Molecular and Structural Characterization of the *Leishmania* RNA Virus LRV1-4

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INTRODUCTION. The *Totiviridae Leishmania* RNA virus 1-4 (LRV1-4) has an icosahedral capsid structure composed of a major capsid protein and an RNA polymerase. Recent data indicate the involvement of LRV1-4 in the *Leishmania* pathogenesis in the mammalian host. In this study, we intend to obtain detailed Negative Stain Transmission Electron Microscopy and Cryo-Electron Microscopy images of the capsid for LRV1-4 for structural analysis. **MATERIAL AND METHODS.** For the *in vivo* purification of the virus, we grew a culture of the *Leishmania guyanensis* MHOM/BR/75/M4147 strain. The lysed sample was centrifuged at low speed and then applied to a Nycodenz® gradient differential ultracentrifugation at 350 000 g. Negative stained samples were prepared wtih uranyl acetate. Additionally, LRV1-4 RNA polymerase ORF was cloned in pET28a(+) (Novagen) and transformed in *E. coli* BL21 DE3 pLysS (Novagen) and the recombinant protein was purified by affinity chromatography. LRV1-4 capsid protein ORF was cloned in pLEXSY (Jena Bioscience) and transfected in *Leishmania talentorae* for the heterologous expression of the protein, which was confirmed by SDS-PAGE. **RESULTS AND DISCUSSION.** Micrographs of the native sample were acquired in a Transmition Electron Microscope JEOL 2100 LaB_6 (LNNano, Campinas-SP) and we observed the expression of the capsid protein in *L. tarentolae* cell lisates. Heterologous expression of the capsid and RNA polymerase proteins in *E. coli* is of low efficiency. **CONCLUSIONS.** The LRV1-4 proteins are not expressed with high efficiency in *E. coli* cells, even when the codon content of the ORF is optimized for the prokaryote expression system. Due to such difficulties we employed the *L. tarentolae* expression system and obtained clones expressing the LRV1-4 protein. This result allow for the structural investigation of the LRV1-4 capsid structure and further determination of the virus structural organization.

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