Mortality (%) of zoea 2 larvae[a] of mangrove crab after							
	24 h				48 h			
	10^2	10^3	10^4	Control	10^2	10^3	10^4	Control
GSM-9701	28.0	36.6	53.3	2.0	44.8	60.4	73.0	3.2
GSM-9703	30.5	41.0	58.5	3.5	50.2	62.5	74.4	5.0
GSM-9706	21.9	35.4	46.4	1.5	40.8	55.8	67.6	2.5

a) Thirty larvae were used in each test.

Table 3. Mortality of zoea 3 larvae of mangrove crab, *Scylla serrata*, challenged with different numbers of zoospores of the fungi isolated from *S. serrata*.

Isolate	Mortality (%) of zoea 3 larvae[a] of mangrove crab after							
	24 h				48 h			
	10^2	10^3	10^4	Control	10^2	10^3	10^4	Control
GSM-9701	22.9	34.3	49.1	1.0	37.5	56.0	62.9	3.0
GSM-9703	30.0	40.0	52.9	3.2	41.2	59.3	68.0	6.0
GSM-9706	17.8	32.2	41.8	2.0	36.6	47.2	56.6	2.5

a) Thirty larvae were used in each test.

Table 4. Mortality of zoea 4 larvae of mangrove crab, *Scylla serrata*, challenged with different numbers of zoospores of the fungi isolated from *S. serrata*.

Isolate	Mortality (%) of zoea 4 larvae[a] of mangrove crab after							
	24 h				48 h			
	10^2	10^3	10^4	Control	10^2	10^3	10^4	Control
GSM-9701	19.9	30.9	44.5	2.5	36.9	48.2	60.4	3.9
GSM-9703	23.1	38.7	51.0	2.0	40.1	53.5	62.5	4.1
GSM-9706	16.2	27.4	40.7	1.0	32.4	36.9	55.0	2.5

a) Thirty larvae were used in each test.

Table 5. Mortalities of mangrove crab, *Scylla serrata*, zoea 1 to zoea 4 larvae challenged with 10^4 zoospores/ml.

Isolate	Mortality (%) of larvae[a] of mangrove crab challenged with 10^4 zoosp./ml after							
	24 h				48 h			
	Z1	Z2	Z3	Z4	Z1	Z2	Z3	Z4
GSM-9701	53.6	53.3	49.1	44.5	77.0	73.0	64.9	60.4
GSM-9703	60.5	58.5	52.9	51.0	79.2	74.4	68.0	62.5
GSM-9706	51.9	46.4	41.8	40.7	68.1	67.6	56.8	55.0

a) Thirty larvae were used in each test.

41.8%. In all cases, the cumulative mortality increased further between 24 h and 48 h. The mortality of the control group was less than 6.0%.

Table 4 shows the pathogenicity of the isolates toward the zoea 4 stage of mangrove crab. For isolate GSM 9701, the cumulative mortality after 24 h in zoeae challenged with 10^2, 10^3, and 10^4 zoospores/ml was 19.9, 30.9, and 44.5%, respectively. For isolate GSM 9703, the corresponding figures were 23.1, 38.7, and 51.0%; and for isolate GSM 9706, 16.2, 27.4, and 40.7%. In all cases, the cumulative mortality increased further between 24 h and 48 h. The mortality of the control group was less than 4.1%.

Discussion

The three fungi isolated from the larvae of mangrove crab, *S. serrata*, with fungal infection had almost the same levels of pathogenicity to the larvae of *S. serrata*. All the fungi were pathogenic to the crab larvae, since the mortality of control groups was lower than those of artificially infected ones, the mortality depended on the number of zoospores. Furthermore, as shown in Table 5, the larvae of zoea 1 stage were the most sensitive to the fungal infection, and the zoea 4 larvae were the most resistant. Of the fungi used in these experiments, *Halocrusticida* sp. GSM 9703 had stronger pathogenicity to the larvae than the other two fungi. The fact that three fungi were isolated from the infected larvae at the same place and in the same period is of interest, because it has been commonly believed that such fungi were epizootic (Nakamura and Hatai, 1995). This means that the environment of the facility for the seed production was already contaminated by several fungi. It is known that the larval stages of marine crustaceans are sensitive to fungal infection (Armstrong et al., 1976; Bian and Egusa, 1980; Lightner, 1981; Lio-Po et al., 1982; Sparrow, 1960).

Although treatment with formalin solution (Kaji et al., 1991) and pH control (Yasunobu et al., 1997) have been used effectively for fungal control in Japan, further development of methods to prevent infection is required.

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