A Reo-like virus associated with high mortality rates in cultured mud crab, *Scylla serrata*, in East China

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ABSTRACT

The discovery of a reo-like virus in cultures of the mud crab *Scylla serrata* (Forsskål, 1755) is reported here. Since May 2004, in Zhejiang Province, Peoples Republic of China, reo-like virus in mud crabs has reached serious epidemic levels, causing mortality rates exceeding 90%. Beginning in May 2004, moribund crabs from different districts and areas were investigated. By using electron microscopy, two different organisms could be detected: reo-like viruses and mollicute-like organisms. The complete viral particle is 60 nm in diameter, icosahedral and non-enveloped. The viruses infect the cytoplasm of connective tissue cells of the gill, stomach, heart and intestine of mud crabs.

Key words: *Scylla serrata,* virus, mollicute, electron microscopy

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INTRODUCTION

Mud crab, *Scylla serrata* (Forsskål, 1755), traditionally called 'green crab', is an economically important marine aquaculture species cultured in the People's Republic of China. In the past few years the aquaculture industry based on *S. serrata* has experienced rapid growth, but in the meanwhile the industry is facing increasing economic losses caused by outbreaks of various diseases. Disease has become one of the main factors inhibiting the development of *S. serrata* aquaculture in Zhejiang Province, China with survival rates less than 10%.

Since summer 2004, an epidemic disease has broken out in Sanmen County and its adjacent areas with symptoms differing from a previous disease outbreak called 'yellow water disease'. These symptoms included debility, weak grip strength of pincers and feet, hydroabdomen, white colour in carapace, drying of gill filaments, and water-like blood after breaking the feet (blood coagulation capacity is weak). Moreover, the disease is widely spread, and is fatal to commercial size crabs, fingerings (40-60 individuals /kg) and breeding crabs. Antibiotic drugs had no effect in relieving the symptoms. The disease is temporarily termed 'clearwater disease' (CD) due to its major symptom. In this study, the ultrastructure of infected crab tissues collected in Sanmen County in 2004 and 2005 were investigated. Two different pathogens in moribund crabs were detected: reo-like viruses and mollicute-like organisms.

MATERIALS AND METHODS

Mud crab

Five moribund mud crabs were obtained from a mud crab farm in Sanmen County, Zhejiang Province, China, and examined by histopathology and by electron microscopy.

Histopathological examination

Hepatopancreas, gill and stomach of both moribund and healthy mud crabs were fixed in Davidson's fixative for 24 hrs. The tissues were routinely processed, embedded, sectioned (5-7 μm) and stained with haematoxylin and eosin (H&E), according to the procedure of Weng et al. (2007). The tissues were examined under light microscopy.

Electron microscopy

After dissection, the heart, stomach, intestine and gill filaments were collected and fixed with 2.5% glutaraldehyde. Sample preparation for electron microscopy followed Yang and Wu (1992).

RESULTS

Necrotic lesions were easily observed in all five mud crabs with CD by staining with H&E (Fig.1A-C). Inclusion bodies, stained light red to deep red near the nucleus of infected cells, were also found in the gills of all specimens (Fig. 1A).

Transmission electron microscopy revealed that the reo-like viruses apparently caused vacuolar degeneration and necrosis of the infected cells in the connective tissues in heart, stomach, gills and intestines. Large numbers of virions with hexagonal shape were observed in the cytoplasm of enlarged gill (Fig. 2A) and intestine cells (Fig. 2B). The capsid measured approximately 60 nm from side to side, consisting of an electron-dense core surrounded by an electron-translucent zone (Fig. 2C), as well as free virions and nuclear structures in developing viruses (Fig. 2D). Crystal-like inclusion bodies produced by a large amount of mature viruses and viral intermediate structures were also present in the intestinal cells (Fig. 2B). However, the inclusion bodies could not be observed in the nucleus of intestinal cells. In stomach cells, a large amount of spherical viral particles aligned in a crystal lattice could be observed (Fig. 2E).

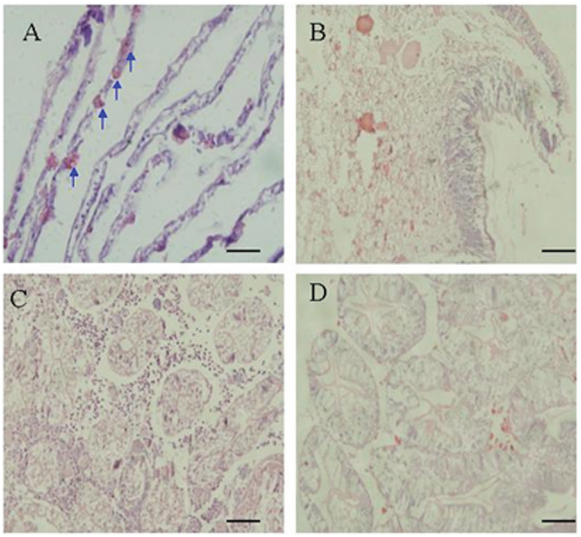

Figure 1. Histology of mud crab. Reovirus-infected tissues of mud crab (H&E). A. Necrosis of gill. Arrows show viral inclusions (bar=50μm). B. Necrosis of stomach (bar=50μm). C. Necrosis of connective tissue of hepatopancreas (bar=50μm). D. Hepatopancreas tissue of healthy mud crab (bar=50μm).

In some samples, gill tissue examined by TEM showed infection with mollicute-like organisms (Fig. 2F). The mollicute-like organisms, located in the cytoplasm of the host cell, varied in form from filate-shaped (3-5 μm) to flask-shaped with a head-like structure (2-3 μm). Another feature is the absence of a cell wall and they were only embedded by a plasma membrane.

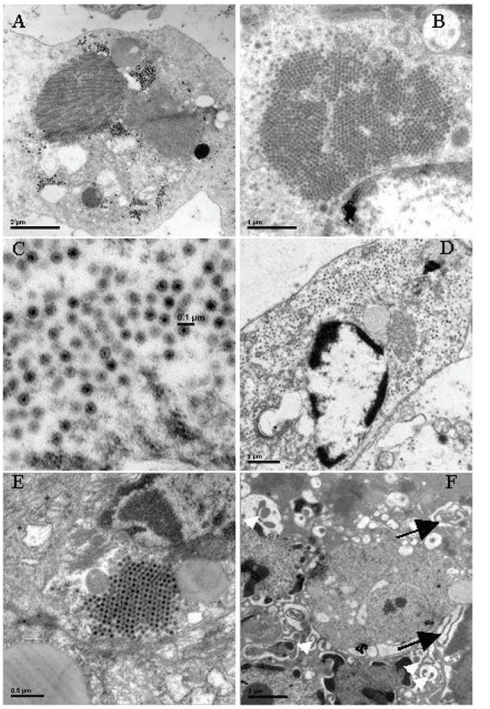

Figure 2. Ultrathin section of mud crab. Reo-like viruses and mollicute-like organisms infecting tissue of gill, intestine, stomach and heart. A. Gill (bar=2μm); B. Intestine (bar=1μm); C. Stomach (bar=0.1μm); D. Stomach (bar=1μm); E. Heart (bar=0.5μm). F. Large arrows indicate mollicute-like organism with filate-shaped form(bar=2μm); Small arrow indicate mollicute-like organism with flask-shaped form.

DISCUSSION

Since the viral isolation from *Macropipus depurator* L. (Vago *et al*., 1966), baculovirus, reovirus, parvovirus, rotavirus, and herpesvirus have been isolated from other species of crabs (Seidel *et al*., 1983; Mari and Bonami.,1988a; Mari and Bonami., 1988b; Montanie *et al*., 1993; Kanchanaphum et al., 1998; Sun and Guo., 1999., Gong *et al*., 2000; Zhang *et al*., 2002; Weng et al., 2007).

Several viruses lead to the diseases in *Scylla serrata,* such as white spot syndrome virus (WSSV) (Chen *et al*., 2000; Hameed *et al*., 2003), muscle necrosis virus (Song *et al*., 2003) and reovirus (Weng *et al*., 2007). The virus observed in the present study is similar to the members of the family Reoviridae, with respect to virus features, form and distribution, especially to the reovirus as reported by Weng *et al*. (2007). Based on these features, the virus is preliminarily identified as an aquatic reovirus. In order to show the difference to the reovirus found in *Eriocheir sinensis*, the present virus is termed temporarily as *Scylla serrata* reovirus (SsRV) on the basis of its host.

Previously studies have revealed that the reovirus was highly pathogenic and lead to various diseases in crabs, e.g. tremble disease in *Eriocheir sinensis* and sleep disease in *S. serrata* (Zhang *et al*., 2002). In terms of disease symptoms, it is proposed that the main pathogen of 'SD' in *Scylla serrata* may be SsRV, which was probably also the pathogen causing the sleeping disease in Fujiang Province and Guangdong Province. Further studies are required to confirm this conclusion.

Mycoplasmas of aquatic animals, especially those of Crustacea, have not been studied extensively. Krol *et al.* (1991) first reported a filamentous mollicute-like bacterium that appeared to be associated with necrotizing hepatopancreatitis lesions in cultured penaeid shrimp. Shortly after, Yang and Wu (1992) reported mollicute-like organisms that appeared to be associated with the gut-node disease, also in penaeid shrimps. Mollicute-like organisms would enter the perinuclear space, when the membrane of mollicute in the cytoplasm began to interact with the membranes in the perinuclear space. Moreover, the spherical nature and size of the mollicutes in the perinuclear space may result from nutritional factors or from environmental parameters (Yang and Wu, 1999). However, this described pathogenesis in shrimp still remains unclear. Isolation and culture of mycoplasma from penaeid was first reported by Ghadersohi and Owens (1999), in association with an epizootic of midcrop mortality syndrome in *Penaeus monodon*.

A tentative identification of the bacterium in *S. serrata* can be obtained based on morphology in preliminary electron micrographs. The intracellular microorganisms in gill cells of *S. serrata* lack a cell wall and are surrounded only by the plasma membrane. This is an indication that these microorganisms are mollicutes (Choi *et al*., 1996). The intracellular microorganisms are highly pleomorphic, and apparently an obligate intracellular pathogen. All of these facts are indications for mollicutes. The organism is now named 'gill mollicute-like organisms' (GMLO) to distinguish them from other mollicutes until their relationship is clarified.

We detected GMLO only in gill cells, not in other tissues. No evidence was found that gill cell necrosis in affected areas have been associated with MLO. Therefore, the virulence of mollicute-like organisms from *S. serrata* remains uncertain. Further studies are necessary to determine the identity of these bacteria and their pathogenesis in *S. serrata.*

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