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Is there a plastid in *Perkinsus atlanticus* (Phylum Perkinsozoa)?

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Abstract

Perkinsus atlanticus is a pathogenic protist that infects the clam *Ruditapes decussatus*. The recent proposal for the inclusion of the genus *Perkinsus* in a new phylum, Perkinsozoa, in the infra-kingdom Alveolata, gave rise to controversies whether this genus should form a phylum on its own. Molecular analysis of some conserved nuclear genes shows a closer proximity of the genus *Perkinsus* to the dinoflagellates than to the apicomplexans. Studies on extranuclear genomes, however, could also be very helpful for a more precise definition of those phyla. In Perkinsozoa, there have been until now no reports about the isolation of mitochondria as well as no conclusive results about the presence of any plastids, therefore a comparison with the data already obtained in Apicomplexa and Dinoflagellata has not yet been possible.

In this work, we identify a plastid in *Perkinsus atlanticus*, using ultrastructural techniques and inhibition growth tests. It will be important to analyze the plastid genome at a molecular level, in order to confirm if the plastid in *Perkinsus* is more similar to those of Dinoflagellata or Apicomplexa. Such information will doubtless contribute to a more precise determination of the phylogenetic position of the genus *Perkinsus*.

Keywords: *Perkinsus atlanticus*; Perkinsozoa; Alveolata; Plastids

Introduction

Protistan parasites of the genus *Perkinsus* are pathogenic microorganisms responsible for great mortality in populations of different species of bivalve molluscs of economic importance. *Perkinsus atlanticus* was first identified in Europe in carpet shell clams (*Ruditapes decussatus*) on the Atlantic south coast of Portugal and described as a new species (Azevedo 1989).

There is a recent proposal for the inclusion of the genus *Perkinsus* in a new phylum, Perkinsozoa, related to both Apicomplexa and Dinoflagellata in the infra-kingdom Alveolata (Nóren et al. 1999). However, there is no consensus about the existence of this new phylum

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(Cavalier-Smith and Chao 2004), consequently its taxonomic characterization remains unresolved. The principal evidence for the relationship between these three phyla comes from molecular analyses of nuclear encoded genes, pointing to a closer relationship of Perkinsozoa to Dinoflagellata (Reece et al. 1997; Saldarriaga et al. 2003). Studies on extranuclear genomes, however, could also be very helpful for a more precise definition of those phyla. In Apicomplexa, it is already well established that they possess one circular 35 Kb plastid genome, in a membrane-enclosed organelle named the apicoplast (Köhler et al. 1997). In Dinoflagellata, the plastids are heterogeneous and have been found to possess a genome composed of 15 mini-circles, each one carrying only one gene (Zhang et al. 1999). Although they possess different characteristics, the plastids in Apicomplexa and Dinoflagellata seem to share a common origin (Fast et al. 2001). In Perkinsozoa, there have been until now no conclusive results about the presence of any plastids.

In this work we identify a plastid-like organelle in *Perkinsus atlanticus*, using electron microscopy and assays of growth inhibition by thiostrepton, a thiazole-containing peptide antibiotic that inhibits protein synthesis and ribosomal GTPase activity by binding to a conserved region in the LSU rRNA of eubacteria and plastids, but not to the corresponding region in nuclear and mitochondrial LSU rRNAs (Rogers et al. 1997).

Materials and methods

Cell cultures

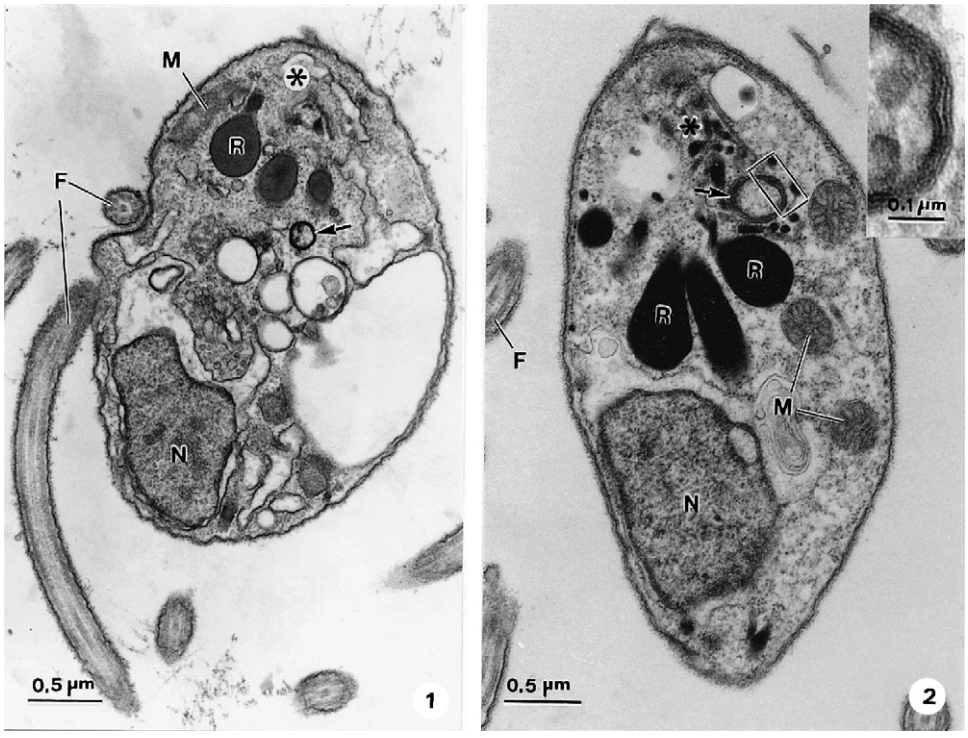
The cultures of *Perkinsus atlanticus* Azevedo, 1989, used in this study were kindly supplied by Prof. Leonor Cancela, University of Algarve, Portugal. They were sub-cultured in our laboratory in DME/Ham's medium (Gauthier and Vasta 1993) with antibiotics at 28 °C.

Electron microscopy

The parasite and the enriched plastid fraction were fixed in 3% glutaraldehyde buffered in 0.2 M sodium cacodylate (pH 7.2) at 4 °C for 10 h, washed overnight in the same buffer at 4 °C, and post-fixed in 2% osmium tetroxide with the same buffer and temperature for 3 h. After dehydration in an ascending ethanol series followed by propylene oxide, the parasites were embedded in Epon. Ultrathin sections were double contrasted with uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM, operated at 60 kV.

Growth inhibition assay by thiostrepton

The assay was carried out as described earlier (McConkey et al. 1997), with the following modifications:



Figs. 1–2. Two ultrastructural sections of a *Perkinsus atlanticus* zoospore, showing the nucleus (N), mitochondrion (M), the plastid (arrow) and the apical end (*) containing such apical complex elements as rhoptries (R). Near the zoospore some transverse sections of the flagella (F). Fig. 2, Insert: a detail of the plastid membranes.