

## Abstract

Amastigotes are forms of *Leishmania*, naturally occurring in vertebrate hosts within phagocytic cells – especially the macrophages. The aim of this project was to compare three types of amastigotes of *Leishmania* that can be used for experiments under laboratory conditions – namely the axenic amastigotes, cultured extracellularly (without vertebrate phagocytic cells), amastigotes isolated from macrophages infected *ex vivo*, and "true" amastigotes isolated from lesions of the infected BALB/c mice.

Amastigotes were compared with respect to the development in the natural vector and at the proteome level. *L. mexicana*, the causative agent of cutaneous leishmaniasis in the New World, was chosen for this comparison. In experiments comparing the development of *Leishmania* in the natural vector *Lu. longipalpis* we found significantly weaker infections in the sand flies infected with axenic amastigotes compared to other types of amastigotes. In addition to the intensity of infection, we compared the localization of promastigotes in the digestive tract of the phlebotomine sand flies. The following localizations were observed: the abdomen, the thorax, the cardia and the stomodeal valve, which is crucial for infectivity of the sand fly. There was no significant difference in localization in any of the groups of amastigotes.

We used quantitative proteomic analysis of LC/MS with quantification by TMT<sup>10</sup>-plex isobaric labeling and collection of quantification spectra in MS<sup>3</sup> mode to compare proteomes of individual types of amastigotes. The result of proteomic analysis was a selected set of significantly different proteins with importance for virulence of amastigotes. Furthermore, significantly differing metabolic enzymes were selected, which allowed us to compare differences in metabolism of individual types of amastigotes. The amastigotes from lesion showed upregulation of some virulence factors and metabolic enzymes. In axenic amastigotes, there was a higher expression of some of the glycolytic enzymes compared to the other types of amastigotes. Amastigotes isolated from macrophages were the middle link in most cases.

**Key words:** amastigote, *L. mexicana*, *Lu. longipalpis*, proteome, TMT<sup>10</sup>-plex