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Lauren Dulik
Cleveland State University

Raghavendra Yadavalli
Cleveland State University

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Morphological study and biochemical characterization of the Alveolate flagellate Colpodella sp. (Apicomplexa) in a diprotist culture with Bodo caudatus

College of Sciences and Health Professions

Student Researchers: Lauren Dulik and Raghavendra Yadavalli

Faculty Advisor: Tobili Y. Sam-Yellowe

Abstract

Plasmodium falciparum, the causative agent of severe human malaria shares the presence of apical complex organelles with the free-living predatory alveolate, Colpodella sp. In this study we investigated morphological, biochemical and molecular characteristics of Colpodella sp. in a diprotist culture containing Bodo caudatus as prey. Colpodella attaches to its prey using the apical end. Attachment lasted for approximately 20 minutes while the cytoplasmic contents of the prey were aspirated into the posterior food vacuole of Colpodella in a process known as myzocytosis. Indirect immunofluorescence assay (IFA) using P. falciparum rhoptry specific antibodies showed intense reactivity with cytoplasmic vesicles of Colpodella but not Bodo caudatus. DNA isolated from a pellet of the diprotist culture was used in polymerase chain reaction (PCR) with oligonucleotide primers designed to target the P. falciparum (strains 3D7, DD2, FC27 and FCR8) rhoptry genes Rhop-3, Rhop-1 and RAMA. An approximately 2,906 bp single fragment was amplified from P. falciparum (strains 3D7 and FCR8) and diprotist DNA using RAMA primers. Similarly, DNA fragments of a similar size were amplified from the same DNA templates using primers targeting a highly conserved fragment of the18S rRNA used to identify a colpodellid associated with a human infection. Primers targeting conserved regions of the 18S rRNA of kinetoplastid species amplified a DNA fragment of 650 bp in P. falciparum (strains 3D7, DD2, FC27 and FCR8) and diprotist DNA. In addition, the kinetoplastid primers amplified a second fragment of approximately 2 kb from the diprotist DNA. Primers targeting the P. falciparum Rhop-1 gene amplified a 690 bp DNA fragment in all four P. falciparum strains but amplified a fragment of approximately 2 kb from diprotist DNA. Primers for the P. falciparum Rhop-3 gene target amplified a 660 bp DNA fragment in all four P. falciparum strains while DNA fragments of 2 kb, 800 bp and 500 bp were amplified from the diprotist DNA template. DNA sequence analysis of PCR amplified diprotist DNA identified the Rhop-3 gene demonstrating conservation of the Rhop-3 gene in Colpodella sp.