academicJournals

Vol. 7(16), pp. 1569-1573, 16 April, 2013
DOI: 10.5897/AJMR12.1872
ISSN 1996-0808 © 2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Virus-like particles detected in postlarvae of abalone (*Haliotis diversicolor supertexta* Lischke) potentially associated with mass mortalities

Jing Zhou[1]*#, Renjun Wang[1#] and Junpeng Cai [2]

[1]College of Life Science, QuFu Normal University, QuFu, 273165, China.
[2]College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, 510640, China.

Accepted 25 march, 2013

Mass mortalities among cultured postlarvae of abalone, *Haliotis diversicolor supertexta* Lischke of between 10 and 30 days post fertilization, characterized by a white appearance and falling of the diatom films on which they grow, has occurred since 2002 on the south coast of China, including Taiwan. However, its causes remain debated. In this paper, we reported our latest findings on two different types of virus-like particles that are potentially associated with mass mortalities of postlarvae of abalone. Fourteen-day-old moribund postlarvae collected during an outbreak of postlarval disease in Shenzhen, China on November 10th, 2010 were examined by transmission electron microscopy. Besides showing severe damages in the postlarval tissues, two different types of virus-like particles were detected. These two virus-like particles were detected within both nuclei and cytoplasms of diseased abalone tissues. As they were detected in the diseased postlarval tissues, it is therefore suggested that they were potentially pathogens for postlarvae in that particular outbreak.

Key words: Postlarvae, abalone, *Haliotis diversicolor supertexta*, mass mortality, virus-like particles.

INTRODUCTION

Abalone, *Haliotis diversicolor supertexta* Lischke is a commercially important and artificially cultured species of the South coast of China, including Taiwan. Culture of *H. diversiocolor supertexta* has expanded tremendously since 1986 due to successful artificial propagation and development of multiple-tier basket systems in grow-out farms (Ringø et al., 2012, 1986; You et al., 2010). However, between 1999 and 2001, farms experienced mass mortalities of grown abalone (Lee et al., 2001). And since 2002, outbreaks of mass mortalities of postlarvae of between 7 and 30 days post fertilization have become epidemic in the South coast of China, causing huge economic losses. Among the causative agents of mass mortalities of grown abalone, vibrios (Liu et al., 2000; Cheng et al., 2004) and spherical viruses have been recognized as the pathogens of the disease (Song et al., 2000). Two forms of spherical virus were involved in the pathogenesis of the disease, viz. enveloped virus of 100 to 150 nm in diameter (Song et al., 2000; Wang et al., 2004) and non-enveloped virus of 50 to 70 nm in diameter (Chen et al., 2005). More recently, Chang et al. (2005) reported that herpes-like virus was also a causative agent of mortality of cultured abalone in Taiwan. Nevertheless, little is known about the possible involvement of virus(es) in the mass mortalities of postlarvae despite the fact that we have known *Vibrio campbellii* (Ma et al.,1996), *Vibrio parahae-*

*Corresponding author. E-mail: jingzhou-2004@163.com.

#These authors contributed equally to this work.

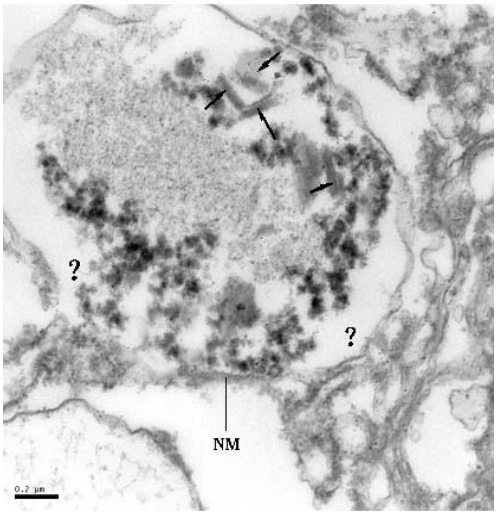

Figure 1. Electron micrograph showing nucleocapsids of rod-shaped virus (black arrows) in the nucleus of a diseased postlarvae abalone (*H. diversicolor supertexta*). Asteroid/spherical virus-like particles (question marks) were also detected (100 nm in diameter); NM = nuclear membrane; bar, 200 nm.

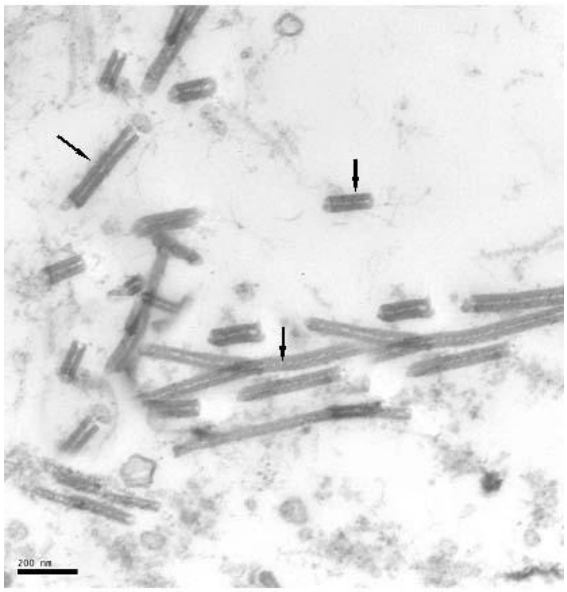

Figure 2. Electron micrograph showing capsids of the rod-shaped virus in different lengths (100 to 300 nm in lenght and 30 nm in diameter). They were detected from another location of the diseased abalone tissue; bar, 200 nm.

molyticuss (Cheng et al., 2004) and *Shewanella alga (*Cai et al., 2006) to be pathogenic to postlarval abalone. In this paper, we reported our latest findings on two different types of virus-like particles that are potentially associated with mass mortalities of postlarvae of abalone.

MATERIALS AND METHODS

Moribund postlarval of abalone, *H. diversicolor supertexta* of 14 days post fertilization and with an average shell length of ca. 1 mm were collected from an abalone farm that was experiencing an outbreak of mass mortality on November 10th, 2010 in Shenzhen of Guangdong Province, China. Water temperature was between 22 and 23°C. Moribund abalone displayed clinical signs of a whitened body color, a lag in reaction time, shrunken body muscles (resulting in shells larger than their meat bodies) and diatom films fell off in the end. Diseased postlarval abalones were divided into two parts. One part was examined by transmission electron microscopy and the second part was used for the extraction of possible viral particles that would be used in challenge tests later on. The second part's tissues were removed from moribund abalone, minced, resuspended in MEM and homogenized for 1 to 2 min in a virus blender. The homogenate was clarified by cemtrifugation at 1500 g for 20 min for at 4°C. The supernatant fluid was collected and passed through 0.45 µm membrane filters. Three groups of 10 abalones each obtained from an abalone farm in Shenzhen, Guangdong Province that had not experienced an epizootic were divided into virus and control groups. Experiments were carried out in 100 L glass aquaria containing 70 L of salt water. Aquaria were aerated by water recirculating through in-tank, glass-wool filters at 17 to 20°C.

Materials for transmission electron microscopic examinations were fixed on site in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After been washed in the same PBS buffer for 1 h at 4°C, samples were post-fixed in 1% osmium tetroxide in the same buffer at 4°C and dehydrated in ascending concentrations of ethanol (50 to 100%), and then embedded in Epon 812 resin. Ultra-thin sections (70 nm) were cut and directly examined without further staining under a Tecnai 12 (PHILIPS-FEI，Holland) electron microscope at an accelerating voltage of 80 kV.

RESULTS AND DISCUSSION

Tissues of postlarval abalones were severely degenerated, leaving only some organelles such as nuclei recognizable at some places. Due to this, it is therefore rather difficult to locate the exact types of tissues or cells where the viruses were found in some cases. However, from the electron micrographs shown below, virus or virus-like particles could evidently be seen on the thin sections and the location of virus-like was judged to be mainly located within the epithelial cells on the ground that microvilli were present near/on the cells where viruses were found. Here, we described two morphologically distinct viral particles.

One virus-like particles, clear rod-shaped nucleocapsids were observed within the nucleus of an infected cell (Figure 1). They measured 100 to 300 nm in lenght and 30 nm in diameter. Capsids were also detected as shown in Figure 2. Their sizes varied from 100 nm in length and 30 nm in diameter to 300 nm in length and 30 nm in diameter. These capsids were similar to the capsids reported by Moser et al. (2001). The whole life cycle of this virus-like particle was not detected due to the severe damage of the tissues. Its precise taxonomic position and pathogenicity will be further investigated when sufficient infected postlarval materials could again be collected from hatchery farms since virus-infected postlarvae are hard to come by.

Other virus-like particles, which were more abundant

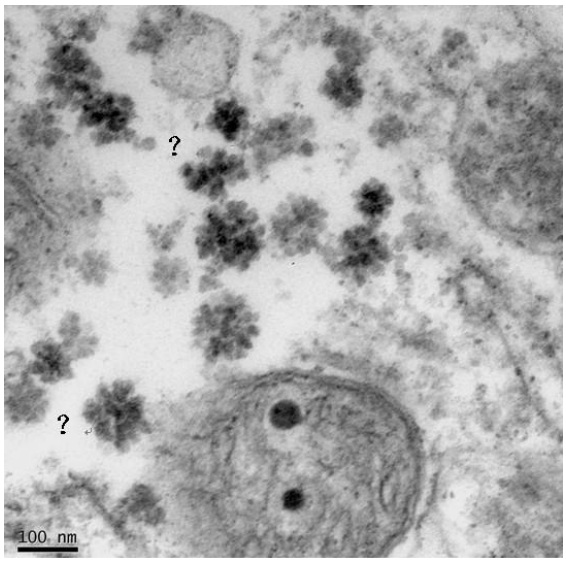

Figure 3. Electron micrograph showing asteroid / spherical virus-like particles in the cytoplasm in higher magnification (question marks); bar, 100 nm.

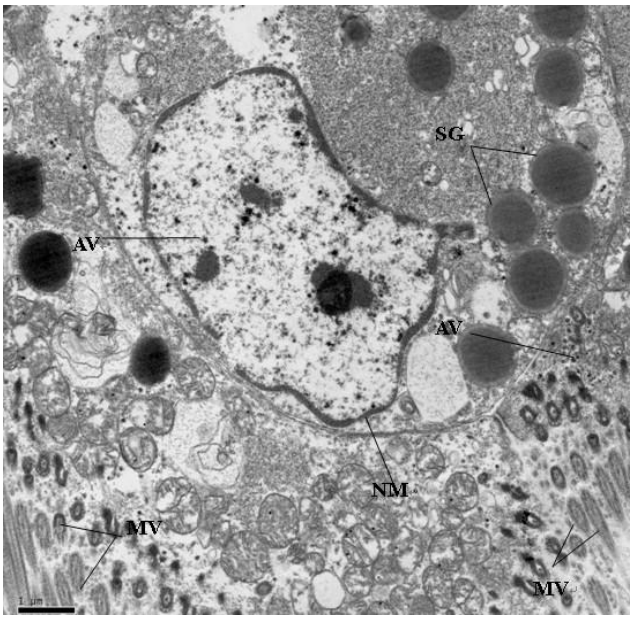

Figure 4. Electron micrograph showing spherical virus-like particles both inside and outside an epithelial cell. AV = asteroid/spherical virus-like particles; SG = secretory granules; NM = nuclear membrane; MV = microvilli; bar, 1 µm.

and frequently appeared in the tissues than the rod-shaped particles, were also detected within both nuclei and cytoplasms of the cells that were judged to be epithelial cells of diseased abalone on the basis that microvilli were clearly shown on several micrographs (Figures 1 to 7). They were spherical in shape, about 100 nm in diameter, and possessed no obvious envelopes. They were rather uniform in size and shape and were apparent-

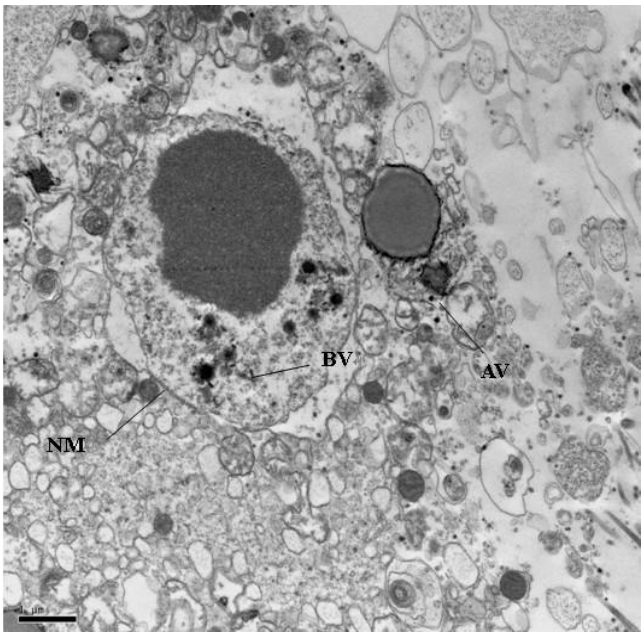

Figure 5. Electron micrograph showing asteroid/spherical virus-like particles both inside and outside an epithelial cell. Several tiny rod-shaped virus particles close to the big spherical bodies within the nucleus were also obser-ved. Judging from the presence of microvilli at the bottom right, this cell should be located at the epithelium of post-larvae. BV = rod-shaped virus; AV = asteroid/ spherical virus-like particles; NM = nuclear membrane; bar, 1 µm.

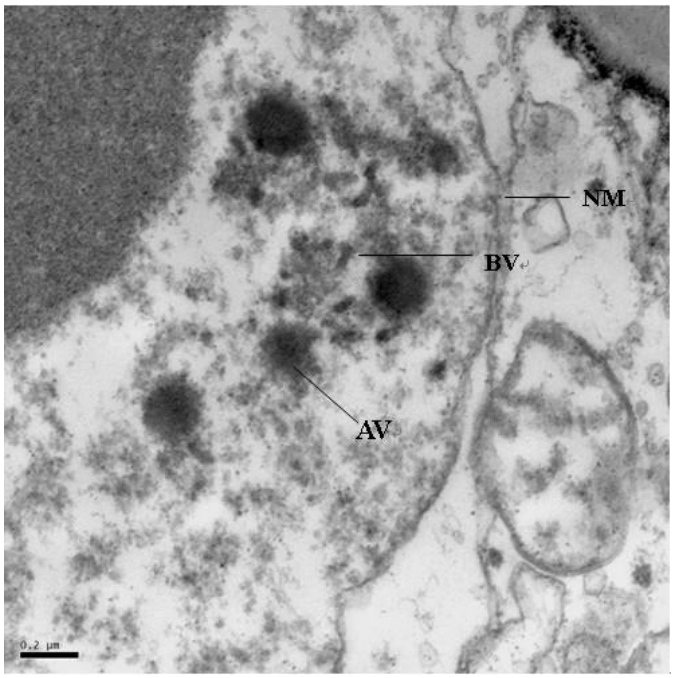

Figure 6. Electron micrograph showing both rod-shaped and asteroid/spherical virus-like particles at the right hand side of the nucleus (Figure 5) in higher magnification. AV = asteroid/spherical virus-like particles; BV = rod-shaped virus; NM = nuclear membrane; bar, 0.2 µm.

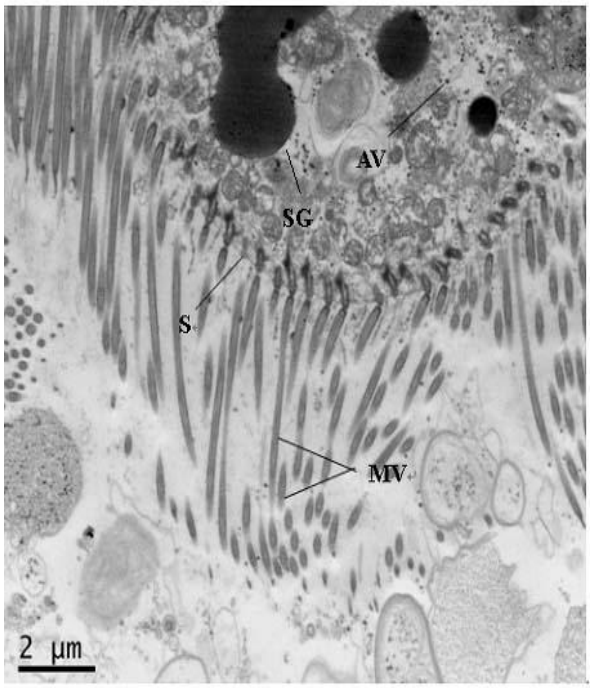

Figure 7. Electron micrograph showing microvilli and asteroid/spherical virus-like particles within tissues just under the epithelium. AV = asteroid/spherical virus-like particles; MV = microvilli; SG = secretory granule; S = possibly the surface of abalone digestion tract; bar, 2 µm.

ly different from synaptic vesicles, elastic fibers or microvilli of the diseased postlarval tissues. However, they were similar to one of the two types of spherical virus reported by Chen et al. (2004), who detected a non-enveloped type of spherical virus in the cytoplasm of the liver cells of the diseased grown abalone, *H. diversicolor aqualitis* and different in shape but same in size for virus particles reported by Song et al. (2000), who detected a large amount of enveloped, hexagonal virus particles of about 100 nm in diameter in the cytoplasm of the cells, where microvilli were based, for postlarval abalone of 28 days post fertilization (2 mm shell length).

Unfortunately, no meaningful results were obtained as nearly 100% postlarvae in both test and control groups died within 24 h of the experiments. This could be due to the fact that these postlarvae were already infected and was about to show clinical symptoms as the same batch of postlarvae from the same hatchery farm suffered an outbreak of mass mortality during the same period. Therefore, a second challenge test would need to be run once sufficient virus-infected materials are available.

Spherical virus is recently discovered to be a pathogen of grown abalone, *H. diversicolor* in South China and would cause outbreaks of mass mortality when water temperature is at or lower than 20°C (Wang et al., 2004). Two different types of spherical virus were noticed so far, viz. enveloped virus of 100 to 150 nm in diameter (Song et al., 2000; Wang et al., 2004) and non-enveloped virus of 50 to 70 nm in diameter (Chen et al., 2005). However, it is still not clear if the spherical viruses found in post larval abalone by Song et al. (2000) (100 nm, enveloped) and in this study (100 nm, non-enveloped) are the same with any of the viruses found in the grown abalone (Song et al., 2000; Wang et al., 2004) even though transmission of spherical virus can be both vertically and horizontally (Handlinger and Berth, 2004) as two facts are against this notion: 1) postlarvae are normally reared at the temperature of above 22°C and the outbreaks of postlarval disease are more severe when the temperature is higher. Some of the farmers even found that they could better successfully rear postlarvae when water temperature dropped down to as low as 18 or 19°C for several days during the postlarval rearing process when a spell of cold wind came from Russian Siberia: 2) spherical virus would only cause outbreaks of grown abalone disease when temperature is equal to or lower than 20°C (Wang et al., 2004). Anyway, the role of the two virus-like particles in the mass mortality of postlarvae needs to be determined. Again, this could not be performed until postlarval disease outbreaks caused by virus infections are encountered and sufficient virus-infected materials are obtained.

With the development of abalone culture industries, the impact of diseases on abalone has become more and more serious. We described, for the first time, the two viruses infection in abalone, *H. diversicolor supertexta* L. associated with the mass mortalities in Shenzhen, China.

Other Chinese colleagues often failed to detect any pathogens in all their endeavor, except one who also detected a spherical virus (100 nm, enveloped) in the diseased postlarvae (Song et al., 2000). This could mean that virus infection in postlarvae is relatively rare and hence infected samples are rather hard to collect. Despite the fact that currently, we are not able to draw a firm conclusion on the pathogenicity of these two types of virus-like particles due to lack of materials, we are the first to report the finding of two different types of virus-like particles in the tissue of diseased postlarvae. This new finding would at least alert abalone farmers to broaden their prevention measures to cover possible virus infection. As for abalone scientists, this could possibly attract their attention to the possible roles of virus in the mass mortalities of postlarval abalone that has devastated abalone industry in Southern China since 2002.

Taking this into consideration, we suspected that the outbreak which occurred on November 10, 2010 in Shenzhen, China could well be a special case in association with this two virus-like particles. However, much works needs to be carried out before concluding on the possible involvement of the two viruses in the mass mortality of abalone postlarvae in Southern China.

ACKNOWLEDGEMENTS

This work was surported by three funds: The Science and

Technology Project of Institutions of Higher Education of Shandong (No. J12LE02), Qufu Normal University Youth Fund (No.XJ201107), Natural Sciences Fund of China (No.NSFC-40776091).

REFERENCES

Cai J, Chen H, Thompson KD, Li C (2006). Isolation and identification of *Shewanella alga Simidu* and its pathogenic effects on post-larvae of abalone Haliotis diversicolor supertexta. J. Fish Dis. 29: 1-4.

Chang PH, Kuo ST, Lai SH, Yang HS, Ting YY, Hsu CL, Chen HC (2005). Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan. Dis. Aquat. Org. 65:23-27.

Chen Y, Huang W, Chen M (2005). The pathogeny and epidemiology of fulminating epidemic disease of abalone in Fujian and Guangdong. Marine Sci. 29: 100-1004 (in Chinese).

Cheng W, Hsiao IS, Hsuc CH, Chen JH (2004). Change in water temperature on the immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemo-lyticus*. Fish Shellfish Immunol. 17: 235-243.
diversicolor supextexta, with withering syndrome. Lett. Appl. Microbiol.31: 433-437.

Handlinger J, Berth F (2004). Virus diseases in abalone. Network of Aquaculture Centres in Asia-Pacific (http://www.enaca.org), pp.1-2.

Lee KK, Liu PC, Chen CY, Huang CY (2001). The implication of ambient temperature with outbreak of vibriosis in cultured small abalone *Haliotis diversicolor supertexta Lischke*. J. Therm. Biol. 26: 585-587.

Liu PC, Chen YC, Huang CY, Lee KK (2000). Virulence of *Vibrio parahaemolyticus* isolated from cultured small abalone, *Haliotis*

Ma JM, Wang Q, Liu MQ (1996). A pathogen of septicopyaemia in the abalone *Haliotis Discus* Hannai Ino. *Dalian*, China. 20(4):332-336.

Moser BA, Becnel JJ, White SE, Afonso C, Kutish G, Shanker S, Almira E (2001). Morphological and molecular evidence that Culex nigripalpus baculovirus is an unusual member of the family Baculoviridae. J. Gen. Virol. 82: 283-297.

Ringø E, Olsen RE, Vecino JLG, Wadsworth S, Song SK (2012). Use of immunostimulants and nucleotides in aquaculture: a review. J. Marine Sci. Res. Dev. 2:1-22.

Song ZR, Huo ZH, Ni ZM (2003). Study on the pathogenicity of mass mortality of abalone *Haliotis diversicolor aquatilis*. J. Fujian Fisher. 4: 1-3 (in Chinese).

Song ZR, Ji RX, Yan SF, Chen CS, Znong YP, Jiang YH, Ni ZM (2000). A sphereovirus resulted in mass mortality of *Haliotis diversicolor aquatilis*. J. Fisher. China. 24(4): 463-467 (in Chinese).

Wang JY, Guo ZX, Feng J, Liu GF, Xu LW (2004). Virus infection in cultured abalone *Haliotis diversicolor Reeve* in Guangdong province, China. J. Shellfish Res. 23 (4): 1163-1168.

You W, Ke C, Luo X, Wang D (2010). Genetic correlations to morphological traits of small abalone Haliotis diversicolor. J. Shellfish Res. 29:683-686.