Withering Syndrome of the Small Abalone, *Haliotis diversicolor supertexta*, Is Caused by *Vibrio parahaemolyticus* and Associated with Thermal Induction

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The susceptibility of the small abalone *Haliotis diversicolor supertexta* to *Vibrio parahaemolyticus* 880915 strain and its extracellular products (ECP) at different temperatures was investigated. The strain was previously isolated from the haemolymph of the moribund small abalone with withering syndrome during an outbreak of mass mortality among the cultured animals in September 1999 in I-Lan, Taiwan. The bacterium and its ECP were lethal to the small abalone. Onset of the withering syndrome in the moribund or dead animals could be observed at 4–7 d post-bacterial challenge. The same bacterial strain could be isolated from the haemolymph of the moribund animals with or without the syndrome post-bacterial challenge. This syndrome could not be observed in the moribund or dead animals post-ECP challenge. The animals were more susceptible to the bacterium and ECP challenge at higher temperature (28 °C) indicating that the outbreak of the disease in warmer season is associated with thermal induction.

Introduction

Vibriosis is one of the major threats in marine shellfish aquaculture worldwide (Lightner, 1988; Austin and Austin, 1989; Lane and Birkbeck, 2000). A few reports regarding this disease have been published on abalone (Elston and Lockwood, 1983; Dixon *et al.*, 1991; Anguiano-Beltran *et al.*, 1998; Nishimori *et al.*, 1998; Liu *et al.*, 2000, 2001), and the causative agents were identified or suggested as *Vibrio alginolyticus*, *V. cararchiae* and *V. paraheamolyticus*.

Furthermore, a withering syndrome of wild and farmed abalone has been reported in California, USA, and demonstrated to be associated with temperature, food availability, parasites and/or bacteria (Lafferty and Kuris, 1993; Gardner *et al.*, 1995; Altstatt *et al.*, 1996; Friedman *et al.*, 1997; Moore *et al.*, 2000). In a further study, a new Ricketttsiales bacteria, *Candidatus Xenohaliotis californiensis*, has been described as the pathogen causing withering syndrome in abalone, *Haliotis* spp., along the west coast of North America (Friedman *et al.*, 2000).

Recently, outbreaks of mass mortality among the cultured small abalone *Haliotis diversicolor supertexta*, manifesting withering syndrome, have occurred in Taiwan (Liu *et al.*, 2000, 2001). In the present study, we report the reproduction of withering syndrome and the implication of ambient water temperature with the outbreak of the disease in cultured small abalone using a *V. parahaemolyticus* 880915 strain originally isolated from the haemolymph of the diseased animals with the syndrome.

Materials and Methods

*Bacterium and extracellular products (ECP)*

*Vibrio parahaemolyticus* 880915 strain originally isolated from the haemolymph of diseased small abalone (*Haliotis diversicolor supertexta*) with withering syndrome during an outbreak of mass mortality among the cultured animals in I-Lan, Taiwan in September (water temperature 26 °C) 1999, was used in this study. The pure stock cultures were stored in phosphate buffered saline (PBS, pH 7.2) supplemented with 10% glycerol at −70 °C.

Stock cultures of the 880915 strain were grown on tryptic soy agar (TSA; Basingstoke, Oxoid, UK; supplemented with 3% NaCl) for 24 h at
25 °C and two swabs of the bacteria were suspended in 5 ml PBS. The suspension was spread onto TSA (+3% NaCl) overlaid with sterile cellophane and grown for 24 h at 25 °C. The ECP was harvested following a method previously described (Lee and Ellis, 1990). In brief, 10 ml of PBS was added to the surface of the cellophane overlaying TSA (+ 3% NaCl) and spread completely. The harvested bacterial suspension was then centrifuged (25,000 g for 60 min at 4 °C) and the pellet discarded. The supernatant was passed through a 0.22-μm filter (Millipore, Bedford, UK) and the ECP stored in 1-ml aliquots at −70 °C. Total protein was measured by the method of Bradford (1976) with bovine serum albumin as a standard.

Abalone and virulence tests

Small abalone (H. diversicolor supertexta) weighing between 10 and 14 g were held in tanks (2,500 litre) supplied with air-lifted 33 ppt salinity sea water at 25–26 °C, and were acclimated at 18, 23 or 28 °C for 24 h prior to bacterial or ECP challenge. The LD50 tests (Trevors and Lusty, 1985), with batches of five abalone per treatment, were conducted by intramantle injection of 0.1 ml bacterial suspension (107, 106, 105 or 104 colony forming unit g−1 abalone body weight) or ECP (15.0, 7.5, 5.0 or 1.5 μg protein g−1 abalone body weight) into the animals at the right side of mantle (Liu et al., 2000, 2001). Sterile PBS was injected into the controls. Mortality of the animals was recorded daily for 1 week post-injection. Isolation and identification of the bacteria from the haemolymph of moribund abalone with or without withering syndrome injected with bacterial cells and reconfirmed to be the same species. No mortality or withering syndrome was observed in the controls injected with PBS.

The bacteria were reisolated from the haemolymph of the moribund abalone with or without withering syndrome injected with bacterial cells and reconfirmed to be the same species. No mortality or withering syndrome was observed in the controls injected with PBS.

Results

Extracellular products

The ECP of V. parahaemolyticus 880915 strain was harvested after 24 h of incubation of the culture at 25 °C. The total protein of the ECP was 1500 μg protein ml−1.

Virulence tests

The injection of the bacterial cells or the ECP into small abalone was lethal to the animals held at different temperature treatments (see Table 1). Moribund animals could be observed within 7 d post-challenged with bacterial cells or ECP. In addition, occurrence of withering syndrome (Fig. 1) (exhibiting gross signs of shrunken foot muscle, discoloration of the epipodium and retraction of visceral tissues, and reduced activity and inability to tightly adhere to the substratum), as seen in natural outbreak, in the moribund or dead animals could be observed at 4–7 d post-bacterial challenge but was not observed within the first 3 d. This syndrome could not be observed in the moribund or dead animals injected with ECP within 7 d.

The bacteria were reisolated from the haemolymph of the moribund abalone with or without withering syndrome injected with bacterial cells and reconfirmed to be the same species. No mortality or withering syndrome was observed in the controls injected with PBS.

Discussion

Aquaculture for the small abalone (H. diversicolor supertexta) has been practiced for more than two decades in Taiwan (Chen, 1990). However, only recently mass mortality of the animals associ-

Table I. Virulence tests of bacterial cells and extracellular products (ECP) of V. parahaemolyticus 880915 strain injected in a volume of 0.1 ml into small abalone Haliotis diversicolor supertexta weighing 10–14 g, held at 18, 23 or 28 °C.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Sample</th>
<th>LD50 value* (g−1 abalone body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>bacterial cells</td>
<td>3.3 × 10⁵ colony forming units</td>
</tr>
<tr>
<td>18</td>
<td>ECP</td>
<td>9.3 μg protein</td>
</tr>
<tr>
<td>23</td>
<td>bacterial cells</td>
<td>5.7 × 10⁵ colony forming units</td>
</tr>
<tr>
<td>23</td>
<td>ECP</td>
<td>3.5 μg protein</td>
</tr>
<tr>
<td>28</td>
<td>bacterial cells</td>
<td>3.7 × 10⁵ colony forming units</td>
</tr>
<tr>
<td>28</td>
<td>ECP</td>
<td>2.5 μg protein</td>
</tr>
</tbody>
</table>

*The virulence tests, with batches of five abalone per treatment, were conducted by intramantle injection into the animals and observed for one week; dead animals were all observed within 7 d of challenge.
Fig. 1. (A) Normal small abalone Haliotis diversicolor supertexta (B) Withering syndrome reproduced in the moribund or dead small abalone observed at 4–7 d post-challenged with V. parahaemolyticus 880915 strain. The gross signs of the syndrome were shrunken foot muscle, discoloration of epipodium and retraction of visceral tissues.

Acknowledgements

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Lane E. and Birkbeck T. H. (2000), Species specificity of some bacterial pathogens of bivalve molluscs is correlated with their interaction with bivalve haemocytes. J. Fish Dis. **23**, 275–279.


