Metabolic changes of pathogenic and nonpathogenic Leishmania species during host cell infection by integration of mathematical models, quantitative proteomics and untargeted 1H-NMR

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The current treatment of visceral and cutaneous leishmaniasis is based on a very limited number of drugs with variable efficiency and many side effects. Thus, the discovery of new drugs and parasite targets is considered a priority strategy to disease control. During infection of mammalian macrophages including humans, Leishmania species changes morphology and biochemical profile in a process named amastigogenesis and blocking key enzyme of this process might interrupt the infection. The mainly goal of this work is the identification of important enzymes to metabolic network changes during in vitro amastigogenesis by comparative analysis of two visceral Leishmania species (*L. donovani* and *L. infantum*), one cutaneous disease (*L. major*) and one non-pathogenic specie (*L. tarentolae*). The fist step was the reconstruction and simulation of metabolic model based on the proteins encode in the genome of four Leishmania species. Since the disease phenotype depends of differential genome content and differential expression, we performed time-course quantitative proteomic analysis for all species during axenic amastigogenesis. The proteomic data were integrated to mathematical metabolic models to predict metabolite concentration changes during the process. The congruence of model was evaluated by comparison of predict metabolite concentration changes with experimental values obtained by untargeted 1H-NMR metabolomics with Pearson correlation coefficient between 0.66 and 0.79 (p-value < 0.0001). Interestingly, visceral parasites have the highest metabolic changes followed by cutaneous disease organism. Specifically, pathway associated to protection to oxidative macrophage response and nutritional requirement of intracellular parasite stage were enriched in infective species compared to apathogenic *L. tarentolae*. We identified the proteins homoserine kinase and trypanothione synthase as key enzymes responsible to control metabolite concentration in important pathways associated with *Leishmania* infection. The importance of these proteins have been validated using mutant parasites super-expressing each enzyme and we have been identified small molecules able to block their functions. Acknowledgements: CAPES, CNPq and FAPEMIG.