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Proteomic analysis of *Taenia hydatigena* metacestode by high performance liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS)

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ABSTRACT

This study determined the profile of proteins in *T. hydatigena* metacestode by LC-MS/MS. Furthermore, comparison of 6 tapeworms revealed the presence of *Taenia*-specific filamin proteins and *Echinococcus*-specific UGPase proteins. These data provide clues for better understanding of *T. hydatigena* biological characteristics, which provides a new choice for screening of new diagnostic antigens for differential diagnosis of diseases by coinfections of various tapeworm metacestodes.

Key words: *Taenia hydatigena,* Metacestode, Proteome, LC-MS/MS

Taenia hydatigena is a canine tapeworm and its metacestodes, *Cysticercus tenuicollis*, are usually found in the omentum, mesentery, peritoneum, pleura and pericardium. This parasite is widely distributed all over the world, including India (Chhabra and Pathak 2013), and its definitive hosts are dogs and wild canids, while intermediate hosts include pigs, wild boars, sheep, goats, cattle and deer. *T. hydatigena* metacestodes are not infective to humans, but their infections cause appetite loss, weight loss and even death in ruminants (Gomez-Puerta *et al.* 2015, Mansouri *et al.* 2016, Morais *et al.* 2017, Nguyen *et al.* 2016, Scala *et al.* 2016). It will result in large economic losses in milk and meat industry (Scala *et al.* 2015). Proteomic research methods have been applied for revealing the biology of several tapeworms (Laschuk *et al.* 2011, Santivanez *et al.* 2010, Wang *et al.* 2015). This study determined the proteome of *T. hydatigena* by LC-MS/MS, and the data would provide clues for better understanding of *T. hydatigena* biological characteristics.

MATERIALS AND METHODS

C. tenuicollis was obtained from a naturally infected yak in Qinghai Province, China. The metacestode without the cyst fluid was used to prepare for a protein pool for sequencing (Fig. 1). Peptides were analyzed by LC-MS/MS, and raw data were obtained using Thermo Fisher Q Exactive Mass Spectrometer. As the genome of *T. hydatigena* is not yet available, raw data were analyzed using Proteome Discoverer by Mascot engine (Matrix Science, version 2.3) against the *Taenia solium* database (UniProt). Annotations of GO, KEGG and COG were also used to identify proteins. In order to analyze protein families, *T. solium*, *Hydatigera taeniaeformis* (*Taenia taeniaeformis*), *Taenia aisatica*, *Echinococcus multilocularis*, *Echinococcus granulosus* and *Echinococcus Canadensis* genomes were inquired. Copy numbers of protein families were checked by WormBaseParaSite (version WBPS8), using the name of protein families and the Gene Tree module of the same database. Both filamin and UTP-glucose-1-phosphate uridylyltransferase protein sequences were compared using Clustal X (version 1.83), and output alignment files were used to draw a phylogenetic tree by MrBayes (version 3.2.5). *Clonorchis sinensis* filamin protein (csin101494 in WormBaseParaSite) and *Schmidtea mediterranea* UTP-glucose-1-phosphate uridylyltransferase protein (mk4.000070.19 in WormBaseParaSite) were used as outgroup.

Fig. 1. *Taenia hydatigena* metacestode from a yak.

RESULTS AND DISCUSSION

A total of 3090 unique spectra were obtained, which constituted 2433 unique peptides. These peptides led to identification of 594 proteins, 62.3% (370/594) of which

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Table 1. Top 50 abundant proteins in *Taenia hydatigena* metacestode

Protein	Molecular weight	Number of unique peptides	Number of unique spectra	Probability
Enzymes				
Enolase	47 kDa	28	62	100%
Phosphoenolpyruvate carboxykinase	70 kDa	39	67	100%
Cytosolic malate dehydrogenase	37 kDa	21	47	100%
Fructose-bisphosphate aldolase	40 kDa	23	53	100%
Glucose-6-phosphate isomerase	62 kDa	23	50	100%
Glyceraldehyde-3-phosphate dehydrogenase	36 kDa	18	42	100%
Ornithine aminotransferase	27 kDa	11	25	100%
Putative phosphoglucomutase	69 kDa	24	35	100%
Bisphosphoglycerate mutase	29 kDa	14	28	100%
UTP—glucose-1-phosphate uridylyltransferase	57 kDa	22	30	100%
Peptidyl-prolyl cis-trans isomerase	18 kDa	11	26	100%
Lysosomal alpha-glucosidase	102 kDa	16	19	100%
Aldo-keto reductase family 1 member C-like protein 1	34 kDa	15	18	100%
beta D xylosidase 2	95 kDa	14	18	100%
Tropinone reductase	28 kDa	13	18	100%
Delta-aminolevulinic acid dehydratase	39 kDa	10	17	100%
2-amino-3-ketobutyrate coenzyme A ligase	46 kDa	16	19	100%
Phosphoglycerate kinase	42 kDa	15	19	100%
Adenosylhomocysteinase	52 kDa	17	20	100%
Aldehyde dehydrogenase	52 kDa	15	16	100%
Dipeptidase B	56 kDa	14	17	100%
S-methyl-5'-thioadenosine phosphorylase	34 kDa	10	19	100%
Triosephosphate isomerase	26 kDa	9	13	100%
Aldo-keto reductase family 1 member C-like protein 1	19 kDa	7	12	100%
Succinate dehydrogenase [ubiquinone] flavoprotein subunit	72 kDa	19	22	100%
Adenylosuccinatesynthetase	48 kDa	13	15	100%
Dihydrolipoyl dehydrogenase	50 kDa	13	16	100%
Glutathione transferase	25 kDa	10	13	100%
Glycogen phosphorylase	67 kDa	16	18	100%
Prolyl endopeptidase	81 kDa	14	15	100%
Phosphoglucomutase	65 kDa	13	15	100%
Structural proteins				
Hsp-70 protein A	71 kDa	44	69	100%
Hsp-70 protein F	70 kDa	22	28	100%
Hsp-70 protein 6	42 kDa	9	14	100%
Actin	42 kDa	23	37	100%
Filamin-A	130 kDa	25	33	100%
Filamin-related	183 kDa	31	35	100%
Regulatory proteins				
Growth regulator 14-3-3	28 kDa	18	30	100%
14-3-3 protein epsilon	28 kDa	22	37	100%
Sarcoplasmic calcium-binding protein	46 kDa	15	20	100%
Filamin-binding LIM protein 1	115 kDa	27	29	100%
Calpain-A	93 kDa	18	24	100%
Tegumental protein	12 kDa	7	13	100%
2-cys peroxiredoxin	22 kDa	11	18	100%
Calponin	50 kDa	15	17	100%
Elongation factor 1-alpha	49 kDa	14	17	100%
Collapsin response mediator protein	65 kDa	18	26	100%
Other proteins				
P29	27 kDa	13	22	100%
H17g protein, tegumental antigen	68 kDa	16	22	100%
Family S33 non-peptidase homologue	45 kDa	13	28	100%

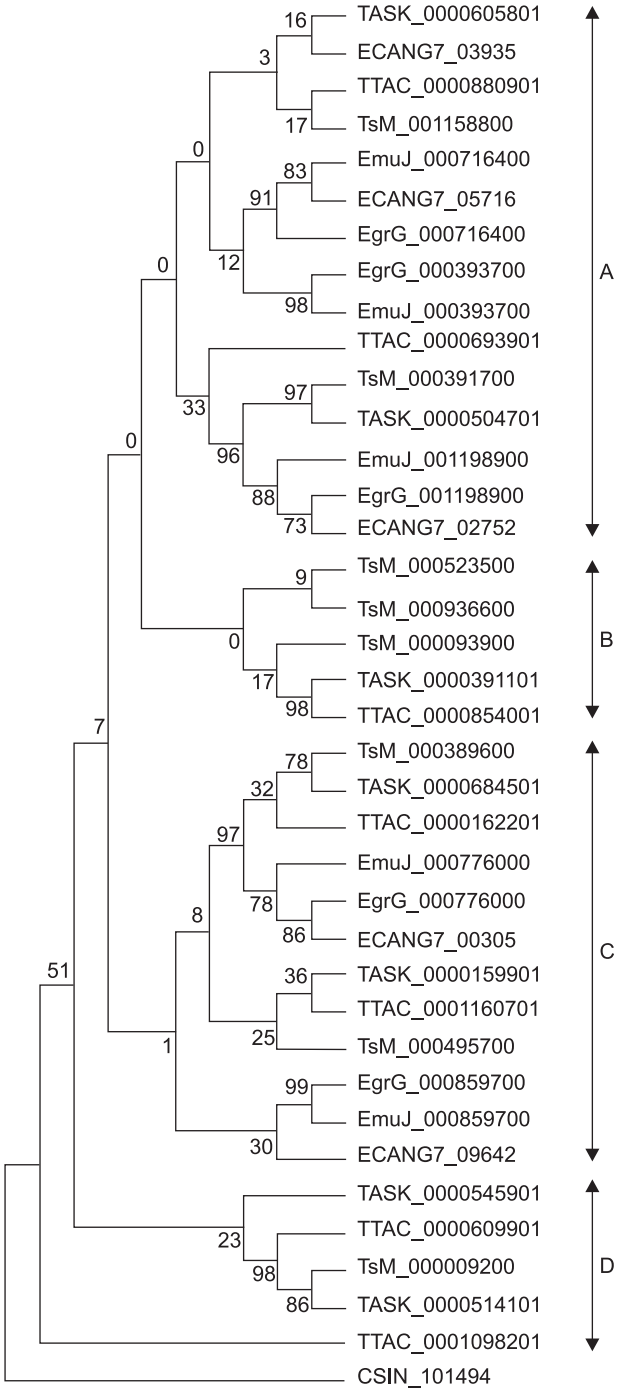

Fig. 2. The phylogenetic tree of tapeworm filamin proteins. All tapeworm filamin proteins were branched into four clades A, B, C and D. TsM, *Taenia solium*; TTAC, *H. taeniaeformis* (known as *Taenia taeniaeformis*); TASK, *Taenia asiatica*; EmuJ, *Echiococcus multilocularis*; EGR, *Echiococcus granulosus*; ECANG7, *Echiococcus canadensis*; CSIN, *Clonorchis sinensis*. A series of numbers followed by the abbreviation of tapeworms are the gene number in WormBase ParaSite.

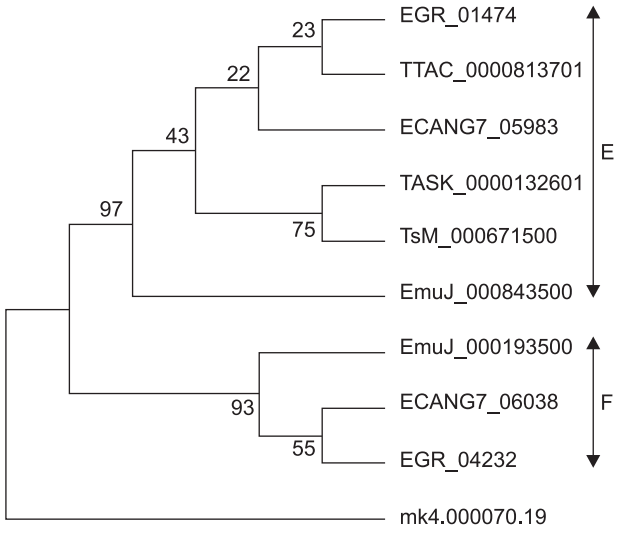

Fig. 3. The phylogenetic tree of tapeworm UGPase proteins. All tapeworm UTP—glucose-1-phosphate uridylyltransferase (UGPase) proteins were branched into two clades E and F. TsM, *Taenia solium*; TTAC, *H. taeniaeformis* (known as *Taenia taeniaeformis*); TASK, *Taenia asiatica*; EmuJ, *Echiococcus multilocularis*; EGR, *Echiococcus granulosus*; ECANG7, *Echiococcus canadensis*; mk4, *Schmidtea mediterranea.* A series of numbers followed by the abbreviation of tapeworms are the gene number in WormBaseParaSite.

were identified with high probability (>98%) (Supplementary File 1). KEGG analysis showed that 506 proteins were annotated into 257 pathways, most of them being involved in metabolic pathways (24.7%) (Supplementary File 2). Consistently, GO analysis also returned metabolic process (16.03%) as the main biological process terms. Some of these proteins, for example enolase, actin, P29, paramyosin, 14-3-3, tubulin, and annexin, were identified to be highly expressed in other tapeworms (Hidalgo *et al.* 2016, Monteiro *et al.* 2010, Santivanez *et al.* 2010, Wang *et al.* 2015). Fifty abundant proteins were classified as enzymes (enolase, carboxykinase, dehydrogenase, aldolase, aminotransferase, mutase, glucosidase, synthetase and so on), structural proteins (Hsp, actin, and filamin), regulatory proteins (14-3-3) and the other proteins (P29) (Table 1). Interestingly, almost all 31 enzymes were related to carbohydrate and amino acid metabolism. It may be explained by the parasite being in the fast growing period or against host's immune system.

Another interesting proteins were Hsp70s. It is well known that Hsp70s are widely distributed in numerous organisms and play an important role in essential cellular processes and also in stress-related processes (Yu *et al.* 2015). Unlike most organisms, Hsp70s of parasites play a central role in the process of differentiation, adaptation and protection from the host's killing mechanisms such as reactive oxygen metabolites, low pH and so on (Maresca and Kobayashi 1994). Up to now, many studies have reported the Hsp70 proteins in tapeworms (Laschuk *et al.* 2011, Monteiro *et al.* 2010, Santivanez *et al.* 2010, Wang *et al.* 2015). Moreover, several studies revealed that Hsp70 of parasites could induce protective immunity (Duan *et al.* 2015, Fang *et al.* 2014, Hartmann *et al.* 2014, Zhuo *et al.* 2016). Whether the high expression of Hsp70s in *C. tenuicollis* will also induce the protective immune response in yaks against further continuous infection is unknown,

Table 2. Comparative analysis of six protein families abundant in *Taenia hydatigena* metacestode.

Protein family	Number of protein family members in different tapeworms					
	Taenia species			*Echinococcus* species		
	T. solium	*H. taeniaeformis**	*T. asiatica*	*E. multilocularis*	*E. granulosus*	*E. canadensis*
Fructose-bisphosphate aldolase	4	4	4	4	4	4
Glyceraldehyde-3-phosphate dehydrogenase	3	3	3	3	3	3
Actin	73	56	63	69	69	42
Filamin	8	7	7	5	5	5
UTP—glucose-1-phosphate uridylyltransferase	1	1	1	2	2	2
Peptidyl-prolyl cis-trans isomerase	21	21	19	21	21	20

**Hydatigera taeniaeformis* (known as *Taenia taeniaeformis*).

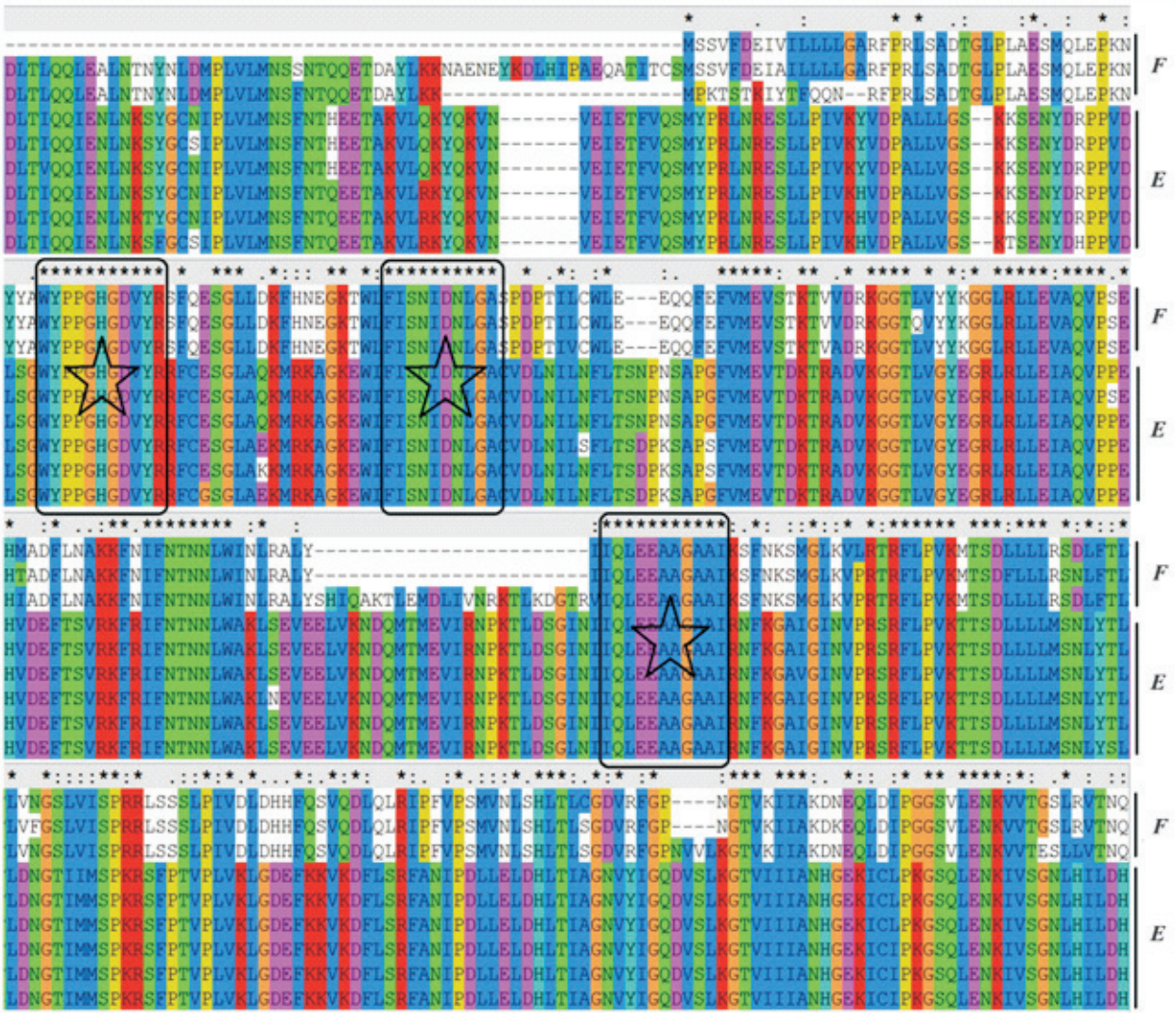

Fig. 4. Amino acid sequence analysis of tapeworm UGPase proteins. Clade E contained six proteins while clade F contained three proteins. The big ☆ indicates a conserved region.

and further experimental verification is needed.

Six high expression protein families in *C. tenuicollis* were chosen for further analysis, including fructose-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, actin, filamin, UTP-glucose-1-phosphate uridylyltransferase, and peptidyl-prolyl cis-trans isomerase (Table 2). In six parasitic cestodes, all the protein families had multiple members, except UTP-glucose-1-phosphate uridylyltransferase in T*aenia* species, and two protein families (fructose-bisphosphate aldolase and glyceraldehyde-3-phosphate dehydrogenase) had the equal number of members. Actin protein family had the most members in each species, being 42~73. Moreover, filamin family had more members, while UTP—glucose-1-

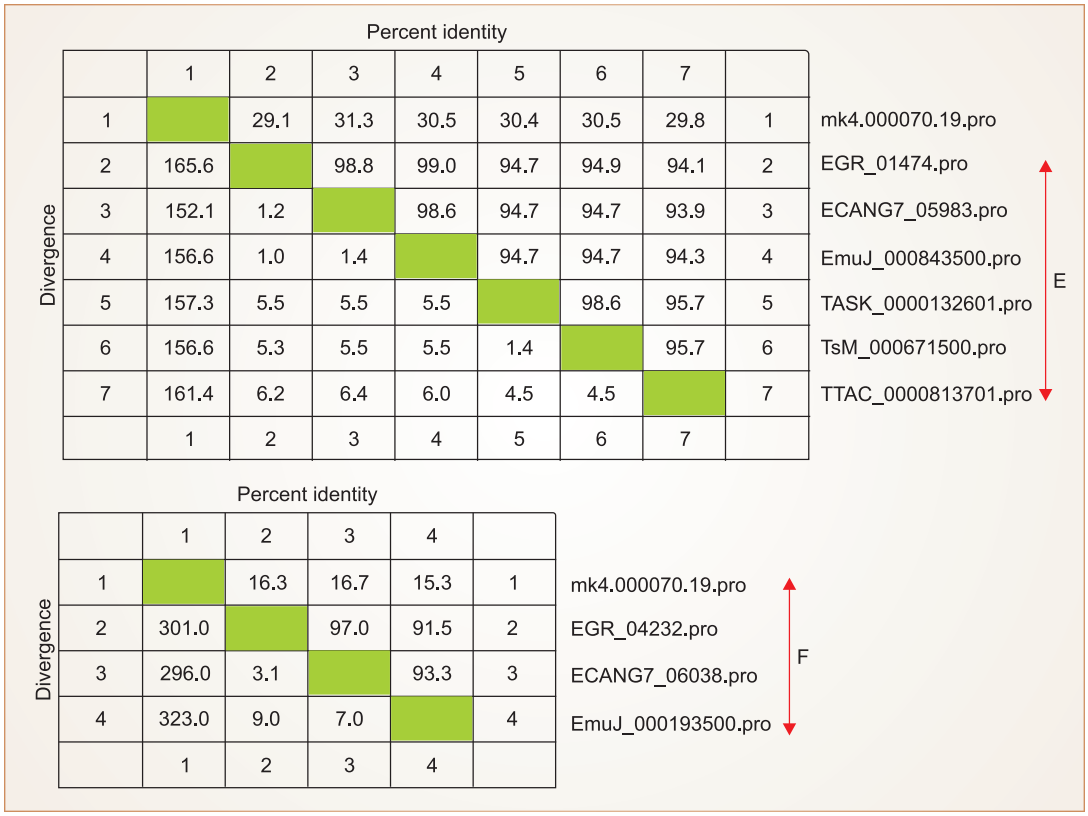

Fig. 5. Amino acid sequence homology percentages of UGPase clade E and F proteins. TsM, *Taenia solium*; TTAC, *H. taeniaeformis* (known as *Taenia taeniaeformis*); TASK, *Taenia asiatica*; EmuJ, *Echiococcus multilocularis*; EGR, *Echiococcus granulosus*; ECANG7, *Echiococcus canadensis*; mk4, *Schmidtea mediterranea.* A series of numbers followed by the abbreviation of tapeworms are the gene number in WormBaseParaSite.

phosphate uridylyltransferase family had fewer in *Taenia* species than *Echinococcus* species (Table 2).

Protein family analysis indicated that filamin proteins might play an important role in *Taenia* species, while UTP—glucose-1-phosphate uridylyltransferase protein functions might be shrinked. Filamin protein interacts with many proteins and is crucial for the cytoskeleton in eukaryotes (Light *et al.* 2012). Many studies showed that it was involved in parasite infection processes and inducing host's immune protection responses, and was also associated with the adult worm tegument (Cook *et al.* 2004, Ludolf *et al.* 2014, Mohamed *et al.* 2008). Thirty seven filamin protein sequences were found by searching the database. *Taenia* species had 7~8 copies and *Echinococcus* species had 5. After evolutionary analysis of these protein sequences, the results showed that there existed 4 clades (A to D), and clades B and D were specific for *Taenia* species (Fig. 2). It would be interesting to evaluate their roles in *Taenia* species.

In total, 9 UTP-glucose-1-phosphate uridylyltransferase protein sequences were also retrieved from the database. *Taenia* species had only 1 copy but *Echinococcus* species had 2. UTP-glucose-1-phosphate uridylyltransferase (UGPase) is an enzyme involved in carbohydrate metabolism, also known as glucose-1-phosphate uridylyltransferase (or UDP–glucose pyrophosphorylase), and has a prominent role in carbohydrate metabolism in both prokaryotes and eukaryotes. It participates in the synthesis of activated sugars, which ultimately serve as precursors for the synthesis of cell-surface structures (Sivaraman *et al.* 2003). The results showed that there were 2 clades (E to F) and clade F was specific for *Echinococcus* species (Fig. 3).

Amino acid sequence analysis showed that the proteins in these two clades had three common conserved regions with ten consecutive conserved amino acids (Fig. 4). Interesting, the six proteins in clade E were highly conserved and identity was 93.9%~99% (Fig. 5). Similarly, amino acid homology of 3 proteins in *Echinococcus* species was also high, being 91.5%~97% (Fig. 5). It is interesting to reveal their biological functions in the lifecycle of *Echinococcus* species in the near future.

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