Ultrastructural description of new Rickettsia-like organisms in the commercial abalone *Haliotis tuberculata* (Gastropoda: Haliotidae) from the NW of Spain

Carlos Azevedo¹,²,*, Ramón F. Conchas³, Jessica Tajdari², Jaime Montes⁴

¹Department of Cell Biology, Institute of Biomedical Sciences, University of Porto (ICBAS/UP), Lg. A. Salazar no. 2, 4099-003 Porto, Portugal
²Centre for Marine and Environmental Research, University of Porto (CIIMAR/UP), Rua dos Bragas no. 177, 4050-123 Porto, Portugal
³Instituto Tecnolóxico para o Control do Medio Maríno de Galicia (INTECMAR), Consellería de Pesca e Asuntos Marítimos, Peirao de Vilaxoán, 36611 Vilagarcia de Arousa, Spain
⁴Centro de Investigaciones Marinas (CIMA), Consellería de Pesca e Asuntos Marítimos, Pedras de Corón, 36620 Villanova de Arousa, Pontevedra, Spain

ABSTRACT: Rickettsia-like organisms (RLOs) were found in the commercially farmed abalone *Haliotis tuberculata* in the northwestern region of the Atlantic Coast of Spain and are described from light and transmission electron microscopy observations. The RLOs measured ~1.6 × 0.9 µm and were found in intracytoplasmic, spherical to ellipsoidal vacuoles (up to 8 µm) in the epithelial cells of the digestive diverticulae. The morphological ultrastructure of these organisms was typically prokaryotic, including a plasmalemma and a thin Gram-negative type cell wall. Several ultrastructural changes were observed in the epithelial cells of the host containing the RLOs. The nuclei became pycnotic and several basophilic dense inclusions appeared in the cytoplasm. In addition, the host cell appeared lysed and was ruptured in advanced stages of infection. It was impossible to ascertain whether the RLOs are responsible for this disease, as a haplosporidian infection was also present. We can only conclude that the presence of RLOs simultaneously with a haplosporidian parasite may contribute to the mortality of the abalone host.

KEY WORDS: Ultrastructure · Rickettsia-like organisms · Abalone · Spain

INTRODUCTION


*Email: azevedoc@icbas.up.pt © Inter-Research 2006 · www.int-res.com
RESULTS

Of the 10 specimens of the abalone *Haliotis tuberculata* parasitized by a haplosporidian identified as *Haplosporidium montforti* (Azevedo et al. 2006), only 6 revealed a simultaneous infection by rickettsia-like organisms (RLOs) (Fig. 1). In addition, of the 5 specimens not parasitized by the haplosporidian, 3 contained RLOs. In both cases, RLOs occurred within large intracytoplasmic vacuoles (up to 8 µm in diameter) in the epithelial cells of the digestive diverticulae, where they were responsible for the formation of spherical to ellipsoidal microcolonies (Figs. 1 to 4). By observation of ultrathin transverse sections made at the level of the largest vacuoles, it was possible to count ~75 RLOs per section (Figs. 3 & 4). The total volume of the largest vacuoles was occupied by ~280 juxtaposed RLO cells.

The RLOs were polygonal in shape, and packed into vacuoles with no intercellular spaces. The chromatin was organized into several compacted and irregular patches (Figs. 3 & 4). Infected cells were hypertrophied and the cytoplasm was largely replaced by the developing RLO microcolony (Figs. 2 to 4). In favourable longitudinal sections it was possible to observe that the RLO vacuoles occupied a basal position beneath the nuclei of the epithelial cells (Fig. 4). The histopathological changes caused by the presence of RLOs in the epithelial cells of the digestive diverticulae included an increase in the volume of the vacuole and substantial ultrastructural alterations of the nucleus that became pycnotic. Additionally, several vacuoles and dense bodies appeared in the cytoplasm of the host cells (Figs. 3 & 4). The most evident alterations were observed at the apical periphery of the host cells where the luminar microvilli disappeared (Fig. 4). Sometimes disrupted vacuoles and free RLOs were observed among host tissues where the cell appeared lysed and the epithelial tissues of the host were disrupted (Fig. 1).
DISCUSSION

The ultrastructural morphology of the prokaryotic microorganisms reported here is similar to that of other RLOs previously described in several species of aquatic fauna (Lauckner 1983, Sparks 1985, Gardner et al. 1995). RLOs differ from bacteria because they lack a true bacterial wall (Elston 1986, Azevedo & Villalba 1991, Bower 2004). These prokaryotes have been reported from marine animals (Lauckner 1983), mainly crustaceans (Bonami & Pappalardo 1980, Fryer & Lannan 1994, Nunan et al. 2003) and bivalves (Buchanan 1978, Elston 1986, Azevedo & Villalba 1991, Gardner et al. 1995, Hine & Diggles 2002, Hine et al. 2002, Azevedo et al. 2005). Previous studies on bivalves infected with rickettsia or RLOs have demonstrated that these prokaryotes can cause important losses in commercially farmed bivalves (Gardner et al. 1995, Friedman et al. 2000, Hine et al. 2002, Bower 2004).

The ultrastructural organization of the compacted chromatin of the RLOs studied in the present work differs from all the other rickettsia and RLOs previously described in molluscs (Elston 1986, Comps et al. 1987, Azevedo et al. 2005). The RLOs described in the present paper were found coincidentally in tissues of cultured Haliotis tuberculata which had experienced high mortality possibly due to the presence of a haplosporidian infection identified as Haplosporidium montforti (Azevedo et al. 2006). During an ultrastructural study of a recently identified haplosporidian found among cultured abalone Haliotis iris, rickettsiae were also encountered and associated with the mortality of the host (Hine et al. 2002). In addition, epizootic mortalities associated with amyotrophia have been observed in hatcheries of the Japanese black abalone Norodosiscus discus discus (Otsu & Sasaki 1997, Nakatsugawa et al. 2000). The appearance of RLOs in the same hosts where a high mortality was associated with the infection of H. montforti may indicate that RLOs contribute to mortality of the abalone. Moribund and dead abalones contained these 2 types of infections simultaneously. The mortality that occurred in the abalone H. tuberculata could not be attributed to the RLO infection alone, because of the presence of another pathogen (H. montforti) (Azevedo et al. 2006) in the same samples. In spite of the fact that the abalones showed symptoms corresponding to those caused by ‘withering syndrome’, it was not possible to establish a definitive connection between the presence of the RLOs and the disease, because of the haplosporidian co-infection.

Acknowledgements. We thank Mr. J. Carvalheiro for the microscopic work (ICBAS/UP). This work was partially supported by the Eng. Antonio de Almeida Foundation (Porto, Portugal), Antonio DB, Andree KB, Moore JD, Friedman CS, Hedrick RP (2000) Detection of Rickettsia-like prokaryotes (RLPs) by in situ hybridization in black abalone Haliotis cracherodii with Withering Syndrome. J Invertebr Pathol 75:180–182


Hine PM, Wakefield S, Diggles VL, Maas EW (2002) Ultrastructure of a haplosporidian containing rickettsiae, asso-

Submitted: December 17, 2005; Accepted: May 14, 2006