Proteomic characterisation of *Schistosoma mansoni* secreted molecules for identification of novel diagnostic targets for schistosomiasis

Leandro Xavier Neves[1], Gustavo Gonçalves Silva[1], R. Alan Wilson[2], William Castro-Borges[3]

[1] Programa de Pós-Graduação em Biotecnologia, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brazil

[2] Centre for Immunology and Infection, Department of Biology, University of York, Heslington, York, United Kingdom

[3] Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto, Campus Morro do Cruzeiro, Ouro Preto, Minas Gerais, Brazil

Schistosomiasis occupies the 4th position on the list of DALYs for tropical neglected diseases and affects over 240 million people in underdeveloped countries worldwide. Despite the efforts to control the disease, public health approaches rely on low sensitive diagnostic tests combined with chemotherapy often employed when the major organ disabilities have become irremediable. Therefore, the identification of molecular markers and development of sensitive methods for early diagnosis are imperative. Molecules located at the host-parasite interfaces such as tegument and gut secreted proteins are constantly released on the host's bloodstream representing targets of particular interest. In this context, the objective of this work is to identify molecules released from living *Schistosoma mansoni* worms in culture using sensitive shotgun proteomics. To achieve that, 28-days old schistosomes recovered from experimentally infected Balb/C mice were incubated in 10mM HEPES buffered RPMI-1640 medium supplemented with L-Glutamine and 1X penicillin/streptomycin without FBS. After 72 hours at 37°C/5% CO_2 the culture supernatant was recovered from the wells of incubation plates in which no damaged or dead parasites were visualised. Spin concentrated culture supernatant (CS) and a PBS-soluble extract of the worm's body (SWAP) were subject to 1D-SDS PAGE and protein trypsinolysis prior to LC-MS analysis. High-resolution and sensitive shotgun analysis was performed on an Ultimate 3000 UHPLC in line with a Q Exactive mass spectrometer (ThermoScientific) operating in high-resolution and sensitive Top12 Data-Dependent Acquisition. Spectral data was interrogated using PEAKS DB (PEAKS Studio, v8. Bioinformatics Solutions Inc.) and label-free quantitation performed at MS1 level by PEAKS Q. The gel electrophoresis revealed a distinct pattern of bands for CS preparation in comparison to SWAP, supporting the enrichment of a distinct sets of proteins secreted by living worms. The LC-MS analysis resulted on the identification of a total of 1674 proteins at 1% False Discovery Rate so that 280 are common to both samples and 79 exclusive to the CS preparation. The later subset is comprised mainly by gut secreted proteins some already found in schistosome vomitus preparations such as saposins, peptidases and cathepsins. Additionally, our analysis detected two MEG families known to be secreted by the oesophageal gland and 14 *S. mansoni* specific entries. Together, the above mentioned proteins were responsible for ~80% of ions signal exclusive to the CS preparation. Tegument signatures were also detectable in both samples indicating that these surface-located proteins are also potential markers for *S. mansoni* infection. Upcoming experiments are intended to refine the list of secreted molecules in order to achieve a number of potential molecular markers of the parasite infection. At last, MS-based diagnostic tests will be performed using plasma of experimentally infected mice. Financial Support: Fapemig (APQ-00829-15) / UFOP (23109.003209/2016-98).