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Transcripts analysis of the entomopathogenic nematode *Steinernema carpocapsae* induced *in vitro* with insect haemolymph☆

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Abstract

Steinernema carpocapsae is an insect parasitic nematode widely used in pest control programs. The efficacy of this nematode in controlling insects has been found to be related to the pathogenicity of the infective stage. In order to study the parasitic mechanisms exhibited by this parasite, a cDNA library of the induced *S. carpocapsae* parasitic phase was generated. A total of 2500 clones were sequenced and 2180 high-quality ESTs were obtained from this library. Cluster analysis generated a total of 1592 unique sequences including 1393 singletons. About 63% of the unique sequences had significant hits ($e \leq 1e\text{-}05$) to the non-redundant protein database. The remaining sequences most likely represent putative novel protein coding genes. Comparative analysis identified 377 homologs in *C. elegans*, 431 in *C. briggsae* and 75 in other nematodes. Classification of the predicted proteins revealed involvement in diverse cellular, metabolic and extracellular functions. One hundred and nineteen clusters were predicted to encode putative secreted proteins such as proteases, proteases inhibitors, lectins, saposin-like proteins, acetyl-cholinesterase, anti-oxidants, and heat-shock proteins, which can possibly have host interactions. This dataset provides a basis for genomic studies towards a better understanding of the events that occur in the parasitic process of this entomopathogenic nematode, including invasion of the insect haemocoelium, adaptations to insect innate immunity and stress responses, and production of virulence factors. The identification of key genes in the parasitic process provides useful tools for the improvement of *S. carpocapsae* as a biological agent.

☆*Note:* Nucleotide sequence data reported in this paper are available in GenBank under accession numbers GR977153–GR979332.

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Appendix A. Supplementary data: Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molbiopara.2009.10.002.

Keywords

Entomopathogenic nematode; *Steinernema carpocapsae*; EST; Nematode transcripts; Secreted proteins; Virulence factors

1. Introduction

Steinernema carpocapsae (Nemata: Rhabditida) is an entomopathogenic nematode (EPN) produced and commercialized worldwide to control a large number of insect pests with large economical impact [1,2]. This nematode is an obligate parasite completing its entire life cycle in an insect host. The infective juvenile (IJ) is the resistant third juvenile which is encased in a double external cuticle with the digestive tract closed carrying into a specific part of the gut the symbiotic bacterium *Xenorhabdus nematophila* [3]. IJ moves freely in the environment and is able to localize and contaminate the host in response to different insect cues [4] entering through natural openings, principally anus and mouth. After IJ comes in contact with the insect tissues, it develops a parasitic phase that is able to invade the insect haemocoel and kill susceptible hosts [5].

Though *S. carpocapsae* is believed to be pathogenic to a large number of insects its efficacy is quite variable. Experimental assays showed that efficacy depends on the target insect and moreover it depends on the specific nematode strain used against each insect [6,7]. These findings support the assumption that the efficacy of *S. carpocapsae* is related to the efficiency of the parasitic phase in promoting parasitism of the target insect, which includes the ability to overcome insect defences, to invade and produce virulence factors.

Upon contact with the host, the nematode faces the insect defences that are suggested to be highly potent and includes hummoral and cellular effectors like reactive oxygen species [8,9]. It is generally accepted that the nematode has the ability to survive insect defences [5], however, encapsulation have been reported in some species of four insect orders [10], suggesting a host parasite dialog. *Pseudalaetia unipuncta* larvae exposed to *S. carpocapsae* are able to develop cellular encapsulation of invasive nematodes, blocking their development and probably the release of the symbiotic bacteria, thus preventing the success of parasitism [11].

The ability of *S. carpocapsae* to invade insect haemocoelium is demonstrated by the fact that more than 50% of a susceptible insect like *Galleria mellonella* had nematodes inside 12 h post-exposure [12]. However, in resistant *P. unipuncta* larvae the number of nematodes in the haemocoelium is reduced (unpublished data). These finds suggest that invasion ability must contribute to the efficacy of these parasites.

In most of the infections caused by *S. carpocapsae*, susceptible insects died 48–72 h after contamination. Experimentally it has been shown that either the associated bacteria or the axenic nematode are able to kill insects despite the efficacy was reduced when each pathogen was applied individually [12]. The associated bacteria are able to cause a generalized septicaemia and express a large set of toxic factors including enzymes and insecticidal toxins, thereby inducing insect mortality [13,14]. The nematode itself depletes

host tissue by feeding also releases toxic factors of peptidic nature that cause insect mortality [15–17]. So far the relevance of the toxic factors produced by the nematode is not known, however, it was shown that a strain with low virulence was secreting and excreting less protein with lower proteolytic activity than a high virulence strain [12].

Taken together these findings suggest that nematode parasitic mechanisms are related to the efficacy of these biological agents. Different aspects in host-parasite relationships have benefited from genomic approaches in other parasitic nematodes including the entomopathogenic nematode *Heterorhabditis bacteriophora* [18–20], however, very little is known about the genomics of *S. carpocapsae*. In this work we constructed a cDNA library with transcripts from the parasitic phase of the nematode and we sequenced and analyzed 2500 ESTs that resulted in the identification of a set of genes that are putatively involved in parasitic mechanisms.

2. Materials and methods

2.1. Induction of nematode parasitic phase

S. carpocapsae (Breton strain) used in this work was grown in an artificial medium according to Bedding [21]. The infective juveniles (IJ) were conserved in tap water for 1 month at 10 °C. To induce recovery of the parasitic phase, IJ were superficially disinfected with 0.5% sodium hypochlorite, rinsed abundantly with sterilized water and transferred to a Petri dish containing 7 ml of the Tyrode's solution with 10% haemolymph of the natural host, *G. mellonella* larvae. To avoid contamination 1% penicillin-streptomycin-neomycin (Sigma) was added. Nematodes were incubated under agitation at 25 °C for 6 h. These nematodes were harvested in a filter paper, rinsed several times with sterilized water and immediately used for RNA extraction.

2.2. cDNA library construction

Total RNA was extracted using Trizol reagent following the manufacturer's recommendations (Invitrogen). The cDNA library was constructed from total RNA using SMART approach (BD Biosciences, Clontech). Briefly, first-strand cDNA synthesis was performed with total RNA in 10 μl of final volume and 100 units of PowerScript reverse transcriptase. All other components as well as the conditions of reaction were in accordance with the recommendations of the supplier. First-strand cDNA was amplified by PCR with Advantage 2 polymerase mix (BD Biosciences, Clontech) using 5′ PCR primer and CDS III/3′ PCR primer. Amplified cDNA was purified with YorBio PCR purification kit (Yorkshire Bioscience Ltd., UK). cDNA library normalization was performed using the Trimmer kit (Invitrogen) to correct for over abundance of highly expressed transcripts according to supplier instructions. Fifty micrograms of ds-cDNA were treated with protease K, digested with *Sfi* restriction endonucleases and fractionised on CHROMA SPIN-400 columns. cDNA was ligated to pDNR-LIB (CmR) vector digested with *Sfi*. The product of ligation was then transformed into 2 ml of XL10-Gold KanR (Stratagene) electrocompetent cells prepared in YorBio. Titre was determined by plotting the pooled transformants on LB agar plates supplemented with chloramphenicol (30 μg/ml), incubated at 37 °C over night and colony forming units were counted.

2.3. Plasmid isolation and DNA sequencing

Clones were transferred to LB medium with 50 μg/ml chloramphenicol and grown for 20 h prior to plasmid isolation. Plasmid DNA was isolated from 2500 randomly selected clones using JETQUICK Plasmid Purification Spin Kit (Genomed, Germany). Sequencing was performed using M13 forward primer in STABVIDA facilities service.

2.4. EST processing, contig assembly and analysis

Vector sequences, adapter regions, and poly(A) tails were trimmed. High-quality ESTs (at least 100bp) were then assembled into clusters of contiguous sequences and subsequently into clusters as previously described [22]. The consensus sequences of contigs and singletons comprised the unique sequences, which were compared against the National Center for Biotechnology Information (NCBI) non-redundant protein database using BLASTx [23] (*E*-value cut-off $E \leq 1e{-}05$) and summarized on cluster level.

2.5. Gene ontology annotation

Gene ontology annotation was performed using BLASTx through NCBI with the unique sequences (consensus sequences of assembled contigs and the singletons). Sequences with BLASTx hits were annotated according to gene ontology terms (GO) using Blast2GO software [24]. Hits with $E>1e{-}05$ were discarded. The remaining hits were grouped by organism. To assign putative functions to the unique sequences, the GO hierarchical terms of homologous genes from the Interpro protein databases were extracted. In addition, the unique sequences with homologs to enzymes participating in metabolic pathways were mapped in accordance with the Kyoto Encyclopedia of Genes and Genomes (KEGG). Enzyme commission (EC) numbers were acquired for the unique sequences by WU-BLASTx searching ($E \leq 1e{-}05$) the KEGG database (v43) [25]. The EC numbers were then used to putatively map unique sequences to specific biochemical pathways.

2.6. Secreted protein identification

All ESTs were conceptually translated into peptides. Secreted proteins were predicted using a combination of programs, to minimize the number of false positive predictions. Firstly, a WoLF PSORT analysis (http://wolfpsort.org/) [26] was performed to predict the sub-cellular localization. Blast analysis was then conducted on the NR database at NCBI to identify similarity and to evaluate the probability for secretion. Only ESTs that contained the N-terminal sequence were analyzed using SignalP prediction (http://www.cbs.dtu.dk/services/SignalP/) [27]. A signal sequence was considered present when it was predicted both by the artificial neural network and the hidden Markov model prediction approaches (SignalP-NN and SignalP-HMM, available as options within SignalP).

3. Results and discussion

3.1. Overview of ESTs sequence analysis

The cDNA library was constructed with the transcripts of the parasitic phase of *S. carpocapsae* induced for 6 h with insect haemolymph *in vitro*. After normalization of this library a total of 2500 ESTs were sequenced producing 2354 readable sequences

representing 94.6% success rate. After removal of clones with poor quality or short inserts (100 bp cut-off) 2180 high-quality ESTs were produced with an average length of 563±246 bp. The cumulative length of all high-quality EST sequences was 1,227,707 bases. Assembling the 2180 ESTs resulted in 1592 unique transcripts consisting in 199 contigs (787 ESTs) and 1393 singletons. The average length of each unique transcript was 575±259 bp, a total length of 915,881 bases that represents about 0.4% of the entire *S. carpocapsae* genomic DNA [28]. One hundred and twenty-four of the 199 contigs contained 2 ESTs (62.3%), 29 contained 3 ESTs (14.6%), 19 contained 4 ESTs, 10 contained 5 ESTs and the remaining 17 contained 6–18 ESTs. Clearly most of the contigs were formed by a reduced number of ESTs, thus reflecting efficiency in normalization and subtraction.

BLASTx analysis against the non-redundant (NR) protein sequences in GenBank indicated that 999 (62.8%) of the unique transcripts had significant match to known proteins, whereas the remaining 593 had no significant matches (*E*>1e−05) in publicly available databases, probably representing new genes. 6.4% of the hits had an *E*-value of 1e−100, 66.1% between 1e−20 and 1e−99, and 27.5% between 1e−19 and 1e−05. Comparative analysis with other complete and partial nematoda genomes revealed that 37.7% clusters have identities in *C. elegans* (Additional file 1), which is the most well-characterized nematode in many respects, particularly in its genome, genetics, biology, physiology, and biochemistry. Moreover 51.5% of these homologs correspond to *C. elegans* genes that have been silenced by RNAi (Additional file 3), thus providing useful information on function of the orthologous genes in *S. carpocapsae* [29–31]. 43.1% clusters had identities in *C. briggsae* and 7.5% in other nematodes (Additional files 2 and 3).

3.2. Annotation and functional classification

Transcripts were categorized by functions based on the gene ontology (GO) classification (www.geneontology.org). Inter-ProScan (ftp://ftp.ebi.ac.uk/pub/software/unix/iprscan) was used to match *S. carpocapsae* clusters to protein domains and subsequently to the three organizing principles of the GO hierarchy(Fig. 1 and Additional file 4). Of the 999 NR hits, 842 (84.3%) clusters matched InterPro domains, and 995 (99.6%) mapped to GO. Complete listing of *S. carpocapsae* assignments can be viewed through the AmiGo browser at http://www.nematode.net [32].

Unique sequences with best matches to EC numbers were also assigned to a specific KEGG pathway, as an alternative functional classification. These sequences were classed into 11 functional categories (Table 1 and Additional file 5). Carbohydrate metabolism (14.4%), amino acid metabolism (9.6%), and cofactors and vitamins metabolism (9.6%) are the best-represented pathways. Complete listing of all KEGG mappings including graphical representation is available at http://www.nematode.net [32].

3.3. Transcripts analysis

Table 2 summarizes the most representative clusters found in the analyzed transcripts, comprising genes encoding ribosomal proteins, elongation factor 1-gamma, cytochrome *c* oxidase subunit I and III, to list a few, which have been also identified as the most abundant transcripts in other nematode EST projects [22,33]. Ribosomal proteins have been shown to

play roles in stress tolerance in yeasts, plants and nematodes [34–36]. Cluster SC00194.cl with 10 ESTs had significant identity to lysine-rich arabinogalactan protein 18 precursor in *Arabidopsis thaliana* [37,38]. So far, arabinogalactan protein in nematodes has only been reported in the plant nematode *Heterodera schachtii* [39]. Genes encoding anti-oxidant factors such as glutathione S-transferase (cluster SC00184.cl) and an oxidation resistance protein (cluster SC00193.cl) were identified. Anti-oxidant factors had been reported in several parasitic nematodes playing important roles by counteracting ROS produced by host [18,40,41].

Cluster SC00179.cl encodes for transthyretin-like protein (ttl), which is one of the abundant nematode-specific domains [34]. Nematode ttl protein was described in the free-living nematodes *C. elegans* [42], in the plant parasitic nematode *Radopholus similis* and in the animal parasitic nematode *B. malayi* [43,44]. However, the number of ttl-ESTs available in public databases is higher in parasitic nematodes, particularly in libraries constructed from the parasitic phase, than in free-living nematodes, thus suggesting its involvement in parasitism.

Cluster SC00139.cl is another abundant cluster and matches trypsin-like serine protease from *S. carpocapse*. Serine proteases are the major proteolytic enzymes expressed by parasitic nematodes and are frequently suggested to be involved in host–parasite interactions [45].

Finally, 3 clusters with no significant similarity with any sequence in the non-redundant protein database were identified. These last clusters have a GC content of 31.2% which is different from that of 50.6% found in sequences that have homology, thus indicating they are probably part of new genes specific to *S. carpocapsae*.

3.4. Secretome analysis

Based on a combination of different programs, 119 unique sequences were predicted encoding putative secreted proteins in the present dataset (Additional file 6). Seventy-four putative secreted proteins had similarity to known proteins in the NCBI database, 35 had similarity in *C. elegans*, 23 in *C. briggsae*, 14 in *B. malayi*, 10 in *S. carpocapsae* and 10 in other plant and/or animal parasitic nematodes. Twenty-eight of putative secreted proteins matched hypothetical proteins and 18 had no similarity to any sequences available in current databases.

Proteases are the most represented among the predicted secreted proteins identified in *S. carpocapsae* ESTs (Table 3). Nine clusters were identified with homology to diverse serine proteases including trypsine-like and elastases. At present two serine proteases were purified from the secreted–excreted products of the parasitic phase of *S. carpocapsae* that were shown to be interacting with insect host defences and with insect mid-gut cells, thus probably helping in the parasitic process [46,47]. Also an elastase-like serine protease was up-regulated in the parasitic phase of this nematode [48]. Five clusters had similarity with members of the metalloprotease family. This protease family has been suggested to be involved in the hydrolysis of extracellular matrix components like type I collagen [49]. The cluster SC00878.cl has a particularly interesting match to metridin-like ShK toxin domain

(SMART accession number: SM0254). This toxin domain was first identified in a family of sea anemone potassium channel toxins [50] although a search on GenBank reveals the presence of this toxin domain in a wide variety of organisms including *C. elegans* and *C. briggsae*. Five aspartic proteases and 3 putative cysteine proteases belonging to family C1 papain-like with homologs in *C. elegans* were also identified. Aspartic proteases have been identified in other parasitic nematodes and suggested to be participating in tissue invasion and in extracellular protein digestion [51–53], whereas cysteine proteinases have been implicated in invasion, tissue destruction, anticoagulation, nutrition, and immune evasion in many helminths [54].

Ten clusters were identified encoding proteins with similarity to serine protease inhibitors and 3 clusters were identified as homologs to cystatins, one of which (SC00822.cl) had very high homology to *S. carpocapsae*. Cystatin has been reported to be up-regulated in the parasitic phase of this nematode [55]. Parasite-derived protease inhibitors are recognized to play a variety of roles in the survival of the parasite by modulating exogenous host proteases [56–59].

Other clusters found related to parasitism were lectins (5 clusters) and acetylcholinesterase (4 clusters). Lectins and AchE are speculated to play important roles in immunomodulation, namely in nematodes inhabiting alimentary tract [60–63].

Cluster SC00264.cl had 49% similarity to a fatty acid retinoid binding protein (FAR) inthenematode *O. ostertagi*. FARs are thought to be involved in host–parasite interactions and were also described in the animal parasitic nematodes *A. caninum* and *B. malayi* and in the plant parasitic nematode *Globodera pallida* [64–66].

Two clusters (SC00346.cl and SC00926.cl) sharing homology to saposin-like protein in *Entamoeba invadens* and *B. malayi*, were interesting. The saposin-like protein family comprises pore-forming peptides, which have been identified in a variety of organisms including the secreted products of blood-feeding nematodes *H. contortus* and *A. caninum* [67,68]. In *C. elegans*, a family comprising 29 genes of saposin-like protein and saposin-like domain containing protein has been identified. The gene *spp-1* in this family (Gene ID: T07C4.4) was expressed as a recombinant in *E. coli* and proved to have antibacterial activity [69]. The genes *spp-1* and *spp-7* in the same family were reported to belong to the innate defence system of *C. elegans* [70]. In *S. carpocapsae* saposin-like proteins could potentially be involved in the modulation of monoxenic relation with the symbiotic bacteria.

3.5. Stress-related proteins

Twenty-six clusters encoding different families of heat-shock proteins (HSPs) were identified in the present transcripts analysis, including transcripts of 90, 70, 60, 40 and 20kDa, alpha-crystallin-type heat-shock proteins, heat-shock factor DnaJ and TCP, and binding proteins (Table 4). HSPs are known to be expressed in response to stress conditions including those caused by hosts in parasites [71]. In *Steinernema* species Hsp40 expression was reported to be related to desiccation tolerance of infective juveniles [72].

In *S. carpocapsae* parasitic transcripts, thioredoxin oxidase, glutathione S-transferase and peroxiredoxin were also predicted. Anti-oxidant proteins of parasitic nematodes have been suggested to be involved in protection against reactive oxygen and nitrogen species generated by the host immune responses [73–76].

4. Conclusions

This study presents the first analysis of cDNA transcripts expressed in the parasitic phase of the entomopathogenic nematode *S. carpocapsae*. Though most of the genes identified were predicted to encode products involved in metabolic activities, a significant number of genes are putatively related to pathogen–host interactions. Putative secreted proteins that could act as virulence factors against insects are part of these parasitism-related genes. Among these are proteases belonging to serine, cysteine, aspartic and metalloproteases families are hypothesised to be participating in invasion of the host and evasion from the host defences. Protease inhibitors were also identified and are suggested to be interacting with host defences. Other identified proteins such as lectins and AchE have a role in immunomodulation and saposin–like proteins are suggested to have antimicrobial activity. Moreover, a large number of expressed genes were related to stress survival revealing the high investment of this specific phase of the nematode to adapt to the conditions imposed by the host.

About 32% of transcripts analyzed had no homology with any known gene in publicly databases. These genes probably are unique to this nematode and likely related to its particular way of life characterized by the alternation of a symbiotic with a parasitic phase. This EST collection opens new avenues in improving the efficacy of entomopathogenic nematodes for pest control by the use of recombinant DNA. It also represents a significant addition to the existing EST resources of nematode species, and serves as a valuable tool for functional genomic analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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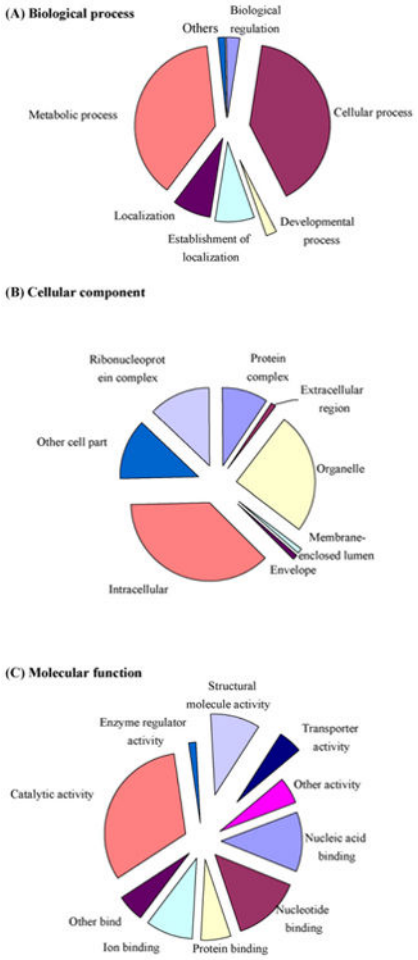

Fig. 1.

Gene ontology (GO) mapping for *Steinernema carpocapsae* clusters by biological process (A), cellular component (B), and molecular function (C). There were 444, 295 and 576 unique clusters that mapped to the three categories, respectively. (For details see Additional file 1. Notice that individual categories can have multiple mappings resulting in a sum greater than 100%.)

Table 1

KEGG biochemical pathway mapping for *S. carpocapsae* clusters.

KEGG categories	Unique No. of clusters	Unique No. of enzymes	No. of ESTs	Representative level (%)[a]
1.1 Carbohydrate metabolism	229	126	488	14.38
1.2 Energy metabolism	77	50	113	4.84
1.3 Lipid metabolism	115	89	274	7.22
1.4 Nucleotide metabolism	126	45	190	7.91
1.5 Amino acid metabolism	153	149	367	9.61
1.6 Metabolism of other amino acids	56	41	73	3.52
1.7 Glycan biosynthesis and metabolism	91	28	146	5.72
1.8 Biosynthesis of polyketides and nonribosomal peptides	48	8	64	3.02
1.9 Metabolism of cofactors and vitamins	152	66	196	9.55
1.10 Biosynthesis of secondary metabolites	80	49	147	5.03
1.11 Xenobiotics biodegradation and metabolism	97	48	257	6.09

[a]The representative level (%) was calculated from [(number of clusters/total number of clusters)×100%].

Table 2

The most abundant transcripts in *S. carpocapsae* cDNA library.

Cluster	Number of ESTs	Accession	*E*-Value	Identity (%)	Description	Organism
SC00199.cl	19	YP_026087	4.8e−193	91.9	Cytochrome *c* oxidase subunit I	*S. carpocapsae*
SC00198.cl	17	CAE70046	6.00e−123	61	Hypothetical protein CBG16478	*C. briggsae*
SC00197.cl	16	XP_518669	6.00e−120	72	Similar to ribosomal protein L3	*Pan troglodytes*
SC00196.cl	13	CAE56763	1.3e−93	94.6	Hypothetical protein CBG24566	*C. briggsae*
SC00195.cl	10	NP_741371	2.9e−89	67.8	Ribosomal protein (rpl-7A)	*C. elegans*
SC00194.cl	10	NP_568027	1.9e−06	40	Arabinogalactan protein 18	*A. thaliana*
SC00193.cl	10	NP_505175	2.9e−09	65.1	F52E1.13b/Oxidation resistance protein	*C. elegans*
SC00192.cl	11	ABK80760	9.8e−38	87.9	60S ribosomal protein L37a	*Dictyocaulus viviparus*
SC00184.cl	8	CAE75137	4.7e−73	57.4	Glutathione S-transferase	*C. briggsae*
SC00191.cl	8	No hit	–	–	–	–
SC00190.cl	7	XP_395309	9.6e−117		Similar to nop5 CG10206-PA	*Apis mellifera*
SC00150.cl	10	CAE74467	5.1e−65	60.7	Hypothetical protein CBG22213	*C. briggsae*
SC00187.cl	8	No hit	–	–	–	–
SC00186.cl	7	NP 957146	8.5e−66	85.3	Ribosomal protein S9	*Danio rerio*
SC00183.cl	7	CAA21030	1.6e−20	69.5	Hypothetical protein Y39A1A.21a	*C. elegans*
SC00182.cl	6	YP_026085	6.2e−100	93	Cytochrome *c* oxidase subunit III	*S. carpocapsae*
SC00189.cl	5	AAK95186	3.7e−45	81.5	40S ribosomal protein S4	*Ictalurus punctatus*
SC00181.cl	5	CAE73419	2.9e−37	78.7	Hypothetical protein CBG20862	*C. briggsae*
SC00180.cl	5	YP_026084	2.8e−166	92.6	Cytochrome *b*	*S. carpocapsae*
SC00179.cl	6	CAE62570	1.5e−50	69.	Transthyretin-like family	*C. briggsae*
SC00178.cl	5	NP_490676	2.2e−79	72.4	Ribosomal Protein, Large subunit family member (rpl-7)	*C. elegans*
SC00139.cl	8	ABY74341	2.00e−29	34	Trypsin-like serine protease	*S. carpocapsae*
SC00176.cl	5	ABR87485	1.5e−82	80	Small subunit ribosomal protein 8	*Koerneria sp. RS1982*
SC00175.cl	8	EDP32835	2.00e−102	71	60S ribosomal protein L5	*B. malayi*
SC00174.cl	5	No hit	–	–	–	–
SC00173.cl	6	CAE58485	9.0e−44	49	Hypothetical protein CBG01629	*C. briggsae*

Table 3

Proteins predicted with a potential role in *S. carpocapsae* parasitism.

Cluster	Residues	Start	SP	*E*-Value	AA-identity (%)	Accession	Homology	Organism
Serine-type peptidases								
SC00074.cl	205	–	–	4.00e−118	199/199 (100%)	ABY74341	Trypsin-like serine protein	*S. carpocapsae*
SC00145.cl	201	–	14	2.00e−96	168/201 (83%)	ABY74341	Trypsin-like serine protein	*S. carpocapsae*
SC00177.cl	202	M	16	4.00e−21	61/176 (34%)	ABY74341	Trypsin-like serine protease	*S. carpocapsae*
SC00306.cl	236	M	16	1.00e−40	95/230 (41%)	ABI18379	Elastase precursor	*S. carpocapsae*
SC00808.cl	268	–	–	2.00e−81	169/263 (64%)	ABI18379	Elastase precursor	*S. carpocapsae*
SC01033.cl	99	–	–	2.00e−18	42/62 (67%)	ABI18379	Elastase precursor	*S. carpocapsae*
SC00590.cl	248	–	–	4.00e−07	29/57 (50%)	CAE61256	Peptidase_S10/Serine carboxypeptidase	*C. briggsae*
SC00993.cl	273	M	18	9.00e−104	174/276 (63%)	CAA88947	Peptidase_S10/Serine carboxypeptidase	*C. elegans*
SC01031.cl	99	–	–	2.00e−20	48/80 (60%)	P52715	Serine carboxypeptidase F13S12.6 precursor	*C. elegans*
Metallopeptidases								
SC00974.cl	248	–	–	100e−118	206/256 (80%)	EDP34514	Metallopeptidase family	*C. briggsae*
SC00777.cl	207	–	–	1.00e−35	82/205 (40%)	XP_001633564	Similar to zinc metalloprotease	*Nematostella vectensis*
SC01262.cl	872	M	–	1.00e−79	142/240 (59%)	NP_509528	Metalloprotease	*C. elegans*
SC01069.cl	280	–	–	4.00e−105	179/259 (69%)	XP_001902361	Zinc carboxypeptidase family protein	*C. briggsae*
SC00878.cl	104	–	–	4.00e−17	41/65 (63%)	CAE61804	Astacin-like metalloprotease with metridin-like ShK toxin domain	*C. briggsae*
Aspartic-type peptidases								
SC00110.cl	393	M	15	1.00e−98	179/284 (63%)	CAE72985	Aspartyl protease family member (asp-1)	*C. briggsae*
SC00113.cl	210	–	17	3.00e−63	122/207 (58%)	CAE58212	Aspartyl protease family member	*C. briggsae*
SC00155.cl	229	M	21	1.00e−50	109/204 (53%)	AAO25989	Aspartyl protease protein 2	*C. elegans*
SC01368.cl	197	–	–	3.00e−53	106/198 (53%)	AAO25989	Aspartyl protease protein 2, isoform a	*C. elegans*
SC01223.cl	169	–	–	2.00e−51	95/166 (57%)	AAO25989	Aspartyl protease family member (asp-2)	*C. elegans*
Cysteine-type peptidases								
SC00304.cl	97	–	–	3.00e−45	82/97 (84%)	NP_507199	Cathepsin L cysteine proteinase	*C. elegans*
SC00750.cl	199	–	–	4.00e−15	46/121 (38%)	CAJ58500	Cathepsin L cysteine proteinase	*C. elegans*
SC01394.cl	196	–	–	2.00e−50	94/156 (60%)	NP_506318	Cathepsin X	*C. elegans*
Serine proteases inhibitors								
SC00031.cl	231	–	–	8.00e−21	45/90 (50%)	AAA92314	Trypsin Inhibitor	*C. elegans*

Cluster	Residues	Start	SP	*E*-Value	AA-identity (%)	Accession	Homology	Organism
SC00230.cl	125	–	18	8.00e−16	31/63 (49%)	CAE59191	Trypsin Inhibitor	*C. elegans*
SC00121.cl	222	M	20	5.00e−05	48/143 (33%)	AAA92314	Inhibitor of serine protease like protein	*C. elegans*
SC00324.cl	251	–	23	5.00e−23	63/216 (29%)	ABY28279	Serpin protein 7, isoform d	*C. elegans*
SC01506.cl	76	–	–	3.00e−04	30/79 (37%)	AAZ38762	Serine protease inhibitor 1	*D. immitis*
SC00861.cl	192	–	–	4.70e−13	43/113 (38%)	AAI02748	Serpin peptidase inhibitor, clade C (antithrombin), member 1	*Bos taurus*
SC01586.cl	80	M	22	2.00e−04	24/71 (33%)	CAL36507	Trypsin Inhibitor like cysteine rich domain	*C. elegans*
SC00959.cl	133	M	16	3.00e−08	48/130 (36%)	CAE72085	Inhibitor of serine protease like protein 2	*C. briggsae*
SC01464.cl	215	–	–	1.00e−50	103/246 (41%)	NP_497206	BPTI/Kunitz family of serine protease inhibitors.	*C. elegans*
SC00107.cl	184	M	19	2.00e−13	54/162 (33%)	CAE64052	BPTI/Kunitz family of serine protease inhibitors	*C. briggsae*
Cysteine protease inhibitor								
SC00022.cl	104	M	18	9.00e−06	28/93 (30%)	CAK50389	Cystatin-like peptide	*A. simplex*
SC00262.cl	92	–	–	1.00e−16	40/90 (44%)	ABI18378	Cysteine protease inhibitor precursor	*S. carpocapsae*
SC00822.cl	72	–	–	1.00e−56	108/111 (97%)	ABI18378	Cysteine protease inhibitor precursor	*S. carpocapsae*
Other known proteins								
SC0061.cl	285	–	–	4.00e−125	206/281 (73%)	ABQ58117	Acetylcholinesterase 1	*Ditylenchus destructor*
SC00553.cl	62	–	–	2.00e−90	152/216 (70%)	ABQ58115	Acetylcholinesterase 3	*D. destructor*
SC00758.cl	264	–	–	9.00e−91	152/216 (70%)	ABQ58115	Acetylcholinesterase 3	*D. destructor*
SC01570.cl	218	–	–	3.00e−28	69/175 (39%)	AAA96175	Acetylcholinesterase precursor (AChE)	*C. elegans*
SC00081.cl	146	–	–	6.00e−05	26/72 (36%)	AAR06853	C-type lectin-3	*Bitis gabonica*
SC00475.cl	188	–	–	5.00e−25	60/145 (41%)	BAA09794	Galectin	*C. elegan*
SC00938.cl	164	–	–	5.00e−35	77/163 (47%)	NP_504629	Legume lectin	*C. elegans*
SC00949.cl	227	M	15	9.00e−82	151/214 (70%)	BAB11971	Galectin LEC-5	*C. elegans*
SC01309.cl	144	–	–	1.00e−57	103/144 (71%)	EDP34209	Lectin C-type domain containing protein	*B. malayi*
SC00264.cl	161	–	–	1.00e−34	79/161 (49%)	CAD20464	Fatty acid retinoid binding protein(Gp-FAR 1)	*Ostertagia ostertagi*
SC00336.cl	164	M	22	4.00e−37	77/160 (48%)	AAA98565	Fatty acid-binding protein homolog precursor	*Ascaris suum*
SC00346.cl	93	M	19	3e−06	33/98 (33%)	AAP80379	Saposin-like	*Entamoeba invadens*
SC00926.cl	257	M	17	2e−59	102/220 (46%)	EDP31692	Saposin-like type B	*B. malayi*
SC00377.cl	173	–	–	8e−28	61/164 (37%)	NP_499625	CD36	*C. elegans*

SP, length of the signal peptide; M, putative start codon.

Table 4

Stress-related proteins predicted in *S. carpocapsae* cDNA library.

Cluster	*E*-Value	AA-identity (%)	Accession	Homology	Organism
Heat-shock proteins					
SC00399.cl	5.00e−108	197/261 (75%)	CAE62006	Hsp90	*C. briggsae*
SC00159.cl	0	393/479 (82%)	CAA06694	Hsp90	*Brugia pahangi*
SC00101.cl	1.00e−57	114/190 (60%)	CAE57585	Hsp70	*C. briggsae*
SC00487.cl	4.00e−43	86/105 (81%)	XP_001900197	Hsp70	*B. malayi*
SC01254.cl	4.00e−60	118/235 (50%)	EDP32131	Hsp70	*B. malayi*
SC00108.cl	6.00e−16	45/74 (60%)	P11141	Hsp70F	*C. elegans*
SC00573.cl	3.00e−123	226/234 (96%)	AAN78300	Hsp70A	*Heterodera glycines*
SC00696.cl	3.00e−95	175/211 (82%)	NP_741117	Hsp60	*C. elegans*
SC01475.cl	5.00e−69	127/169 (75%)	AAM81355	Hsp40	*Steinernema feltiae*
SC01199.cl	2.00e−41	82/147 (55%)	EAT45096	Hsp40	*Aedes aegypti*
SC00870.cl	2.00e−13	44/94 (46%)	XP_001604538	Hsp20	*Nasonia vitripennis*
SC00570.cl	1.00e−08	35/104 (33%)	XP_001668479	Hsp alpha-crystallin-type	*C. briggsae*
SC01013.cl	6.00e−07	33/109 (30%)	CAB03380	Hsp alpha-crystallin-type	*C. elegans*
SC01415.cl	7.00e−17	50/102 (49%)	EDP38262	Hsp20/alpha-crystallin-type	*B. malayi*
Heat-shock protein factors					
SC01220.cl	2.00e−85	167/212 (78%)	CAE59760	Chaperonin TCP-1	*C. briggsae*
SC0696.cl	9.00e−96	176/212 (83%)	NP_741117	Chaperonin TCP-1 family member (cct-5)	*C. elegans*
SC00332.cl	2.00e−128	226/280 (80%)	EDP36116	Chaperonin TCP-1 family, delta subunit	*B. malayi*
SC00013.cl	4.00e−17	44/71 (61%)	EDS36116	Heat-shock factor binding protein 1	*Culex quinquefasciatus*
SC01199.cl	2.00e−41	82/147 (55%)	EAT45096	DNA-J/hsp40	*A. aegypti*
SC00151.cl	1.00e−64	147/331 (44%)	NP_501006	DNaJ domain family member (dnj-11)	*C. elegans*
SC00443.cl	8.00e−86	145/203 (71%)	EDP35424	DnaJ homolog subfamily B member 11 precursor	*B. malayi*
SC00484.cl	3.00e−91	174/276 (63%)	EDP36388	DnaJ homolog subfamily B member 4	*B. malayi*
SC01136.cl	3.00e−56	109/205 (53%)	NP_502126	DNaJ domain family member (dnj-2)	*C. elegans*
SC00534.cl	4.00e−66	118/157 (75%)	CAE60769	Heat-shock chaperonin binding	*C. briggsae*
SC00406.cl	8.00e−45	89/145 (61%)	EDP30208	DnaJ domain containing protein	*B. malayi*
SC00059.cl	3.00e−14	54/131 (41%)	XP_001900052	DnaJ domain containing protein	*B. malayi*

Cluster	*E*-Value	AA-identity (%)	Accession	Homology	Organism
Oxidative defense proteins					
SC00558.cl	7.00e−94	164/194 (84%)	AAT28331	Peroxiredoxin	*H. contortus*
SC01235.cl	9.00e−76	136/179 (75%)	CAE70206	Thioredoxin-like	*C. briggsae*
SC01524.cl	2.00e−58	110/222 (49%)	CAE59167	Glutathione S-transferase (C-terminal-like)	*C. briggsae*
SC00131.cl	2.00e−28	103/206 (50%)	XP_976145	Glutathione S-transferase	*Tribolium castaneum*
SC00359.cl	1.00e−15	55/146 (37%)	XP_001599411	Similar to Glutathione S-transferase	*N. vitripennis*