Proteomic analysis of the excretory-secretory proteins of the *Trichinella spiralis* L1 larva, a nematode parasite of skeletal muscle

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Running title: Excretory-secretory proteins of the *Trichinella spiralis* muscle larva

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Abstract

Trichinella spiralis is an intracellular nematode parasite of mammalian skeletal muscle. Infection of the muscle cell leads to the formation of a host-parasite complex that results in profound alterations to the host cell and a re-alignment of muscle-specific gene expression. The role of parasite excretory-secretory (ES) proteins in mediating these effects is currently unknown, largely due to the difficulty in identifying and assigning function to individual proteins. In this study, a global proteomics approach was used to analyse the ES proteins from *T. spiralis* muscle larvae. Following 2-dimensional electrophoresis (2-DE) of ES proteins, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)-mass spectrometry and liquid chromatography tandem mass spectrometry (LC-MS/MS) were used to identify the peptide spots. Specific *Trichinella* EST databases were assembled and used to analyse the data. Despite the current absence of a *Trichinella* genome-sequencing project, 43 out of 52 protein spots analysed were identified and included the major secreted glycoproteins. Other novel proteins were identified from matches with sequences in the *T. spiralis* database. Our results demonstrate the value of proteomics as a tool for the identification of *Trichinella* ES proteins and in the study of the molecular mechanism underpinning the formation of the host-parasite complex during *Trichinella* infections.

1 Introduction

Profound alterations in skeletal muscle can occur as a result of injury, disease or infection with pathogens. One of the most extreme cases is observed in cells infected with the nematode parasite, *Trichinella spiralis*, an intracellular parasite specific for mammalian skeletal muscle. The newborn L1 larva migrates from the intestine of the host to the skeletal muscle where it infects and encapsulates within a portion of the myofiber and develops into the infective or muscle L1 larva [1]. During this process an intimate host-parasite complex, the nurse cell, is formed. Infection of the terminally-differentiated cell by *T. spiralis* leads to profound changes, including re-entry into the cell cycle with subsequent suspension at the G2/M border, down-regulation of muscle-specific gene [2] and muscle-specific protein [3] expression, dissolution of myofibrils, proliferation of secretory organelles and nuclear enlargement [4]. These changes to the infected muscle cell suggest that the differentiated state in the skeletal muscle cell is repressed and that post-infection a process of de-differentiation is initiated. This is followed by re-differentiation into an alternative cell type, one that is adapted to support the parasite until its transmission to the next host. The molecular mechanisms underlying the re-programming of the skeletal muscle are currently unknown although there is circumstantial evidence implicates larval excretory-secretory (ES) proteins in the formation and/or maintenance of the nurse cell. ES proteins collected from both newborn larvae and muscle larvae elicit morphological and structural changes to cultured primary rat myocytes [5].

Furthermore, antigens sharing epitopes with secreted *Trichinella* proteins have been detected within isolated host cell nuclei where they co-localise with nuclear chromatin complexes [6, 7, 8]. However, the abundance of these proteins has been too low to allow identification and therefore confirmation of the parasite origin of these antigens awaits data connecting them to *Trichinella*-encoded genes. Although several biochemical activities have been observed in the *Trichinella* ES fraction, including DNA-binding [9], DNA endonuclease [10, 11], protease [12, 13] and kinase activities [14, 15], relatively few of the proteins responsible or the genes encoding them have been characterised to date.

The identification and characterisation of ES proteins is of fundamental importance to understanding the mechanism of parasite-induced de-differentiation. Recently, mass spectrometric analysis has led to the identification of two ES proteins, a 67 kDa 5'-nucleotidase [16] and a putative serine protease [17]. This suggests that the application of proteomics may circumvent many of the problems encountered with the identification of ES proteins in the past. Proteomic analysis has been successfully used to identify ES proteins from other helminth parasites such as the nematode *Haemonchus contortus* [18] and the trematode *Fasciola hepatica* [19, 20]. In the current study, we have used data collected from 2-DE and mass spectrometry (MS) to analyse and identify the ES proteins from *T. spiralis* muscle larvae. This represents the first study which attempts to comprehensively identify *Trichinella* ES proteins and, as such, will provide valuable information for further studies.

2 Materials & Methods

2.1 Parasites

T. spiralis (ISS390) parasites were maintained in female ICR/CD1 mice and muscle larvae were isolated from infected mice as previously described [21].

2.2 Collection and preparation of ES proteins

Isolated *T. spiralis* muscle larvae were washed several times in pre-warmed RPMI 1640 medium (Invitrogen) and resuspended at 5000 muscle larvae ml^{-1} in RPMI containing 2 mM L-glutamine, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin. The parasites were maintained at 37°C / 5% CO_2 for up to 20 h after which the larvae were allowed to sediment and the culture medium containing the ES protein was collected [22]. Where necessary, the larvae were resuspended in fresh culture medium and incubated for a further 20 h under the same conditions. ES proteins were concentrated from the culture supernatants by precipitation with methanol / chloroform [23]. Normally, 400 μl of culture supernatant was used for 1-DE and 1.6 ml for 2-DE, although volumes were adjusted to ensure equal loading between successive gels. Protein samples were resuspended in buffer (100 mM Tris-HCl, pH 6.8, 200 mM DTT, 4% SDS, 20% glycerol) prior to 1-DE or re-swelling buffer (7 M urea, 4% CHAPS, 2 M thio-urea, 0.3% DTT, 2% resolyte ampholytes pH 4-7) prior to 2-DE.

2.3 De-glycosylation of ES proteins

ES proteins were de-glycosylated using PNGase F (New England Biolabs) according to the manufacturer's instructions. Briefly, precipitated ES proteins were resuspended in 80 μl 1X denaturation buffer (supplied with enzyme) and heated to 95°C for 10 mins. Following denaturation 8 μl NP-40, 8 μl G7 buffer (supplied with enzyme) and 5 μl PNGase F were added and the samples incubated at 37°C overnight. The control sample was identical except for the omission of PNGase F. The ES proteins were then precipitated as above and resuspended in an appropriate buffer for electrophoresis.

2.4 1-D and 2-D electrophoresis

ES protein samples were analysed by 1-DE using NuPage® Novex® 4-12% Bis-Tris gels (Invitrogen) and NuPage® LDS sample buffer plus Sample Reducing Agent (Invitrogen) were added to the samples prior to electrophoresis. 1-DE analysis was also used to equalise ES protein samples prior to 2-DE. For 2-DE, the ES protein samples were loaded onto pH 4-7 IPG strips (Amersham Biosciences) for separation in the first dimension. The samples were re-hydrated overnight at room temperature followed by step voltage focussing (0.01 h 200 V; 1.5 h 3500 V; 1.05 h 3500 V) at 22°C and the strips were incubated in 7mls of equilibrating buffer (50 mM Tris, 6 M Urea, 30% glycerol, 2% SDS) containing 10 mg ml^{-1} DTT for 30 min. Following this, a further equilibration step was performed with equilibrating buffer containing 25 mg ml^{-1} iodoacetamide for 30 min. For separation in the second dimension, the IPG strips were laid on 10-15%

non-linear gradient polyacrylamide gels and run at 75 V for 1 h and then at 150 V

until 140 volt-hours were reached. After separation, proteins were visualised by

staining with Colloidal Coomassie Blue G250 (Sigma) as described elsewhere

[24].

2.5 Immunoblotting

ES proteins separated by 1-DE and 2-DE were transferred to Hybond-C pure

nitrocellulose membranes (Amersham Biosciences) using a Novex XCell

Surelock™ Mini-Cell blot module apparatus (Invitrogen) for 1 h at 30 V.

Following transfer, the membranes were incubated in blocking solution (TSBT:

20 mM Tris-HCl, 150 mM NaCl, 1% Tween-20, pH 7.6) containing 5% skimmed

milk for 3 h at room temperature. Primary antibody (mAb 18H) was diluted

1:1500 in TBST containing 1% skimmed milk and applied to the membranes

overnight at 4°C. After washing in TBST (4 X 10 min), the anti-mouse-IgG

secondary antibody conjugated to alkaline phosphatase (Sigma) was applied at a

dilution of 1:10000 in TBST for 1 h. The blots were developed using 5-bromo-4-

chloro-3-indolyl phosphate / nitro blue tetrazolium substrate (Sigma).

2.6 Mass Spectrometry

MALDI-TOF-MS was performed at the University of Aberdeen Promteome

Facility using a protocol adapted from a published method [25]. Briefly, protein

spots were excised from the stained 2-D gels and subjected to in-gel proteolytic

digestion with trypsin (Sigma) using an Investigator™ ProGest™ instrument

(Genomic Solutions). The resulting tryptic fragments were guanidylated with methylisourea, desalted and concentrated using an Investigator™ ProMS™ instrument (Genomic Solutions) before analysis using a PerSpective Biosystems Voyager-DE STR MALDI-TOF mass spectrometer, operating in reflectron mode at 20 kV accelerating voltage. Spectra were internally calibrated using trypsin autodigestion products. LC-MS/MS was performed at the Sir Henry Wellcome Functional Genomics Facility, University of Glasgow. After in-gel tryptic digestion, the resulting peptides were solubilized in 0.5 % formic acid and fractionated by nanoflow HPLC on a C18 reverse phase column, eluting with a continuous linear gradient to 40% acetonitrile over 20 minutes. The eluate was analysed by online electrospray tandem MS using a Qstar Pulsar instrument (Applied Biosystems). A 3-second survey scan preceded each MS/MS data collection cycle of four 3-second product ion scans, giving a duty cycle of 15 seconds. For tandem MS performed by the FingerPrints Proteomics Facility, University of Dundee, tryptic peptides were analysed on an Applied Biosystems 4700 Proteomics Analyser (MALDI-TOF-TOF-MS-MS system) in MS reflector mode to generate a peptide mass fingerprint (PMF). Five of the most intense peptide ions were selected for further analysis by MS/MS. The MS and MS/MS data were combined and used by the Applied Biosystems GPS system (Global Proteomics Server) and MASCOT (Matrix Science) to search the databases.

2.7 Database searching

The PMF profiles obtained from MALDI-TOF-MS were used to search databases, run locally using MS-Fit (Protein Prospector) against a custom-made *Trichinella* EST database and the NemaCluster database for *Trichinella* [26]. The following parameters were used: methionine oxidation, cysteine carbamidomethylation and lysine guanidylation. The peptide mass tolerance was initially set at 50 ppm but was increased to 150 ppm when searching EST databases. Similarly, up to 3 missed cleavages were allowed when searching EST databases. Searches were also carried out against all mouse sequences in the NCBInr database in order to identify potential host proteins. Database searches using LC-MS/MS data were carried out using MASCOT (Matrix Science).

3 Results

3.1 2-DE analysis of *Trichinella* ES proteins

ES proteins secreted by isolated *T. spiralis* muscle larvae during *in vitro* cultivation were precipitated from culture supernatants collected following 6 h, 20 h and 40 h incubation in RPMI medium and analysed by 2-DE (Fig. 1A-C). The most prominent ES proteins in the 6 h sample migrated in the 40-60 kDa range. These corresponded to the major glycosylated proteins in the 40 h ES sample as shown by Western blotting and immuno-analysis with mAb 18H, a monoclonal antibody that is specific for tyvelose-containing glycans epitopes [27] found on some *Trichinella* ES proteins (Fig 1D). This was consistent with previous observations that the major immuno-dominant ES proteins of the *T. spiralis*

muscle larva are glycosylated and in this size range [28, 29, 30]. Following later

cultivation times, both the number of protein spots and the overall complexity of

the ES profile increased, notably in the lower molecular mass range (ie 15-40

kDa). The 2-DE profiles shown are representative of *T. spiralis* ES proteins and

gel-to-gel reproducibility was very high between independent samples (for

example, compare Fig. 1C and Fig. 2).

3.2 Identification of Proteins by 2-DE and Mass Spectrometry

Using the current sample preparation method and pH range, approximately 125

Coomassie Blue-stained spots were visible on 2-D gels of *T. spiralis* 40 h ES

proteins (Fig. 1C). In order to identify the major ES proteins, a total of 52

different prominent spots were excised from 2-D gels (Fig 2) and a proteomic

analysis of selected spots was carried out using MALDI-TOF-MS. The resulting

PMF data were used to search databases. As relatively few *Trichinella* sequences

are available in the public cDNA and protein databases, searches using these were

largely unsuccessful. Consequently, a custom-made database was compiled from

all *Trichinella* sequences, both cDNA and EST, deposited in GenBank. Latterly,

the *Trichinella* EST cluster database compiled as part of the Parasitic Nematode

EST project [26] was also used for searches. In line with other studies, a match to

a *Trichinella* protein was considered significant where there were at least 5

matched peptides and 15% coverage of the protein sequence [31]. For matches to

ESTs, fewer peptide matches and a lower percent coverage were tolerated in

making a putative assignment of protein identity. Other criteria used to assign

spots to proteins included matching the calculated theoretical molecular mass and

pI values of the protein, where possible, with the observed position of the peptide

on the 2-D gel. Because of the limited availability of complete cDNA sequence

data all of the spot identifications initially assigned on the basis of PMF data were

considered tentative until confirmation could be obtained by tandem MS. To do

this LC-MS/MS was used to supplement the PMF data and to assign identities to

those proteins un-identified by MALDI-TOF alone. In these cases, searches were

carried out using MASCOT (MatrixScience) and MOWSE scores >70 were

considered to be significant.

Using the above criteria for PMF data, positive matches to *Trichinella* proteins or

ESTs were obtained for 23 peptide spots (Table 1), although in 2 cases (spots 8

and 10) the PMF-based assignment was considered borderline. The identities of

22 were confirmed by LC-MS/MS; spot 12 was excluded as the identification

based on the PMF data was to a complete cDNA and was considered robust. A

further 20 spots were successfully identified using LC-MS/MS alone giving a

total of 43 out of 52 *Trichinella* ES proteins identified using MS. The remaining

9 spots were un-matched to any *Trichinella* sequence currently in the database.

The 43 significant matches corresponded to only 13 different proteins encoded by

Trichinella cDNAs or ESTs; 4 to known *T. spiralis* ES proteins, 3 to predicted ES

proteins and the remaining 6 to putative proteins encoded by novel ESTs (Table

1). Twelve spots (1, 8-12, 26 and 31-35), ranging in size from 44.5 to 69.0 kDa

and with pI values ranging from 5.14 - 6.64, were assigned to a cDNA encoding

the *T. spiralis* serine protease, TspSP-1 [32, 17]. The predicted size of TspSP-1

based on the cDNA is 48 kDa, however, anti-TspSP-1 antibodies detect native

proteins of 50, 64 and 70 kDa by 1-DE and evidence from *de novo* amino acid

sequencing and tandem MS, suggests that several isoforms of the protein are

present in the ES [17]. Two spots (2 and 7) were identified as the major secreted

glycoprotein p43; the identification of two isoforms of this protein differing in pI

was again consistent with previous observations [29]. A further three spots (5, 27

and 28) were identified as the secreted 45 kDa antigen and one spot (13) was

assigned to the secreted 5'-nucleotidase [16, 33]. In these cases, there was close

agreement between the predicted molecular mass and pI with those observed by 2-

DE.

Spots assigned to three different hypothetical *Trichinella* proteins were also

identified, confirming that these proteins are expressed and secreted. Seven spots

(3 and 16-21) were assigned to ORF 9.10. Spots 3 and 16 shared molecular mass

and pI values of 48.5 kDa and 4.94 / 4.86, respectively, whilst the other ORF 9.10

spots formed a distinct group of lower molecular mass (~35 kDa) with pI values

in the range of 4.94 – 5.78. Five spots (39-43) with approximate molecular mass

of 22.5 – 24.6 kDa and pI values ranging from 4.92 – 6.39 were identified as the

ORF 17.20 protein. In addition, spot 46 was assigned to the ORF 11.30 protein.

All of these are novel proteins that have yet to be characterised. The remaining

twelve spots were assigned to proteins encoded by *Trichinella* ESTs, all of which

appeared to be novel. Spots 45 and 49-52 were assigned to two related ESTs (BG353021 and BG353717), which were subsequently found on contig TS01176 in the NemaGene database. A complete ORF was identified within TS01176 encoding a novel 15.7 kDa protein with a putative N-terminal signal peptide. The theoretical molecular mass for this protein matched that observed for this group of spots. The ESTs BG520944 (spot 6), BG520575 (spots 14 and 15), BQ540916 (spots 29 and 30) and BG345905 (spot 38) all encoded protein fragments that included an N-terminal signal peptide, as predicted by the SignalP algorithm [34]. Thus 12 of the identified proteins were predicated to be secretory proteins due the presence of an N-terminal signal peptide. The single exception was EST BQ542714 (spot 36), which encoded a protein fragment lacking the signal sequence though this could be due to the fact that the EST probably derives from a truncated cDNA lacking 5' gene sequence information.

Despite the successful identification of 43 ES proteins, a few notable exceptions were not identified including the 53 kDa secreted glycoprotein [35] and the secreted nucleoside diphosphate kinase [15]. These proteins have predicted pI values of 8.42 and 7.94 respectively and therefore will not resolve on the 2-D gels used in this study.

3.3 Glycosylation of *T. spiralis* ES proteins

The major tyvelosylated *T. spiralis* ES glycoproteins resolved as approximately 30 immuno-reactive peptides following 2-DE and Western analysis with the anti-tyvelose monoclonal Ab18H (Fig 1D). Fifteen of these tyvelose-bearing proteins were identified by MS including the secreted 45 kDa antigen, glycoprotein p43 and the TspSP-1 serine protease. Following treatment with PNGase F, which cleaves *N*-linked glycans many ES proteins, including those not recognised by the mAb 18H antibody, changed position with regard to their molecular weight and pI (Fig 3). Overall, there was a net movement of spots into the 30-40 kDa and 15 kDa molecular mass regions indicating that a number of peptides had decreased in mass following de-glycosylation. Therefore, a large subset of the ES proteins appears to be glycosylated with *N*-linked glycans. These included the secreted 45 kDa and those encoded by the ESTs BG520575, BQ540916, BG353717 and BG353021. Peptide spots that did not change their position following de-glycosylation included those identified as the ORF 9.10 protein, the secreted 5'-nucleotidase, the ORF 17.20 and ORF 11.30 proteins, and those spots encoded by BG354905 and BG520944.

4 Discussion

To date few *Trichinella* ES proteins have been analysed in terms of their biological activity, due largely to problems in identifying individual proteins. Yet these proteins function at the host-parasite interface and are probably crucial for successful parasitism both during the muscle stage and the intestinal stage during which a new infection is established in a host animal. In the current study, we

have successfully used 2-DE and proteomic analysis tools to identify ES proteins from the muscle larva, despite the absence of a genome-sequencing project for *Trichinella*. Using PMF data from MALDI-TOF-MS and LC-MS/MS and a custom-assembled *Trichinella* sequence database, we assigned identities to 43 out of the 52 ES peptide spots analysed. The majority of the ES proteins identified contain a predicted N-terminal signal peptide which supports the identifications and verifies that they are secretory proteins. The use of 2-DE, coupled with protein identification, has also been informative with regard to determining the overall constitution and complexity of the ES. The 43 peptide spots identified represented only 13 different proteins, indicating that several isoforms of many of the proteins were present. There are a number of possible explanations for the different isoforms including post-translational modification, splice variants and protein processing. For example, a large subset of *T. spiralis* ES proteins is glycosylated and differential glycosylation may account for some of the isoforms. The significance of differentially glycosylated variants may become more apparent once the roles of individual ES proteins have been elucidated. For instance, it has been suggested that glycosylation may influence the activity of the TspSP-1 serine protease, as several *N*-glycosylation sites are found near the active site of the protein [17].

The question of the function of the muscle larva ES proteins remains to be determined. Undoubtedly, some of these proteins will play a role in the establishment or maintenance of the nurse cell and the observations that ES

proteins localise to the surface of the parasite [36] and to the infected host nuclei [6, 7, 8]support this conclusion. However, tyvelose-bearing ES glycoproteins may also play a significant role in the intestinal phase. These glycoproteins originate in the muscle larva in a specialised secretory organ, the stichosome and are deposited in the intestinal epithelium during the establishment of a new infection [37, 38]. Furthermore, antibodies raised against Tyvelose are protective and result in rapid expulsion of parasites from the intestine on administration of a challenge infection [39, 40]. The application of proteomics in the present study has provided some much-needed basic data which will allow more focussed studies on the functions of individual *Trichinella* ES proteins to be carried out. These studies are in progress and will be the subject of future communications. The identification of the ES proteins is critical to understanding the host-parasite interaction and may have broader implications to the study of the fundamental biological mechanisms including those underlying maintenance of the differentiated state in mammalian cells.

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Figure Legends

Figure 1. 2-DE analysis *T. spiralis* muscle larvae ES proteins in the pH range 3-7 and separated on 10-15% non-linear gradient polyacrylamide gels in the second dimension. 2-D gels stained with colloidal Coomassie blue G250 of ES proteins collected after 6 h (A), 20 h (B) or 40 h (C) of *in vitro* culture. (D) Immunoblot of 2-D gel of 40 h ES proteins, probed with the anti-tyvelose mAb 18H.

Figure 2. Coomassie-stained 2-D gel of 40 h ES proteins showing the positions of the 52 protein spots subjected to analysis by MS. Spots were numbered and those identified are listed in Table 1.

Figure 3. Comparison of the 2-DE profiles of 40 h ES proteins that have been treated (+PNGase) or mock-treated (-PNGase) with PNGase F. Spots identified by MALDI-TOF-MS following de-glycosylation: (a) secreted 5'-nucleotidase; (b) ORF 9.10; (c) 45 kDa antigen; (d) BG520575; (e) TspSp-1; (f) ORF 17.20. The ORF 17.20 peptide spots that do not change position on treatment with PNGase are marked with arrowheads.
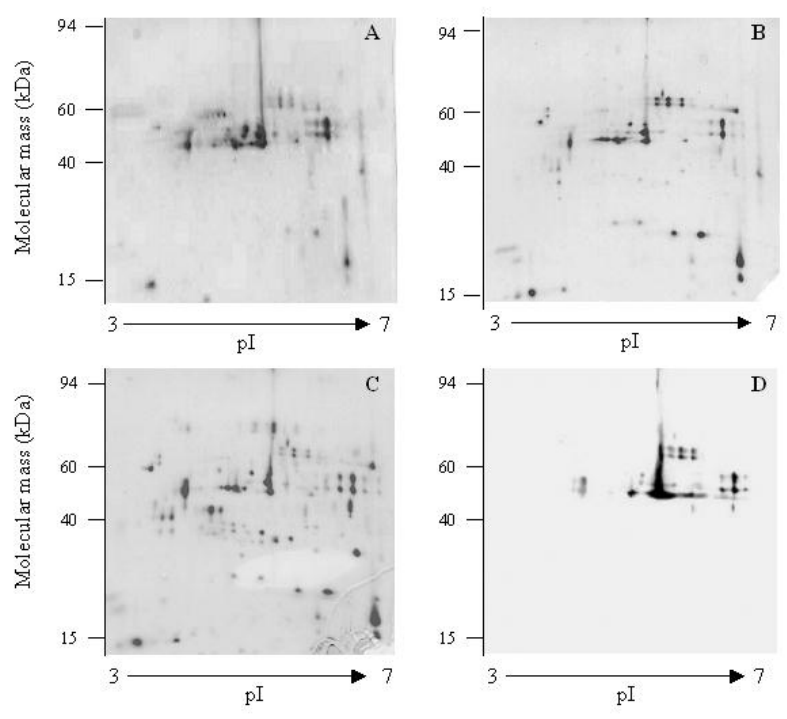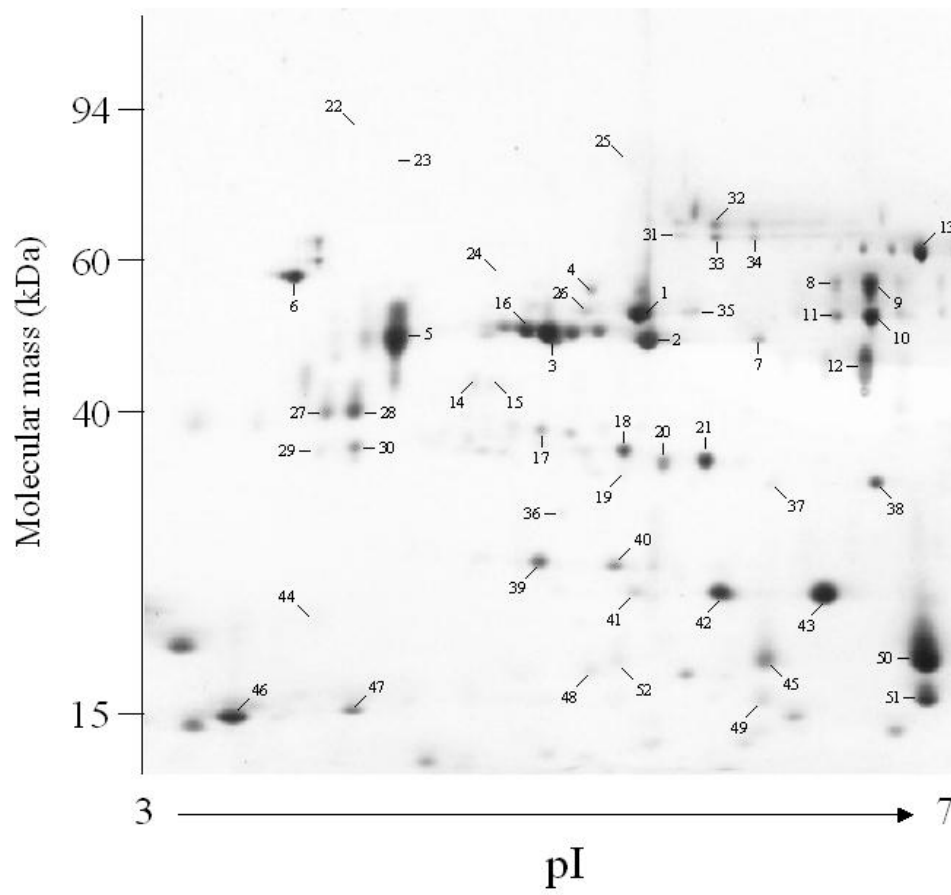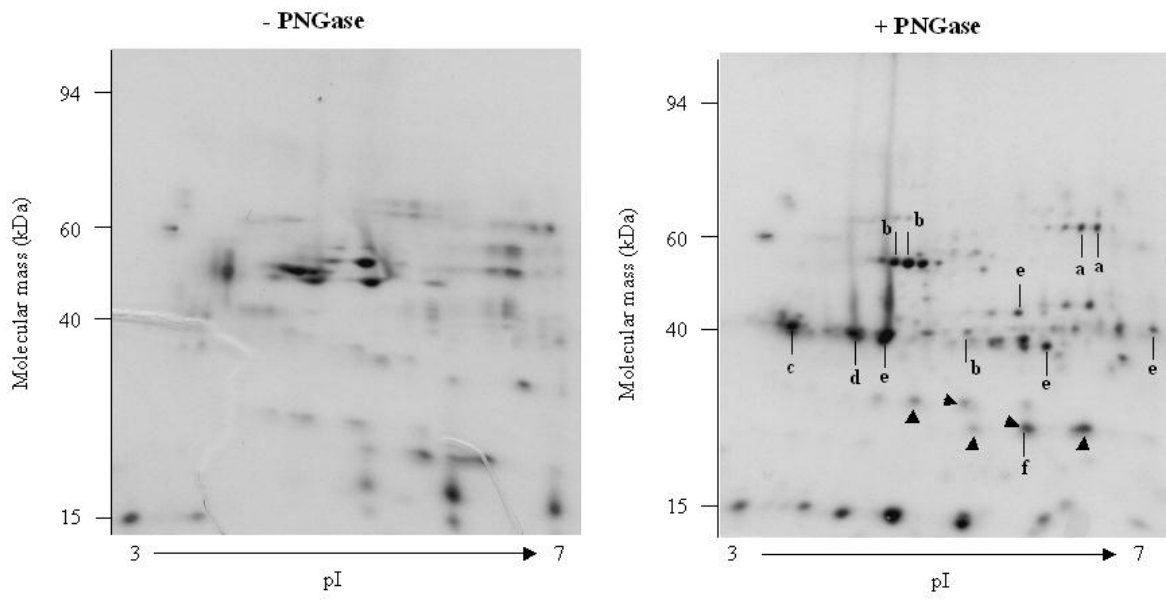
Figure 1

Figure 2

Figure 3

Table 1.   Identification of *T. spiralis* ES proteins identified by MALDI-TOF and LC-MS/MS

Spot	*Trichinella* Protein	Accession Number	Theoretical M$_r$ / pI[1]	Observed M$_r$ / pI	Coverage (%)	Matched Peptides	MOWSE Score[2]
1	Serine protease	AY02897	48.0/6.33	54.0/5.44	24	4	274
2	gp43	M95499	38.0/5.95	47.1/5.48	25	7	433
3	ORF 9.10	U88241	47.6/5.60	48.5/4.94			440
5	45kDa antigen	U01847	31.0/4.76	47.1/4.21	17	5	326
6	Unknown	BG520944		57.0/3.75			362
7	gp43	M95499	38.0/5.95	47.1/6.04	13	5	270
8	Serine protease	AY02897	48.0/6.33	55.8/6.44	7	3	121
9	Serine protease	AY02897	48.0/6.33	55.8/6.64			175
10	Serine protease	AY02897	48.0/6.33	51.0/6.64	12	4	214
11	Serine protease	AY02897	48.0/6.33	51.0/6.44			244
12	Serine protease	AY02897	48.0/6.33	44.5/6.58	42	7	ND
13	5'-nucleotidase	AY12757	62.0/6.13	62.8/6.86	15	6	1117
14	Unknown	BG520575		41.5/4.60	24	6	218
15	Unknown	BG520575		41.5/4.69	25	10	263
16	ORF 9.10	U88241	47.6/5.60	48.5/4.86	32	10	404
17	ORF 9.10	U88241	47.6/5.60	36.4/4.94	38	5	392
18	ORF 9.10	U88241	47.6/5.60	35.0/5.36			423
19	ORF 9.10	U88241	47.6/5.60	32.5/5.36	21	5	405
20	ORF 9.10	U88241	47.6/5.60	33.2/5.55	35	5	336
21	ORF 9.10	U88241	47.6/5.60	33.2/5.78	35	6	543
26	Serine protease	AY02897	48.0/6.33	54.0/5.14			163
27	45kDa antigen	U01847	31.0/4.76	38.0/3.92			87
28	45kDa antigen	U01847	31.0/4.76	38.0/4.00	14	6	106
29	Unknown	BQ540916		35.0/3.89	22	5	496
30	Unknown	BQ540916		35.0/4.00	27	6	417
31	Serine protease	AY02897	48.0/6.33	65.0/5.64			175
32	Serine protease	AY02897	48.0/6.33	69.0/5.83			210
33	Serine protease	AY02897	48.0/6.33	65.0/5.83			211
34	Serine protease	AY02897	48.0/6.33	65.0/6.01			201
35	Serine protease	AY02897	48.0/6.33	47.1/5.69			105
36	Unknown	BQ542714		28.2/5.00			239
38	Unknown	BG354905		31.0/6.67			214
39	ORF 17.20	U88239	19.5/6.13	24.6/4.92			211
40	ORF 17.20	U88239	19.5/6.13	24.6/5.33	47	9	338
41	ORF 17.20	U88239	19.5/6.13	22.5/5.42			179
42	ORF 17.20	U88239	19.5/6.13	22.5/5.89	40	16	208
43	ORF 17.20	U88239	19.5/6.13	22.5/6.39	37	9	320
45	Unknown	BG353021	15.7/6.28[3]	18.2/6.10			256
46	ORF 11.30	U88238	12.7/4.17	15.0/3.44			397
49	Unknown	BG353021	15.7/6.28[3]	16.0/6.06			257
50	Unknown	BG353021	15.7/6.28[3]	18.2/6.89	57	7	387
51	Unknown	BG353717	15.7/6.28[3]	16.3/6.89	39	6	239
52	Unknown	BG353021	15.7/6.28[3]	18.2/5.36			310

[1] Theoretical molecular mass (kDa) and pI
[2] A MOWSE score of >70 was used to assign identity to a protein analysed by LC-MS/MS
[3] Molecular mass and pI of predicted protein encoded on contig TS01176