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Immunoproteomic analysis of the excretory-secretory products of *Trichinella pseudospiralis* adult worms and newborn larvae

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Abstract

Background: The nematode *Trichinella pseudospiralis* is an intracellular parasite of mammalian skeletal muscle cells and exists in a non-encapsulated form. Previous studies demonstrated that *T. pseudospiralis* could induce a lower host inflammatory response. Excretory-secretory (ES) proteins as the most important products of host-parasite interaction may play the main functional role in alleviating host inflammation. However, the ES products of *T. pseudospiralis* early stage are still unknown. The identification of the ES products of the early stage facilitates the understanding of the molecular mechanisms of the immunomodulation and may help finding early diagnostic markers.

Results: In this study, we used two-dimensional gel electrophoresis (2-DE)-based western blotting coupled with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/TOF-MS/MS) to separate and identify the *T. pseudospiralis* adult worms ES products immunoreaction-positive proteins. In total, 400 protein spots were separated by 2-DE. Twenty-eight protein spots were successfully identified using the sera from infected pigs and were characterized to correlate with 12 different proteins of *T. pseudospiralis*, including adult-specific DNase II-10, poly-cysteine and histidine-tailed protein isoform 2, serine protease, serine/threonine-protein kinase ULK3, enolase, putative venom allergen 5, chymotrypsin-like elastase family member 1, uncharacterized protein, peptidase inhibitor 16, death-associated protein 1, deoxyribonuclease II superfamily and golgin-45. Bioinformatic analyses showed that the identified proteins have a wide diversity of molecular functions, especially deoxyribonuclease II (DNase II) activity and serine-type endopeptidase activity.

Conclusions: Early candidate antigens from the ES proteins of *T. pseudospiralis* have been screened and identified. Our results suggest these proteins may play key roles during the *T. pseudospiralis* infection and suppress the host immune response. Further, they are the most likely antigen for early diagnosis and the development of a vaccine against the parasite.

Keywords: *Trichinella pseudospiralis*, Excretory-secretory proteins, Immunoproteomics

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Background

Trichinellosis is an important food-borne parasitic zoonosis that infects humans and other mammals, with outbreaks in many parts of the world [1, 2]. Humans acquire the disease by consuming raw or undercooked meat of pigs and other animals containing the infective larvae of *Trichinella spiralis* [3]. Nine different species and three genotypes have been identified to date. Among them, *T. nativa*, *Trichinella T6*, *Trichinella T9*, *T. murrelli*, *Trichinella T8*, *T. britovi*, *T. patagoniensis*, *T. nelsoni* and *T. spiralis* are encapsulated in the host muscle tissues with the formation of collagen layer. *Trichinella pseudospiralis*, *T. papuae* and *T. zimbabwensis* do not induce formation of the collagen layer in the nurse cell [4]. *Trichinella spiralis* and *Trichinella pseudospiralis* are independent and typical species in the genus *Trichinella*. These two species are similar but differ in certain host responses, such as capsule morphology, gene expression, immunological responses and ES products.

After being ingested with infected muscle tissue, the muscle infective larvae (ML) are released and invade the small intestinal epithelium, where larvae complete four moults in 30–40 h and develop into adult worms. The female begins to release the newborn larvae (NBL) over a period of 5–10 days. The NBL penetrate the intestinal wall through the blood and lymphatic circulation into striated muscle, where they grow and form encapsulated and non-encapsulated forms [5]. *Trichinella pseudospiralis* has a worldwide distribution in Europe, Asia, North America and Australia. It has been detected to infect sylvatic predators such as pigs and rats [6, 7], lynx [8] and red foxes [9]. Moreover, this species can infect humans [10] and is the only species that infects birds [11].

Trichinella spiralis and *T. pseudospiralis* ES products are very similar but are not identical in cDNA sequence, molecular mass, antigenicity and peptide maps of ES products [12–15]. ES products are considered to be directly exposed to the host's immune system, which induces the host immune responses. Consequently, ES products may play a crucial role in the invasion and development of *Trichinella* larvae [16, 17]. The ES products of *T. spiralis* include some functional proteins, such as heat shock proteins [18], endonucleases [19], proteinases [20], protein kinases [21, 22], proteinase inhibitors [23], DNA binding [24], and 5′-nucleotidase [25]. The ES products of *T. pseudospiralis* are likely involved in products that have been published, including gp 38, TppSP-1, 45 kDa antigen, TpSerP and 21 kDa ES [17]. The study of the ES products of *T. pseudospiralis* that modulate the host environment to allow parasite development and survival is of fundamental importance to identify the mechanisms leading to immunosuppression and relieving the host inflammatory response in *T. pseudospiralis*-infected host and may provide good markers for diagnosis and candidates for drug and vaccine development.

Recent advances in technology, such as western blotting, indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA) and proteomics have been utilized to identify the ES proteins of *Trichinella* spp. [26, 27]. Proteomics-based analyses involve the simultaneous separation, visualization and quantification of thousands of proteins. More importantly, the combination of proteomics with immunoblotting assays may discover more species-specific antigens than one-dimension analysis can. 2-DE and western blotting combined with MALDI-TOF/TOF-MS/MS are an effective approach for the high-resolution analysis and identification of complex groups of ES products.

Methods

Parasites and animals

Trichinella pseudospiralis preserved in Food-Borne Parasitology Laboratory of Key Laboratory for Zoonoses Research, Ministry of Education, Institute of Zoonoses, Jilin University were genotyped and proved by OIE Collaborating Center on Foodborne Parasites in Asian-Pacific Region in August 2014. *Trichinella pseudospiralis* (ISS13) ML were isolated by pepsin-HCl digestion from infected mice at 30 days. Adult worms and NBL were isolated from the infected mice intestines at 6 days post-infection (dpi) [28].

Collection and preparation of *T. pseudospiralis*-infected animal sera

Trichinella pseudospiralis-infected pig sera were collected from 4 pigs orally infected with 10,000 worms/pig for 26 days. Uninfected sera from the same pigs before infection were collected as negative controls.

Collection of *T. pseudospiralis* adult worms and NBL proteins sample preparation

The collected adult worms and NBL were washed several times with sterile PBS and then were cultured in pre-warmed RPMI-1640 supplemented with 100 U penicillin/ml and 100 μg streptomycin/ml. The adult worms and NBL were incubated at 5000 worms/ml for 20 h at 37 °C in 5% CO_2. After incubation, the media containing the ES proteins were centrifuged at 1000× *rpm* at 4 °C for 5 min and the supernatant containing the ES products were filtered through a 0.2 μm membrane into ultrafiltration device. The ES products were centrifuged at 5000× *rpm* at 4 °C and were concentrated to 100 μl. The total protein concentration was determined by Bradford assay [29].