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Infection of an intrahemocytic paramyxean parasite from tunicate *Halocynthia roretzi* in Korea

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Introduction

Paramyxians, a group of protistan parasites that infect marine invertebrates and cause significant diseases in cultured marine molluscs, are characterized by the formation of spores. These spores consist of several cells, enclosed inside one another, which result from internal cleavage of a stem cell (Desportes and Perkins 1990). In the phylum Paramyxea, species of the genus *Marteilia* and *Marteilioides* have been extensively studied due to their detrimental effect on commercially exploited bivalves. *Halocynthia roretzi* is an important aquaculture species in Korea and Japan. It is an edible tunicate as well as a source of collagen products. The mass mortalities of *H. roretzi* have occurred over the last two decades along the southern and eastern coasts of Korea and those have caused serious economic affects. Mortalities of more than 50,000 metric tones of cultured *H. roretzi* was noted from Korea in 2003, which provoked this epidemiological study. We describe histological and ultrastructural characteristics of a paramyxean intrahemocytic parasite that is associated with the mass mortality of *H. roretzi*.

Materials and Methods

Tunicates (n=30) *Halocynthia roretzi* 1 to 2 yr old were collected from private aquaculture farms located in Goje, Korea in June 2004. *H. roretzi* tissues were fixed in Davidson's solution, processed for paraffin-embedding and cut into 4 μm thick sections. Sections were stained with Harris's hematoxylin and eosin for light microscopy examination. *H. roretzi* tissues were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.2 for

1 h at 4°C. After 2 washes in cacodylate buffer, the tissues were post-fixed in osmium tetroxide in the same buffer at 4°C. Samples were dehydrated through graded alcohols, rinsed twice for 15 min in propylene oxide, and embedded in Epon resin compound. Semi-thin sections (200 nm) were stained with toluidine blue, and ultra-thin sections (60 nm) were stained with uranyl acetate and lead citrate. Ultra-thin sections were carefully examined with a Hitachi 7100 transmission electron microscope at 80kV.

Results and discussions

Infected tunicates characteristically possessed a thin tunic that lost elasticity with disease progression. Moribund tunicates had a bad odor, became flaccid and finally died with rupturing of the tunic. Histological observations of infected tunicates, in the connective tissues surrounding the intestines and digestive diverticulae as well as mantle and gill tissues of all tunicates showed severe hemocyte infiltrations. Within the hemocytic infiltrate, enlarged hemocytes were observed that contained several vacuoles in the cytoplasm. Electron microscopy showed infection within enlarged hemocytes scattered in the connective tissues of the intestine and digestive diverticula. The earliest stage was a primary cell of the parasite which was found in the cytoplasm of the hemocyte. The secondary cells developed from the primary cell by internal cleavage of the stem cell. During sporulation, a high density of cytoplasmic ribosomes was found within the cytoplasm of infected hemocytes. The host nucleus was located peripherally after maturation of the secondary cells. The mature spore was round. The intracellular parasite was bounded by a plasma membrane. No internal structure was observed. Light and electron microscopy have several limitations in the determinations of taxonomic boundaries of these parasites. Sequencing genes of taxonomic value may help to resolve the taxonomic position of IPP. In addition, the use of DNA sequences, DNA probes and techniques such as in situ hybridization in identifying hemocyte type will prove invaluable in future studies of this parasite.

References

Desportes I., Perkins F. O. (1990) Phylum Paramyxea. Margulis L, Corliss JO, Melkonian M, Chapman DJ (ed), Handbook of Prototista. Jones & Bartlett, Boston. P30-35.