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Virus-like particles in *Perkinsus atlanticus* (Apicomplexa, Perkinsidae)

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ABSTRACT: Virus-like particles (VLP) were observed in different phases of the in vitro zoosporulation of *Perkinsus atlanticus* (Apicomplexa, Perkinsidae) which parasitized the gill tissues of *Ruditapes decussatus* (Mollusca, Bivalvia). These VLP were represented by numerous electron-dense bodies (ca 125 nm diameter) not previously described in similar culture processes. The VLP were densely packed in a membrane-lined inclusion of isolated host-cell cytoplasm of parasitic origin. They were also found among prezoosporangia and zoosporangia of *P. atlanticus*, a species that causes recurrent mortalities in the clam *R. decussatus*. The ultrastructural organization of these VLP seems to represent a new and unclassified virus of *P. atlanticus*.

INTRODUCTION

The occurrence of viruses and virus-like particles (VLP) among eucaryotic algae, lower fungi and protozoan species was reported by Lemke (1976), who recorded an extensive list of eucaryotic algae in which viruses and VLP have been observed. In addition several reviews on algal viruses have been written (Sherman & Brown 1978, Dodds 1979, Lauckner 1983).

The isolation and characterization of some viruses has also been described from several types of hosts (Dodds & Cole 1980, Stanker et al. 1981, Van Etten et al. 1981, 1985, Martin & Benson 1982, Schuster et al. 1986a, b).

This paper describes the presence of VLP infecting cultures of the apicomplexan protozoan *Perkinsus atlanticus* (Levine 1978), a new recently described species (Azevedo 1989).

MATERIALS AND METHODS

Several trophozoites of *Perkinsus atlanticus* (Azevedo 1989) isolated from gill tissues of *Ruditapes decussatus*, a clam of great commercial importance in Portugal, were prepared for zoosporulation in fluid thioglycollate medium according to a previously used technique (Chu & Greene 1989), as modified by Azevedo (1989). After centrifugation, the pellet was fixed in 3 % glutaraldehyde in 0.1M cacodylate buffer, at pH 7.8 for 2 h at 4 °C, washed for 2 h at 4 °C and post-fixed in 2 % osmium tetroxide in the same buffer and conditions (Azevedo 1989). The material was dehydrated through a graded series of ethanol solutions and embedded in Epon. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined in a JEOL 100CXII TEM at 60kV.

RESULTS

Among different developmental stages of *Perkinsus atlanticus* zoosporulation, some trophozoites were seen to be infected by numerous membrane-bound electron-dense bodies. These bodies resembled VLP tightly packed in a membrane-lined inclusion in the trophozoite cytoplasm (Figs. 1 to 4). The VLP were regularly distributed in paracrystalline parallel arrays (Figs. 2 and 3) among the different cytoplasmic structures; these included mitochondria, lipid droplets, cisternae of reticulum, free ribosomes, and vacuoles of the trophozoites (Figs. 3 and 4). They had a density of 64 VLP μm^{-2} (Figs. 2 and 3). The periphery of the infected trophozoites showed a dense material that seemed to reinforce the plasmalemma (Figs. 3 and 4).

At high magnification, the VLP appeared different according to the plane of section. They were hexagonal and ca 125 nm in diameter. The internal matrix was

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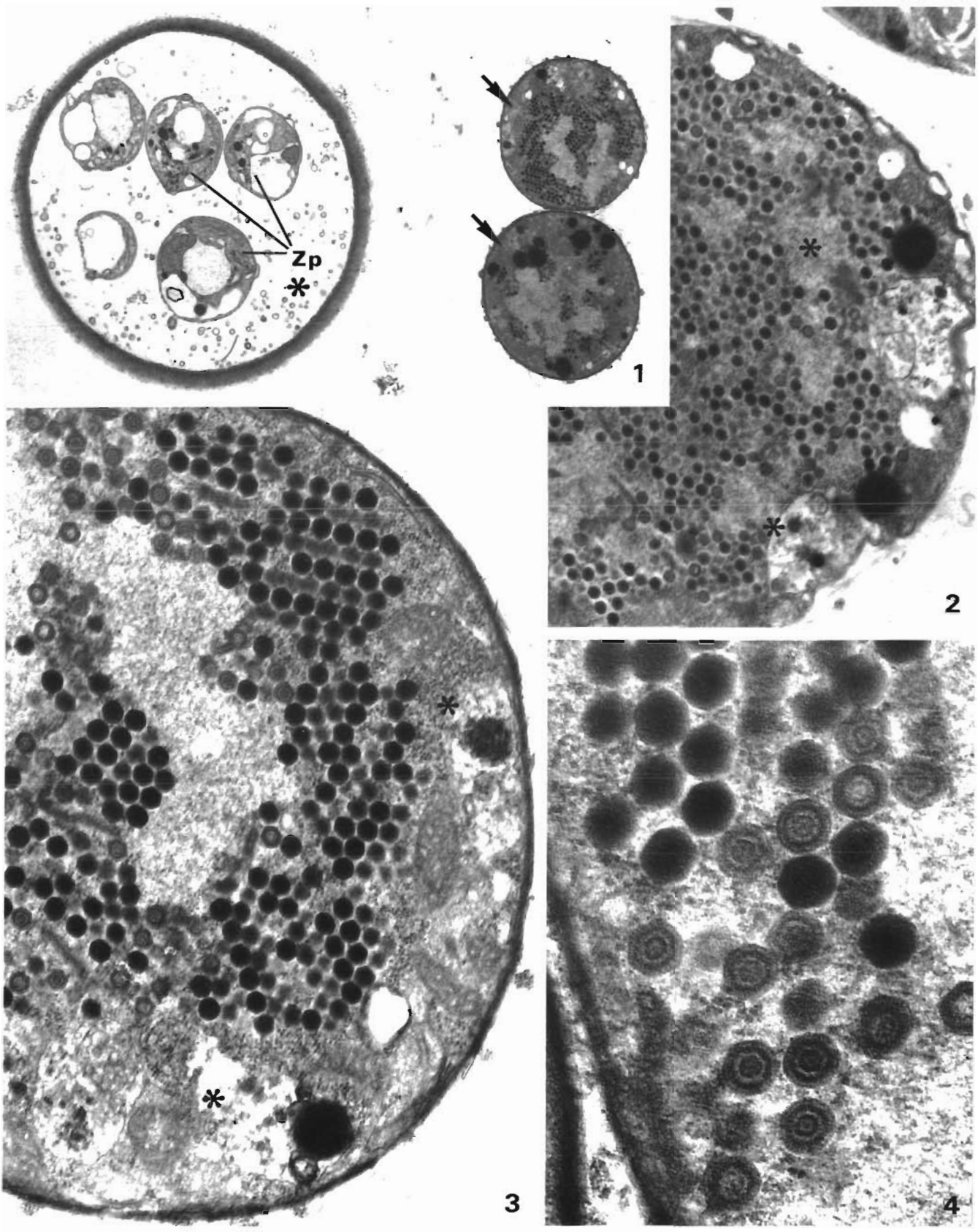

Figs. 1 to 4 *Perkinsus atlanticus* Virus-like particles (VLP) in the parasite *P. atlanticus* which infects the gill tissue of *Ruditapes decussatus* Fig. 1. Right: two free host trophozoites (arrows) containing several VLP; left: an isolated zoosporangium (*) of *P. atlanticus* showing some immature zoospores (Zp), × 5800 Fig. 2. Two free trophozoites from Fig. 1 showing the VLP regularly distributed in the cytoplasm (*). Some signs of lysis are also visible: × 19000. Fig. 3. Host cytoplasm (*) with lytic aspect containing numerous VLP; × 32000. Fig. 4. High magnification of a group of VLP sectioned at different levels; × 80000

electron-dense and its internal organization was hardly visible (Fig. 4). In favorable sections it was possible to observe that the VLP contained a central spherical core measuring ca 50 nm, surrounded by a circular and concentric layer situated between the central core and the external capsid (Fig. 4). Host cells containing VLP appeared degenerate, mainly at the periphery just beneath the plasmalemma. Some dense bodies, possibly lipid droplets, were scattered throughout the cytoplasm (Figs. 1 to 3). The nuclear membranes appeared to be ruptured or fragmented when the VLP were seen in the cytoplasm (Fig. 3).

DISCUSSION

The occurrence of VLP in the earliest phases of the in vitro zoosporulation of *Perkinsus atlanticus* is rare and an incidental finding noted during ultrastructural studies. The origin of these VLP is not clear, but they appear to be derived from host cells associated with high water-temperature during zoosporulation (Chu & Greene 1989).

No evidence of the life cycle of these VLP was observed, but our specimens exhibit a similar hexagonal outline to previously published electron micrographs of VLP in different marine animal groups (Rungger et al. 1971, Farley et al. 1972, Comps & Duthoit 1976, Buchanan & Richards 1982). Some indication of a reduction in the final number of zoospores was found in the in vitro zoosporulation process when some trophozoites contained VLP. The presence of VLP caused disintegration of host cells and may also have interfered with zoospore multiplication when released into culture medium.

Similar polygonal VLP have been observed in eucaryotic algae (Lemke 1976, Dodds 1979, Dodds & Cole 1980, Van Etten et al. 1981). However, the lack of the characterization of these VLP does not permit their classification.

This is the first record of the presence of VLP in the in vitro culture of zoosporulation in this apicomplexan protozoan. In future, attempts should be made to isolate, culture and further study the pathogenicity and transmissibility of these VLP and to determine their relationship if any with clam mortalities.

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