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Short Communication

Preliminary Analysis of *Taenia multiceps* Metacestode Antigens by Two-Dimensional Electrophoresis

Wen Hui LI [1], Zi Gang QU [1], Nian Zhang ZHANG [1], Long YUE [1], Wen Yan GAI [1], Wan Zhong JIA [1,2], Jian Xun LUO [1,2], Hong YIN [1,2], *Bao Quan FU [1,2]

1. *State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Public Health of the Ministry of Agriculture, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China*
2. *Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Disease, Yangzhou, China*

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***Correspondence**
Email:
fubaoquan@163.com

Abstract

Background: *Taenia multiceps* is a cestode parasite with its larval stage (metacestode), *Coenurus cerebralis*, mainly encysts in the central nervous system of sheep and other livestock causing cerebralis coenurosis. Since treatment of coenurosis with chemotherapy showed little effect and surgical removal of cysts is not advisable in field conditions, vaccination is useful to control coenurosis. Previous study indicated that immunization with *T. multiceps* metacestode antigens could induce protection in sheep against coenurosis, so the aim of this study was to identify *T. multiceps* metacestode antigens in order to find potential vaccine development candidates for further study.

Methods: The protein extracts from the larval *T. multiceps* were analyzed by two-dimensional electrophoresis (2-DE) and characterized by mass spectrometry.

Results: A total of 150 protein spots were detected with isoelectric point (pI) value from 4.97 to 9.65 and molecular weight from 14 to 98 kDa. Twenty-two protein identities were determined by mass spectrometry and 15 unique proteins were obtained. Functional annotation revealed that some of these proteins are involved in catalytic activity, binding, metabolic, cellular process and stress response. Among these molecules are antioxidant proteins (peroxiredoxin and glutathione-S-transferase), glycolytic enzymes (malate dehydrogenase and enolase), proteins with chaperone activity (heat shock protein 70 and small heat shock protein), and structural proteins (actin, actin modulator protein and paramyosin).

Conclusion: The identification of *T. multiceps* metacestode protein will provide valuable information to elucidate their specific roles in the parasitism and screen new targets for vaccine development.

Introduction

Taenia multiceps is a taeniid cestode parasite that, in its adult stage, lives in the small intestine of dogs and wild carnivores, the definitive hosts, while most of its intermediate hosts are herbivores (e. g. sheep). After ingestion of parasite eggs passed in the feces of definitive host, sheep become infected and *T. multiceps* oncospheres are hatched and penetrate the intestinal mucosa and migrate via the blood to the nervous system, including brain and spinal cord where they encyst and develop into mature, infective coenurus larva (metacestode) and cause cerebral coenurosis (1). The presence of coenurus cysts typically leads to neurological symptoms that, in the majority of cases, lead the animal to die after some weeks from starvation (2). Thus, the parasite infection is usually lethal and causes significant economic loss in many parts of the world involved in sheep/goat grazing (3).

In China, the infection of *T. multiceps* has been investigated in dogs (4, 5) and recently molecular characterization of *T. multiceps* isolates from sheep and goats were also reported (6,7). Since treatment of coenurosis with chemotherapy showed little effect and surgical removal of cysts in small ruminants is not advisable in field conditions (3), vaccination is useful to control coenurosis in sheep with native antigens or recombinant antigens (7-11).

A previous study indicated that immunization with *T. multiceps* metacestode antigens could induce protection in sheep against coenurosis (12), so two-dimensional gel electrophoresis (2-DE) and mass spectrometry analysis were performed on *T. multiceps* metacestode antigens in the present study to identify potential vaccine development candidates.

Materials and Methods

T. multiceps coenuri were obtained from the brains of naturally infected sheep from Jingtai county, Gansu province, northwest China. The coenuri samples were homogenized using Tissue Ruptor (QIAGEN) and centrifuged at 12,000 *g* for 10 min at 4°C. The supernatant was collected and the pellet was resuspended in PBS and centrifuged. The insoluble proteins containing the proteinase inhibitors were mixed in lysis buffer (8 M urea, 2 M thiourea, 4% CHAPS, 20 mM Tris-base, 30 mM DTT, 2% [v/v] Bio-Lyte 3/10 carrier ampholyte (BioRad). Subsequently, the lysate was sonicated and deposited by trichloroacetic acid (TCA). Finally, the total protein extracts obtained were purified with ReadyPrep 2-D Cleanup Kit (GE Healthcare, UK) to avoid streaking in the subsequent 2DE. The 2-DE and MALDI-TOF/TOF-MS protocol was according to the reference (13). Briefly, the protein sample (200 µg) was suspended in rehydration buffer (8 M urea, 2% [v/v] CHAPS, 0.001% [w/v] bromophenol blue, 45 mM DTT, 0.2%[v/v] Bio-Lyte 3/10 carrier ampholyte by 30 s vigorous agitation combined with 1 min incubation intervals for five times. The mixture was loaded onto a 7 cm IPG strip (immobilized pH gradient, pH 3–10, linear, Bio-Rad). Isoelectric focusing (IEF) was performed (Protean IEF Cell, Bio-Rad) at 18°C according to the following program: 12 h at 50 V; 250 V for 30 min×2 times; 500 V for 30 min; 4000 V for 3 h; 4000 V for 20,000 Vh and 500 V rapid ramp for 30 min at a maximum current of 50 µA/gel. Then, the IPG strips were equilibrated for 15 min in equilibration buffer 1 (6 M urea, 0.375 M Tris-HCl pH 8.8, 20% glycerol, 2% SDS, 2% DTT, and later in equilibration buffer 2 (6 M urea, 0.375 M Tris-HCl pH 8.8, 20% glycerol, 2% SDS, 2.5% iodoacetoamide for 15 min.

After equilibration, the IPG strips were then embedded in a precast gel and sealed into place using 0.5% low melting point agarose in 25 mM Tris, 192 mM glycine, 0.1% SDS and a trace of Bromophenol Blue sealing solution. SDS-PAGE was carried out at 25 mA/gel in a

Mini Protean 3 Cell (Bio-Rad). The gels were fixed overnight in 50% (v/v) ethanol with 10% (v/v) acetic acid, washed in water, and stained with Coomassie brilliant blue R-250 overnight, then the gels were destained in 50% (v/v) ethanol with 10% (v/v) acetic acid, washed in water. The best three runs with good reproducibility from triplicated samples was imaged with UMAX PowerLook 2100XL scanner (UMAX Technologies Inc, USA) and analyzed using PDQuest V 7.4 (Bio-Rad).

The most abundant spots of various isoelectric points (pI) and molecular weights (MW) were selected and cut out from the gel using the manual spot picker. Finally, the spots were washed with 50 mM ammonium bicarbonate and then with acetonitrile. After that, reduction and trypsin digestion was performed. The resulting peptides were processed in an ABI4800 MALDI-TOF/TOF mass spectrometer (Applied Biosystems), then peptide mass fingerprinting (PMF) data were used to perform database searches using MASCOT software. The function, gene name, and Gene Ontology category of each protein were determined using the Mascot v2.1 software protein database search engine.

Results

The whole soluble fraction of the larval stage of *T. multiceps* were separated on a 2-DE gel covering a pH 3-10 nonliner and the protein spots were visualized following Coomasie R-250 staining (Fig. 1). A total of 150 Coomassie Blue-stained spots were reproducibly separated from a 200 µg sample. The overall protein spots were distributed in pI 4.97-9.65/MW>10 kDa areas of the electrophoreogram. Out of the 32 major protein spots, collected for further analysis, 22 were successfully identified by mass spectrometry. Analysis of 22 spots by mass spectrometry and determination of the average position and number of spots with the PDQuest software showed that some proteins were present in multiple spots on the gel, including enolase, actin and 2-cys peroxiredoxin, which possibly corresponded to protein isoforms or post-translational modifications. Therefore, 15 unique proteins were identified including antioxidant proteins (peroxiredoxin and glutathione-S-transferase), glycolytic enzymes (malate dehydrogenase and enolase), proteins with chaperone activity (heat shock protein 70 and small heat shock protein) and so on. Table 1 provides a list of the identified proteins.

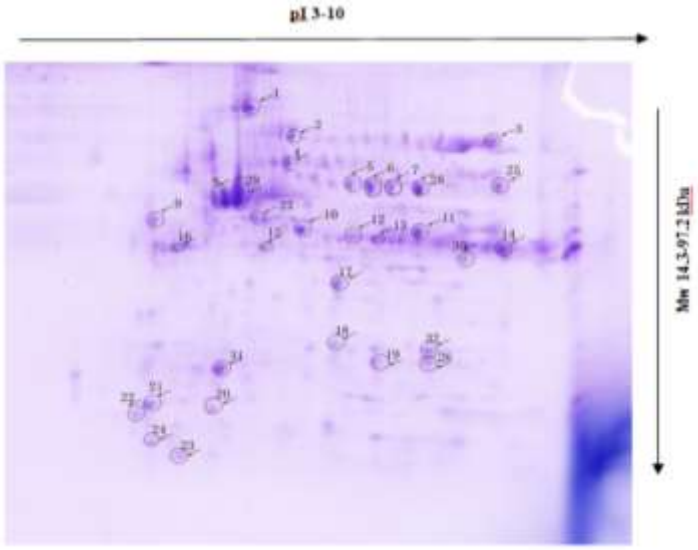

Fig. 1: 2-D electrophoresis pattern for the completely soluble fraction of *T. multiceps coenuri*

Table 1: Mass spectrometry results for proteins isolated from *T. multiceps* coenur

Spot No.	Protein name	Accession No.	Theoretical pI/Mr	Protein score	Matched peptides	Species
1	Paramyosin	gi|42559495	5.28/98.8	783	43	*T. saginata*
2	HSP70	gi|124783175	9.34/21.7	280	7	*T. asiatica*
3	Phosphoenolpyruvate carboxykinase	gi|283466494	8.67/54.2	94	14	*T. multiceps*
4	Actin modulator protein	gi|124783834	6.02/27.2	205	7	*T. asiatica*
5	Enolase	gi|261266611	6.77/46.6	341	10	*T. asiatica*
6	Enolase	gi|261266611	6.77/46.6	464	11	*T. asiatica*
7	Enolase	gi|261266611	6.77/46.6	542	15	*T. asiatica*
8	Actin	gi|55976640	5.30/41.7	581	17	*T. solium*
9	Antigen B	gi|59891334	8.74/95.9	30	4	*E. multilocularis*
11	Myosin heavy chain	gi|189239933	5.73/22.4	56	24	*T. castaneum*
14	Malate dehydrogenase	gi|62178022	8.77/32.6	163	10	*E. granulosus*
17	Glutathione S-transferase	gi|60418504	8.29/25.4	114	10	*T. solium*
18	Cyclophilin	gi|31077167	6.41/17.3	178	4	*E. granulosus*
19	2-cys peroxiredoxin	gi|223403612	6.89/21.6	498	13	*T. solium*
24	Galactokinase	gi|289724628	6.04/36.7	45	6	*G. morsitans*
25	Glutathione S-transferase	gi|241999128	5.46/26.5	46	8	*I. scapularis*
26	2-cys peroxiredoxin	gi|223403612	6.89/21.6	448	12	*T. solium*
27	Small heat shock protein p36	gi|74923842	5.66/35.6	265	11	*T. saginata*
28	Enolase	gi|261266611	6.77/46.6	733	18	*T. asiatica*
29	Actin	gi|55976640	5.30/41.7	711	18	*T. solium*
31	Actin	gi|55976640	5.30/41.7	60	9	*T. solium*
32	2-cys peroxiredoxin	gi|223403612	6.89/21.6	174	10	*T. solium*

Spots 10, 12, 13, 15, 16, 20, 21, 22, 23, 30 were not identified.

Discussion

Identification of proteins by peptide mass fingerprint (PMF) remains difficult for *T. multiceps,* since there was limited information regarding *T. multiceps* gene sequences. Identification of the respective proteins was based on matches to homologous proteins from closely related Taeniid organisms including *T. solium*, *T. asiatica, T. saginata, Echinococcus granulosus* and *E. multilocularis* as well as distantly related organisms such as *Haemonchus contortus*, *Schistoso-*

ma japonicum and so on. However, the increasing number of *T. multiceps* nucleotide sequences in the GenBank will assist the identification of more proteins in the 2-DE gels by PMF in future assessments.

Here we described the analysis of *T. multiceps* metacestode protoscoleces protein extracts by 2-DE and the identification of prominent proteins from the 2-DE gel by PMF. During protoscoleces protein extracting, we found that some protoscoleces proteins were insoluble in PBS following lysis by sonication. The proteins separated by 2-DE reflects only a part of the putative proteome and furthermore membrane proteins have been shown to be particularly difficult to solubilize before separating them by 2-DE (14), suggesting that they are hydrophobic, and might correspond to structural and/or tegument components of the parasite. The elimination of non-protein content after TCA precipitation provided superior resolution of the protoscoleces protein compared to an untreated sample and most mixture proteins soluble in PBS were detected within the neutral or basic end of the 2-DE gel (Fig. 1).

Vaccination with recombinant oncosphere antigens Tm16 and Tm18 could reduce the susceptibility of sheep to infection with *T. multiceps* and field trial confirmed the effectiveness (10, 11). However the protection levels against *T. multiceps* were lower than the high levels of protection that have been demonstrated with other taeniid cestode vaccines (10). The metacestode of *T. multiceps* encysts in the brain, which is an immunologically privileged site and may protect the parasite from serum antibodies and complement. Although vaccination with *T. multiceps* metacestode antigens could partially protect sheep against experimentally coenurosis, the antigens are difficult to prepare in large quantity (12). Therefore, identification of the potent antigenic component of the metacestode antigens is the first step to develop vaccine. In this study, several proteins were identified from *T. multiceps* larval stage by a proteomic approach, some of which are potentially antigenic, such as enolase, paramyosin and antigen B. The protectivity of these recombinant proteins need further studied.

Conclusion

Some potential vaccine candidates were identified from *T. multiceps* coenuri antigens using 2-D electrophoresis. These results provide a framework for further proteomic studies of *T. multiceps* and may be valuable for the identification of new targets for vaccine development.

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