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Manual microdissection of schistosomes for proteomic and transcriptomic characterisation of the worm alimentary tract

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We use this protocol and it's working

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Abstract

Schistosomes are intravenous parasites with ability to survive in the mammalian host for decades, using its blood as source of nutrients. The feeding process is multistep and takes place along the worm's alimentary tract, which comprises an (i) oral cavity opened to a short (ii) oesophagus that is connected to the (iii) gut caecum. The ultrastructure morphology of *Schistosoma mansoni* and *S. japonicum* has revealed the existence of two secretory cell masses surrounding the oesophagus tube, referred to as anterior and posterior oesophageal glands (antESO and postESO, respectively). We recently established that the oesophageal glands have a pivotal role in the first steps of blood processing. For instance, erythrocyte and leucocyte are quickly neutralized along the oesophagus before they are propelled to the lower parts of the intestines for further digestion nutrient uptake. We knowledge that incorrect functioning of alimentary tract is associated with worms death by starvation. This was firstly observed in the self-cure response of Rhesus macaque (*Macaca mulatta*), the only known vertebrate capable of combating the disease through worm elimination once the infection is stablished. Classical immunoproteomics (2D-PAGE and Western blotting) has revealed potential targets in both exposed tegument and secreted gut proteins. Recently, a more detailed investigation using the *S. japonicum* in the Rhesus model shed light on the possible operating mechanisms that prevent parasite feeding on blood. Ultrastructural studies and immunocytochemistry on surviving worms indicated the esophageal lumen and the gland secretions as the primary targets of a potent and protective humoral immune response that ultimately disrupts the oesophageal functions. Therefore, the molecular characterisation of the oesophageal gland constituents is imperative if one intends to emulate the Rhesus self-cure response for therapeutical purposes. However, this is not a trivial task and as challenges are multiple. Perhaps, the most important caveat is that both anterior and posterior parts of the oesophageal gland represent a minor fraction of the whole parasite body (or even of its head), meaning that dominant constituents, such as those derived from muscle tissues, suppress the identification of the unique set of gland products. Indeed, our recent investigation on the soluble protein composition of a *S. mansoni* preparation failed to reveal their presence attesting for their low abundance. Although optimized protocols for chemical/enzymatic dissection are reported for isolation of testes and ovary of adult worms, not a single method has proved feasible for gastrodermal epithelium and the oesophageal gland cells. We tackled these challenges by developing a dissection technique of male and female worms preserved in RNAlater solution that entirely compatible with downstream RNAseq and proteomics analyses. This collection contains all methods developed by our group that have permitted the large-scale characterization of transcripts and proteins expressed in the *S. mansoni* and *S. japonicum* oesophagus and gastrodermis.

Files

SEARCH

Protocol

NAME

Manual dissection of the Schistosoma mansoni esophagus and back end for proteomic analysis

VERSION 1

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Protocol

NAME

Manual dissection of the Schistosoma mansoni and S. japonicum head and back end for transcriptomic analysis

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