シャコから分離されたPlectosporium oratosquillaeとAcremonium sp.のシャコに対する病原性
Pathogenicity of Anamorphic Fungi *Plectosporium oratosquillae* and *Acremonium* sp. to Mantis Shrimp *Oratosquilla oratoria*

Pham Minh Duc¹,² and Kishio Hatai¹*

¹Laboratory of Fish Diseases, Nippon Veterinary and Life Science University, Tokyo 180-8602, Japan
²College of Aquaculture and Fisheries, Cantho University, Can Tho City, Viet Nam

(Received December 29, 2008)

**ABSTRACT**—This study was carried out to determine pathogenicity of anamorphic fungi *Plectosporium oratosquillae* NJM 0662 and *Acremonium* sp. NJM 0672, which were isolated from gills of mantis shrimp *Oratosquilla oratoria* caught in Yamaguchi and Aichi Prefectures in Japan. Cumulative mortality of the mantis shrimp injected with a high dose (5.0 × 10⁶ conidia/mL) and a low dose (5.0 × 10⁴ conidia/mL) of the isolate NJM 0662 reached 100% and 60% at day 25, respectively. Cumulative mortality of the shrimp injected with the high dose and the low dose of the isolate NJM 0672 reached 100% and 80% at day 25, respectively. The gill lesions in the shrimp experimentally infected with the fungi were similar to those of naturally infected shrimp. Histopathologically, the hyphae and conidia were found in the gill filaments and heart, and the hyphae were encapsulated by hemocytes in the gill filaments and the base of gills. The result confirmed that these two anamorphic fungi were pathogenic to mantis shrimp.

**Key words:** *Acremonium* sp., *Plectosporium oratosquillae*, *Oratosquilla oratoria*, mantis shrimp, pathogenicity

In marine environment, anamorphic fungal infections commonly occur in crustacean species (Egusa and Ueda, 1972; Khoa and Hatai, 2005). Some *Fusarium* species cause black gill disease in kuruma prawn *Penaeus japonicus* (Ishikawa, 1968; Hatai and Egusa, 1976; Momoyama, 1987; Khoa et al., 2005). Black gill disease due to *Fusarium* infection also occurred in black tiger shrimp *Penaeus monodon* cultured in Viet Nam (Khoa et al., 2004). Fungal infection caused by the other anamorphic fungi has been previously reported from crayfish *Austropotamobius pallipes* (Alderman and Polglase, 1985), *Astacus leptodactylus* (Diler and Bolat, 2001), red sea bream *Pgrus major* (Hatai et al., 1986a), ayu *Plecoglossus altivelis* fry (Hatai et al., 1986b) and young striped jack (Munchan et al., 2006). In 2005 and 2006, many mantis shrimp *Oratosquilla oratoria* with black or brown gills were found in Yamaguchi and Aichi Prefectures, Japan. Number of mantis shrimp sampled monthly from April 2005 to December 2006 was 2,284 in number. In which 25% of the mantis shrimp was infected with the fungi. As a result, two kinds of anamorphic fungi *Plectosporium oratosquillae* and *Acremonium* sp. were present independently or together in the infected gills of these mantis shrimp. The former fungus was slow growth on all cultured media and an obligatory marine fungus. The latter fungus was fast growth on all cultured media and a wide range of salinity for growth. It was the first report on fungal infection in mantis shrimp (Duc et al., 2009). Experimental infection to confirm whether the fungi are really pathogenic to mantis shrimp was conducted in this paper.

**Materials and Methods**

**Fungal strains**

Two anamorphic fungi *Plectosporium oratosquillae* and *Acremonium* sp. were isolated from the gills of mantis shrimp caught from fishing ground in Yamaguchi and Aichi Prefectures, along the Pacific side of central Japan. The isolates *P. oratosquillae* NJM 0662 and *Acremonium* sp. NJM 0672 were randomly selected from the isolated fungal strains. The former fungus isolated in April 2006 in Yamaguchi Prefecture and the latter fungus isolated in September 2006 in Aichi Prefecture. The fungi were inoculated on PYGS agar [0.125% Bacto peptone, 0.125% Bacto yeast extract, 0.3% glucose, 1.2% Difco agar, and 3.8% artificial seawater (Aqu-Ocean®, Japan Pet Drugs, Tokyo)] and incubated at 25°C for one month in the case of NJM 0662, and for 10 days in the case of NJM 0672. To make a conidial suspension, 10 mL of sterile saline (0.85% NaCl) was poured into the dish, and the surface of colonies was crushed using a sterile loop to suspend conidia. The conidia and mycelia suspension was then filtered.
through two sterile layers of sanitary medical gauze to obtain a conidial suspension. The number of conidia was determined using a hemocytometer and the concentration was adjusted to $5.0 \times 10^6$ conidia/mL for the high dose group and $5.0 \times 10^4$ conidia/mL for the low dose group.

**Mantis shrimp**

Fifty mantis shrimp averaging 20 g in body weight were used in this experiment. The mantis shrimp were caught in October 2008 in Okayama Prefecture, and moved to the Laboratory of Fish Diseases, Nippon Veterinary and Life Science University. The mantis shrimp were kept in a 20 L glass tank for one week before experimental injection. They were fed daily with 2% body weight of commercial food for cultured kuruma prawn. The mantis shrimp were reared in artificial seawater with a salinity of 35% (w/v) (Marine aquarium salt, Nisso Co. Ltd., Tokyo, Japan), at a temperature of 20–24°C and pH 7.5 before and during the experiment. Twenty-five mantis shrimp without clinical signs by naked eye were selected for the experiment.

---

**Fig. 1.** Mantis shrimp intramuscularly injected with 0.1 mL of $5.0 \times 10^5$ conidia/mL of *Plectosporium oratosquillae* NJM 0662. A. Numerous brown spots (arrow) in the gill at day 10, high magnification (left), low magnification (right), bar = 5 mm. B. Hyphae (arrow head) and conidia (arrow) in the gill filament at day 10, high magnification (left), low magnification (right), bar = 10 μm. C. Hyphae (arrow) in the gill filament at day 7. Grocott-HE. D. Encapsulated hyphae (arrow) at the base of gill at day 10. PAS. E. Hyphae in the heart (arrow), high magnification of H (left), but no hyphae in muscle (M), hepatopancreas (He), gut (G) and testis (T) at day 10. Grocott-HE.
Experimental design

The experiment was conducted from October to November 2008 at our laboratory. Twenty-five mantis shrimp were divided into five groups and placed in five separate tanks. Two groups each of mantis shrimp were intramuscularly injected with 0.1 mL of a conidial suspension of either $5 \times 10^6$ (high dose) or $5 \times 10^4$ (low dose) conidia/mL. The conidial suspension was injected at the 4th dorsal abdomen of mantis shrimp according to a method modified from Khoa et al. (2005). In the control group, each of the five mantis shrimp was injected with 0.1 mL of sterile saline. The experiment was conducted for 25 days.

Clinical observation

Mortality was observed daily and then cumulative mortality was calculated at the end of the experiment period. Fresh gills of all dead or moribund mantis shrimp were directly observed under a light microscope to determine whether the fungal hyphae penetrated in

![Fig. 2. Mantis shrimp intramuscularly injected with 0.1 mL of $5.0 \times 10^6$ conidia/mL of Acremonium sp. NJM 0672. A. Numerous brown spots (arrow) in the gill at day 7, high magnification (left), low magnification (right), bar = 5 mm. B. Hyphae (head-arrow) and conidia (arrow) in the gill filament at day 7, high magnification (left), low magnification (right), bar = 5 μm. C. Hyphae and conidia (arrow) in the gill filament at day 7. Grocott-HE. D. Encapsulated hyphae (arrow) in the gill filament at day 13. Grocott-HE. E. Hyphae in the heart (arrow), high magnification of H (left), but no hyphae in the muscle (M), hepatopancreas (He) and testis (T). Grocott-HE.](image-url)
the gills and/or the conidia produced. The fungus was then attempted to re-isolate from the gill lesions. Changes in color of the gills were also checked directly by naked eye.

Histopathological examinations

All dead or moribund mantis shrimp were fixed in 10% phosphate buffered formalin solution (PBF). Fixed gills and cross-sections of the 4th abdomen were decalcified in 10% formic acid-formalin (10% formic acid in 10% PBF) for 3 days, then de-acidified in sodium sulfate solution (5% sodium sulfate in distilled water) for 1 day, after which the specimens were immersed in 10% PBF again for 3 days at room temperature. The gill and abdomen specimens were embedded in paraffin, sectioned at 3–4 μm, and stained with periodic acid Schiff (PAS) or Grocott-H&E.

Results

Clinical signs

The external clinical signs of mantis shrimp challenged with Plectosporium oratosquillae NJM 0662 and Acremonium sp. NJM 0672 were numerous brown spots in gill filaments (Figs. 1A & 2A), but no clinical signs were found in the other parts. The brown spots resembled nodules with hyphae inside. All injected mantis shrimp in the high and low dose groups showed gill lesions. Fungal hyphae and conidia were observed in the gill filaments under a light microscope (Figs. 1B & 2B). In the control group, however, no lesions were observed in the gills.

The cumulative mortality and fungal recovery

The cumulative of mantis shrimp injected with Plectosporium oratosquillae NJM 0662 reached 100% in the high dose group after 10 days and 60% in the low dose group after 25 days (Fig. 3). The cumulative mor-

Fig. 3. The cumulative mortality of mantis shrimp experimentally injected with Plectosporium oratosquillae NJM 0662. □: 0.1 mL of 5.0 × 10⁶ (high dose) conidia/mL, ○: 0.1 mL of 5.0 × 10⁴ (low dose) conidia/mL, ▲: 0.1 mL of physiological saline (control).

Fig. 4. The cumulative mortality of mantis shrimp experimentally injected with Acremonium sp. NJM 0672. □: 0.1 mL of 5.0 × 10⁶ (high dose) conidia/mL, ○: 0.1 mL of 5.0 × 10⁴ (low dose) conidia/mL, ▲: 0.1 mL of physiological saline (control).

Histopathological examination

Histopathological examination of mantis shrimp injected with the isolates NJM 0662 (Fig. 1C) or NJM 0672 (Fig. 2C) revealed numerous hyphae in the gill filaments at day 7 after inoculation. Encapsulated hyphae were observed in the gills and at the base of gills at day 10 or at day 13 after inoculation of the isolates NJM 0662 (Fig. 1D) or NJM 0672 (Fig. 2D), respectively. Numerous hyphae were observed in the heart at day 10 or at day 13 after inoculation of the isolates NJM 0662 (Fig. 1E) or NJM 0672 (Fig. 2E), respectively, but no fungi were found in the internal organs such as midgut, testis, ovary and hepatopancreas in the samples examined. This suggests that the fungi were parasitic in the circulatory system of mantis shrimp.

Discussion

Fungal infections similar to those by the present anamorphic fungi are known among penaeid shrimps in the gills; Fusarium solani and F. oxysporium infection of kuruma prawn Penaeus japonicus (Bian and Egusa, 1981; Khoa and Hatai, 2005; Khoa et al., 2005) and F. incarnatum infection of black tiger shrimp Penaeus monodon (Khoa et al., 2004). Besides, another anamorphic fungus Fusarium tabacinum (=Plectosporium tabacinum) caused gill disease in the crayfish Austropotamobius pallipes in England (Alderman and
Polglase, 1985). The gills showed black color. They described that the encapsulation of hyphae was observed in the gill filaments. Mantis shrimp infected with Plectosporium oratosquillae NJM 0662 or Acremonium sp. NJM 0672 showed similar gill lesions due to the fungal infection even if the fungal species were different.

The mantis shrimp intramuscularly injected with the anamorphic fungi showed many brown spots in the gill filaments, which were similar to the clinical sign of mantis shrimp naturally infected with the fungi. In addition, fungal hyphae and conidia were found in wet-mount preparations of the gill filaments, and identical fungi were re-isolated from the injected shrimp. The infection rates of mantis shrimp, as well as fungal recovery from mantis shrimp challenged with the isolates NJM 0662 or NJM 0672 were 100% in both the high and low dose groups. This indicates that conidia of both fungi germinated and developed in the gills of mantis shrimp. They both caused damage to the gill filaments. The two fungi caused high mortality to mantis shrimp. It indicated that the two fungi were pathogenic to mantis shrimp in experimental infection. On the other hand, histopathological findings showed that numerous hyphae were also found in the heart, but no hyphae in the other internal organs. This suggested that the fungi invaded into the circulatory system, especially the gills, and caused death to the mantis shrimp.

References