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Pathology and physiology of *Haliotis diversicolor* with withering syndrome

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ABSTRACT

Abalone withering syndrome is a serious chronic disease. Ultrastructure and pathological changes were studied using transmission electron microscopy. Myofibers appeared hollow, and the number of inner intact myofibrils was reduced greatly. The morphology of mitochondria in cells appeared abnormal. Crystal lattice-like inclusions in pathological muscle cells were observed. The hepatopancreas was damaged severely; it was full of empty vesicles and devoid of any recognizable cellular structures. The activities of 3 enzymes, acid phosphatase (ACP), alkaline phosphatase (AKP), and total superoxide dismutase (T-SOD), in hemolymph of healthy and diseased abalone showed little differences. ACP activity in the pedal mucus of healthy and diseased abalone was not significantly different. However, in diseased abalone pedal mucus, activities of AKP and T-SOD were significantly lower than in the control group. In pedal mucus of healthy and diseased abalone, the differential (SDS-PAGE) bands were identified as actin and hemocyanin. Protein identification was accomplished with mass spectrometry. A total of 16 2-DE gel spots were identified; 5 gel spots showed upregulation and 11 gel spots showed downregulation in diseased abalone. Proteins involved in energy production and storage, including fructose-1, 6-bisphosphate aldolase, arginine kinase, and triosephosphate isomerase, showed diverse expression patterns in diseased abalone. For stress-responsive proteins, expression of Cu/Zn-superoxide dismutase showed downregulation. For contraction and regulation proteins of muscle, actin showed significant downregulation.

Statement of relevance: Abalone withering syndrome (WS) is a serious chronic disease. However, there is limited information on the physiological performance of infected abalones. The present study was to assess the alterations of *Haliotis diversicolor* caused by WS using transmission electron microscopy as well as assess the immune enzyme activity of hemolymph and mucus, and muscle, mucus proteins changes by 2-DE and SDS-PAGE.

1. Introduction

Abalone (Haliotis spp.) is a marine gastropod that is distributed worldwide along coastal waters in tropical and temperate areas and is an economically important seafood (Li and Yan, 2010). The small abalone, *Haliotis diversicolor*, is a commercially important species cultured along the coast of southern China (Cai and Wang, 2008). China, with more than 50,000 tons per annum (Di et al., 2013), is the most important abalone-producing country in the world. Since 1999, the yields of cultured abalone in China have been severely affected by continual outbreaks of a fatal epidemic disease caused by abalone withering syndrome. Withering syndrome is a serious chronic disease that affects various abalone species in both natural and farmed populations. It was first observed in the 1980s in the Channel Islands southwest of California, where it caused a 99% decline in the population of the black abalone (*Haliotis cracherodii*). Since then, it has been detected in pink (*Haliotis corrugata*), flat (*Haliotis walallensis*), white (*Haliotis sorenseni*), red (*Haliotis rufescens*), green (*Haliotis fulgens*), Taiwanese (*H. diversicolor supertexta*) abalones, as well as in *Haliotis tuberculata* and *Haliotis discus hannai* (Wetchateng et al., 2010; Crosson et al., 2014).

Withering syndrome is caused by infection with the intracellular rickettsial bacterium *Candidatus Xenohaliotis californiensis* (a *Rickettsia*-like organism) (Rosenblum et al., 2008; Crosson et al., 2014; Friedman et al., 2014). Withering syndrome occurs along the eastern Pacific margin of North America in California, USA, and Baja California, Mexico, and abalones in Chile, China, Taiwan, Iceland, Ireland, Israel, Spain, Thailand and Japan have been also infected (Crosson et al., 2014).

Gross signs of the disease include pedal atrophy, a mottled digestive gland, anorexia, weakness, and lethargy (Gardner et al., 1995; Friedman et al., 2000, 2002; Balseiro et al., 2006). Moribund abalones have less elasticity and melanization of the mantle and muscle with recessive feeding, and most fall from the reef and die (Zhuang et al., 2010; Jiang

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et al., 2012). Withering syndrome is manifested by morphological changes in the digestive gland, which yields a loss of functionality. The distribution, impacts, current diagnostic methods, and new findings about abalone withering syndrome have been reviewed (Crosson et al., 2014). It is important to determine the effects of withering syndrome on the physiological performance of abalone. However, there is no such information available (González et al., 2012).

Functional proteomics have become a powerful tool for the identification of sample proteins differentially responding to microorganisms or special stimuli. Use in marine invertebrates is limited and few proteomic analysis studies have been conducted in *H. diversicolor* with withering syndrome. The epidermal mucus is considered an important component of innate immunity. In addition, acid phosphatase (ACP) and alkaline phosphatase (AKP) are important for innate immune defense in small abalone (Wang et al., 2004). Superoxide dismutase (SOD) is an important antioxidant.

This article examines the effects of withering syndrome on the physiological performance of abalone. Alterations of *H. diversicolor* caused by withering syndrome were studied using transmission electron microscopy (TEM), enzyme activities, and 2-dimensional gel electrophoresis.

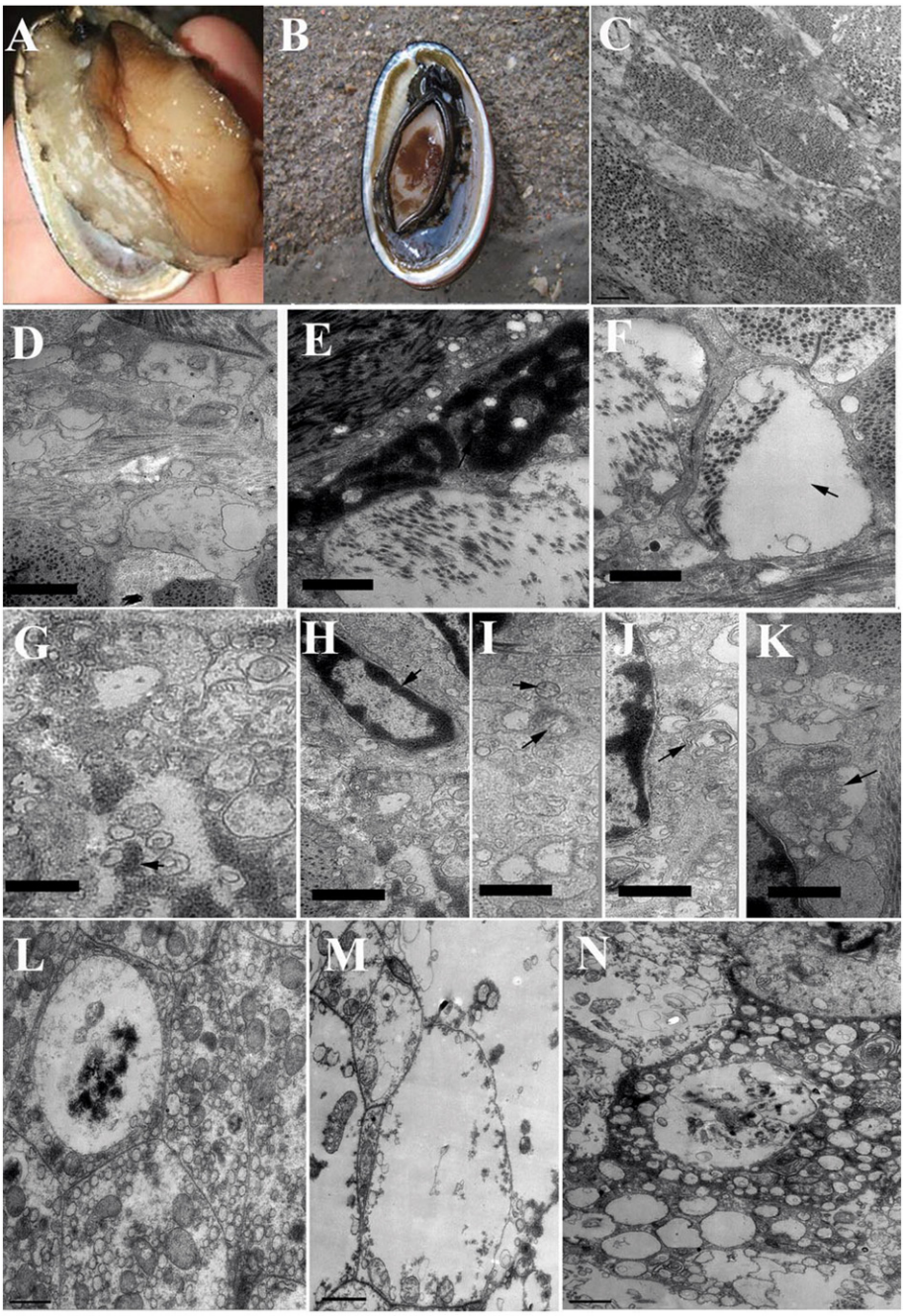

Fig. 1. (A): Healthy small abalone (*Haliotis diversicolor*). (B): Withering syndrome in a small abalone (*H. diversicolor*). (C): Electron microscopy of an abalone with a healthy pedal, the bar = 1 μm. (D–K): Pathological changes in the foot of abalone (*H. diversicolor*) with amyotrophia, the bar = 1 μm. (D): Foot tissue cells were devoid of intracellular organelles and cell components disappeared. (E): Like-inclusions appeared in the nucleus. (F, G): Myofibers appeared hollow, and the number of inner intact myofibrils was obviously reduced (arrow). (H): Karyoplasm gathered at the edge of the nucleus in the foot cells (arrow). (I): Morphology of mitochondria in muscle cells was abnormal and crystal lattice-like inclusion bodies appeared (arrow). (J): The structure of foot cells appeared myelin-like (arrow). (K): Tubular structures proliferated in the foot cells, and the foot cells were full of empty vesicles (arrow). L–N: Pathological changes of the hepatopancreas in abalone (*H. diversicolor*) with amyotrophia, the bar = 1 μm. (L): The hepatopancreas in a healthy abalone. (M): The number of mitochondria greatly decreased. (N): The cells were full of empty vesicles and devoid of any recognizable cellular structures.