Exploring Surface Membrane Proteinases in *Leishmania (Viannia) braziliensis* Thor Strain and Its Subpopulations

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Abstract: *Leishmania (Viannia) braziliensis* is a protozoan parasite that causes American cutaneous leishmaniasis (ACL) and is most prevalent in Brazil and Latin America. The *L. (V.) braziliensis* Thor strain consists of multiple subpopulations with distinct biological features, including varying virulence profiles in vitro and in vivo within murine models. Given that the parasite's surface proteins are the first to interact with the host, this study focuses on comparing the surface membrane proteinase activity of promastigotes and axenic amastigotes of the Thor strain, specifically the Thor03, Thor10, and Thor22 subpopulations, to understand their differential virulence and adaptation strategies. Phospholipase C (PLC) was used in this study as a selective tool for obtaining surface proteases from these parasites. The isolated protease fractions were further characterized by gelatin-SDS-PAGE and with fluorogenic substrates in the presence of specific inhibitors. PLC treatment revealed metalloproteinases, cysteine proteinases, and serine proteinases activities in the protein fractions. The types and amounts of proteinases were very different between the promastigotes and axenic amastigotes of the Thor strain and its subgroups. Proteinase activity assays in solution revealed that metalloproteinases were the most active type, followed by cysteine and serine proteinases on the surfaces of both promastigotes and axenic amastigotes. These findings highlight the biological significance and critical role of surface proteases in the *L. (V.) braziliensis* phenotype, emphasizing the potential of the *Thor* strain and its subpopulations to adapt to different hosts and providing key insights into the parasite's adaptation strategies.

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