International Journal for Parasitology: Parasites and Wildlife 3 (2014) 118–123

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Ortleppascaris sp. and your host *Rhinella marina*: A proteomic view into a nematode–amphibian relationship

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ARTICLE INFO

Article history:
Received 23 April 2014
Revised 27 May 2014
Accepted 29 May 2014

Keywords:
Ortleppascaris
Rhinella marina
Host–parasite
Relationship
Proteomic
Helminth

ABSTRACT

The success of the helminth–host relationship depends on a biochemical molecular arsenal. Perhaps the proteome is the largest and most important set of this weaponry, in which the proteins have a crucial role in vital processes to the parasite/host relationship, from basic metabolism and energy production to complex immune responses. Nowadays, the bioproducts expressed by the parasites are under the "spotlight" of immunoassays and biochemical analysis in helminthology, especially in proteomic analysis, which has provided valuable information about the physiology of the infecting agent. Looking into this point of view, why not turn to the infected agent as well? This study characterised the proteomic profile of fluid-filled fibrous cysts of encapsulated *Ortleppascaris* sp. larvae in the hepatic parenchyma of their intermediate host, the amphibian *Rhinella marina*. The proteins were separated by two-dimensional electrophoresis and identified by MS with the aid of *Peptide Mass Fingerprint*. A total of 54 molecules were analysed in this system, revealing a complex protein profile with molecules related to basic metabolic processes of the parasite, energy production, oxi-reduction and oxidative stress processes as well as molecules related to the host response. This study contributes to proteomic studies of protein markers of the development, infectivity, virulence and co-existence of helminths and their hosts.

1. Introduction

The parasite survival depends on the relationship established between them and the host (McKay, 2009) and this relationship starts to increase in the complexity when the larval helminths and their intermediate hosts have elaborate life cycles and transmission strategies to definitive hosts (Chubb et al., 2010).

The secretome, the set of products excreted/secreted by a cell or organism, can tell us a co-evolutionary life history on the dynamics of a parasite, including how it interacts and carries out its functions in the host "milieu" (Hewitson et al., 2008; Nagaraj et al., 2008; Ranganathan and Garg, 2009; Bourke et al., 2011).

Bioactive molecules found in helminth extracts or in excretion/secretion products are responsible for modulating and suppressing the host immune response to favour co-existence of both organisms (Johnston et al., 2009). According to Craig et al. (2006) and Soblik et al. (2011), these substances are derived from the body surface or from specialised excreting/secreting glands in helminths and are often released during specific stages.

This set of products, especially the proteins, has been intensely studied. They can play an important role in the infection and in host–parasite interactions, assisting the survival of parasites within hosts (Ranganathan and Garg, 2009; Liao et al., 2011).

Proteomics has been producing data on a large scale; however due to the scarcity of helminth genomes, there is a need for the production of more data on "helminth-derived" substances. This is especially true when considering the large number of species and the plasticity of these organisms in terms of their biology, morphology, development in different hosts and infection and transmission modes (Rebello et al., 2011; Mutapi, 2012).

Despite this biological variety, the murines are the current animal models of choice in the biological sciences; however, other biological systems *in vivo* can be promising study models and also to contribute to the elucidation of the host–parasite relationship (Bolker, 2012; Sotillo et al., 2012; Robinson et al., 2013).

Herein we explore the parasitism of *Ortleppascaris* sp. larvae in the liver of the amphibian *Rhinella marina*. These larvae are characteristically found within fibrous cysts filled with a viscous fluid stimulating severe pathological alterations in the tissue infected

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http://dx.doi.org/10.1016/j.ijppaw.2014.05.004
2213-2244/

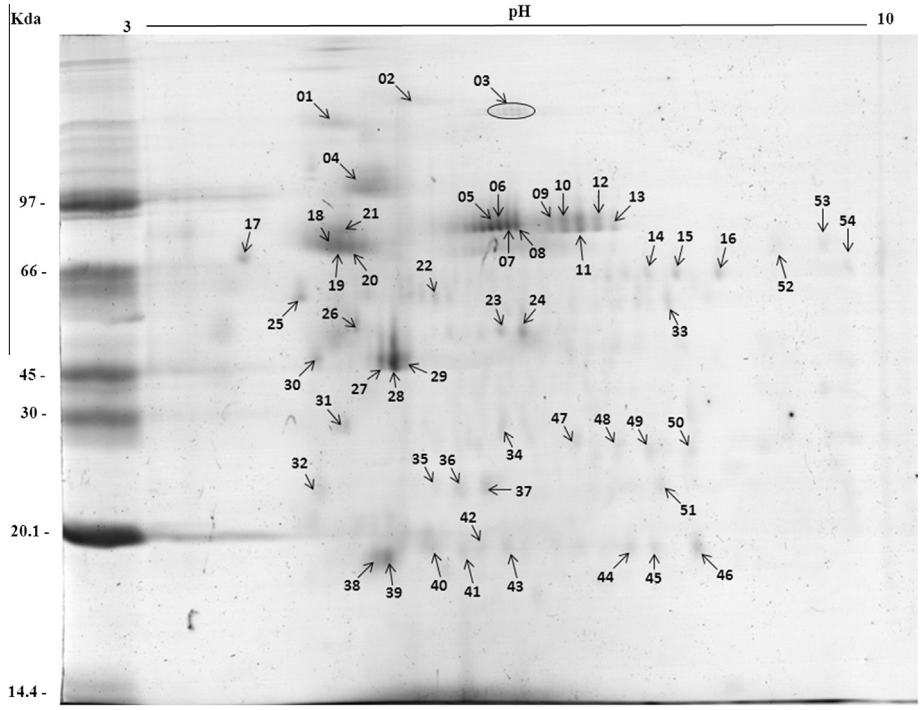

Fig. 1. Two-dimensional gel of proteins originating from cystic fluid of *Ortleppascaris* sp. encystment.

(Silva et al., 2013a,b). The current study used proteomic tools to identify the protein composition of this fluid found within the fibrous cysts and to analyse the biochemical and biological aspects of parasitism by these larval nematodes in the liver of *R. marina*.

2. Materials and methods

The present study was approved by the Animal Research Ethics Committee of the Federal University of Pará (UFPA) through authorisation CEPAEUFPA: BIO010-10.

2.1. Cystic fluid

Cysts of the hepatic parenchyma were removed and the fluid was collected by suction using approximately 200 cysts with the aid of a disposable syringe and stored in extraction buffer (7 M Urea, 2 M Thiourea, 2% CHAPS) at −20 °C.

2.2. Preparation of the cystic fluid proteins

The collected fluids in extraction buffer were centrifuged at 13,000*g* for 15 min at 4 °C to obtain the supernatant, which was used directly for protein analysis.

2.3. Two-dimensional electrophoresis and in-gel tryptic digestion and mass spectrometry

Two-dimensional electrophoresis of the fluid, tryptic digestion procedures and mass spectrometry analysis were performed as described in Silva et al. (2014).

2.4. Database searching and gene ontology analysis

The search for proteins by PMF (peptide mass fingerprint) homology was performed in the NCBI [National Center for Biotechnology Information (NCBInr)] public database with the aid of the online version of Mascot (Matrix Science). The taxonomic parameter was restricted to *Xenopus laevis*. The following search parameters were used: up to two lost cleavage sites, error of 0.1 Da in peptide identification, cysteine carbamidomethylation as a fixed modification and methionine oxidation as a variable modification. To infer the biological process in which the identified molecules are involved, the PMF data analysis was enriched by comparing it with Gene Ontology (GO) terms by means of the UniProt online database. The identified proteins were grouped into functional categories and subcategories according to their ontology.

3. Results

To characterise the fluid's protein profile, the most abundant spots detected with Image Master 2D Platinum software were analysed, shown in Fig. 1 and indicated as spots 1–54.

The spots observed in the two-dimensional gel (Fig. 1) and their respective proteins are summarised in Table 1, according to the database used in this study. Of these spots, 47 were identified, 4 were uncharacterised (03, 13, 34, 36), and 3 spots (35, 37, 49) did not generate good quality spectra for identification because they not presented sufficient similarity to any protein in the database.

The list of marked spots and the respective proteins are summarised in Table 1. These proteins found in the fluid make up a set of molecules that may be grouped into general categories of functions important for cellular functioning (such as redox balance and vesicular traffic), in addition to a group of molecules related to specific processes.

Prolyl endopeptidase-like (spot 01) belongs to the S9A subfamily and has probable serine endopeptidase activity. Subunit beta of the proteasome (spot 15) also has endopeptidase activity and is involved in ATP/ubiquitin-dependent non-lysosomal proteolytic activity.

A protein similar to cyclin-dependent kinase inhibitor, p16, (spot 02) was found, which are involved in the regulation of the eukaryotic cell cycle and is considered a tumor suppressor.