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ORIGINAL PAPER

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Cloning and characterization of the Cu/Zn superoxide dismutase of *Trichinella pseudospiralis*

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Abstract Copper/zinc (Cu/Zn) superoxide dismutase (SOD) activity was identified for the first time in both crude somatic extracts (CE) and excretory/secretory (E/S) products of *Trichinella pseudospiralis*. It was the dominant SOD in infective-stage larvae. Native polyacrylamide gel electrophoresis of CE and E/S products yielded a prominent band, which was cyanide-sensitive and was partly inhibited by hydrogen peroxide in SOD assay. Cytosolic Cu/Zn SOD was cloned. The 471-bp full-length cDNA sequence contained an open reading frame of 157 amino acids. The gene contained three introns. Quantitative reverse transcription–polymerase chain reaction indicated that the expression of cytosolic Cu/Zn SOD was substantially higher in infective-stage larvae than in adult worms. Cluster analysis showed that the sequence of the Cu/Zn SOD of *T. pseudospiralis*, an adenophorean nematode, is related to those of *Brugia pahangi*, *Acanthocheilonema viteae*, *Onchocerca volvulus*, and *Haemonchus contortus* (all belonging to the sercenentean group).

Introduction

Trichinella pseudospiralis, first discovered by Garkavi (1972) in raccoons in the former Soviet Union, belongs to a unique noncapsulated group of trichinellids. Andrew et al. (1995) reported the first infection in humans. The worm can move freely along host myofibers in situ (Al Karmi and Faubert 1981). Although substantial amounts of excretory/secretory (E/S) antigens of the nematode have been observed in host muscles, few cellular infiltrations are elicited (Li et al. 1999). The adaptive mechanisms used by the parasite to survive in a nonsecluded cellular habitat still remain unknown. However, Stewart et al. (1991) suggested that infection of mice by *T. pseudospiralis* was accompanied by a pronounced suppression of the host inflammatory response. Cytotoxic T lymphocyte response, accompanied by down-regulation of cell-mediated reactions, was depressed.

Reactive oxygen intermediates (ROI) of oxygen, including superoxide anion, hydroxyl radical, and hydrogen peroxide, are produced by activated phagocytes and a variety of biological reactions in the mitochondria of many living organisms. These ROI are toxic to microbes. To defend against intracellular oxidative stress and oxidative killing by the host, many endoparasites have developed special protective mechanisms, including antioxidant enzyme systems. One important antioxidant is superoxide dismutase (SOD), which catalyzes the conversion of superoxide anion into hydrogen peroxide and molecular oxygen. It exists commonly in most organisms to protect against endogenous oxidative stress. Three types of SODs have been reported, depending on the metal cofactor [i.e., copper/zinc (Cu/Zn) SOD, manganese (Mn) SOD, and iron (Fe) SOD]. Mn SODs occur in the prokaryotes and in the matrix of mitochondria (Henkle-Duhrsen et al. 1995). Fe SODs occur in prokaryotes and chloroplasts of some plants. Intracellular and extracellular Cu/Zn SODs have been found in the cytosol and E/S products of some eukaryotes (Amemura-Maekawa et al. 1996).

A review of the characteristics of antioxidant enzyme families in parasitic nematodes was performed by Henkle-Duhrsen and Kampkötter (2001). SOD activities have been reported in some sercenentean nematodes (e.g., ascarids such as *Ascaris suum*, *Toxascaris leonine*, *Toxocara canis*, and *Toxocara cati*) (Sanchez-Moreno et al. 1987). Cu/Zn SOD was detected in E/S products of the hookworm *Necator americanus* (Taiwo et al. 1999). Extracellular Cu/Zn SOD activity has also been demonstrated in several tissue-dwelling filarioids (e.g., *Acanthocheilonema viteae*, *Onchocerca volvulus*, *Brugia pahangi*, and *Brugia*

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malayi). Cu/Zn SODs of the latter three species have been cloned (James et al. 1994; Tang et al. 1994; Ou et al. 1995). *Dirofilaria immitis* and *Dictyocaulus viviparous* secrete a substantial amount of Cu/Zn SOD (Sato et al. 1994; Callahan et al. 1993). The extracellular Cu/Zn SOD of the trichostrongyloid *Haemonchus contortus* has been suggested to facilitate its survival in the host gastrointestinal tract (Liddell and Knox 1998).

However, little is known about SODs in adenophorean nematodes to which *Trichinella* belongs. These nematodes have unconventional life cycles. Rhoads (1983) reported the presence of Cu/Zn SOD in both the crude somatic extracts (CE) and the incubation fluid of the infective-stage larvae of *Trichinella spiralis*, an encapsulated species. The SOD exists as a dimer, with a molecular mass of 36 kDa and a p*I* of 5.6. Fukumoto et al. (1987) noted SOD activity in the somatic extracts of the infective-stage larvae of *T. pseudospiralis*, but no details were given. Based on histochemical study, Hadas et al. (1993) observed that the activities of SOD in muscles infected with *T. pseudospiralis* were about twice those harboring *T. spiralis*. However, the dominant type of dismutase of *T. pseudospiralis* and its molecular structure have not been identified. It is also not known whether different levels of SOD gene expression occur at different stages of worm development. Knowledge on the latter may help to elucidate the survival mechanisms adopted by the nematode at intestinal and muscular niches. Therefore, the present study was undertaken to determine the prominent SOD activity in the CE and E/S products of this noncapsulated trichinellid, to clone and characterize its cytosolic Cu/Zn SOD, and to compare the expression level of the gene between the muscle larvae and the adult worms. This work represents part of our study on intracellular adaptations of trichinellid nematodes.

Materials and methods

Parasite and experimental infection

The strain of *T. pseudospiralis* used was kindly provided by Professor Donald Lee, formerly of the School of Biology, the University of Leeds, UK. The worm has since been maintained routinely in the laboratory by serial passages through ICR mice. Each mouse was orally fed about 1,000–1,500 infective-stage larvae using a Pasteur pipette. On day 40 postinfection, the infective-stage larvae were recovered from skeletal muscles of mice by standard pepsin digestion method (Campbell 1983). After recovery, the larvae were washed three times with 0.85% saline.

Isolation of CE and E/S products

To prepare the CE, larvae were washed twice with phosphate-buffered saline (PBS). Worms resuspended in 2 ml of PBS were homogenized six times, using an Ultra-Turrax T25 homogenizer. The sample was centrifuged for 10 min at 2,000×*g*. The supernatant was filtered using a 5-ml syringe with a 0.22-μm pore size membrane. To collect the E/S products, about 100,000 infective-stage larvae were maintained for 24 h at 37°C in a 50-ml canted-neck cell culture flask, containing 15 ml of RPMI 1640 medium, 100 IU/ml penicillin G, 100 μg/ml streptomycin, 0.25 mg of amphotericin, and 1/4 portion of proteinase inhibitor cocktail tablet (Boehringer Mannheim). After incubation, the worms were removed by centrifugation at 800×*g*. The E/S products in the medium were concentrated 100-fold. Protein concentrations were determined by the Bradford dye-binding procedure.

Determination of SOD activity

Electrophoresis of 1.56 μg of CE and 25 μg of E/S proteins in a 12.5% nondenaturing polyacrylamide gel was performed. After electrophoresis, the gel was shaken for 15 min with an SOD assay solution (2.8×10^{-5} M riboflavin, 0.036 M KH_2PO_4, and 0.028 M TEMED) under illumination. After discarding the assay solution, the gel was shaken with 10 ml of nitro blue tetrazolium (NBT; 2 mg/ml 4-nitro blue tetrazolium chloride; Roche). Inhibitors such as hydrogen peroxide or potassium cyanide were added at concentrations of 5 and 2 mM, respectively. The gels were shaken in the dark for 10 min before being placed in beakers for overnight illumination.

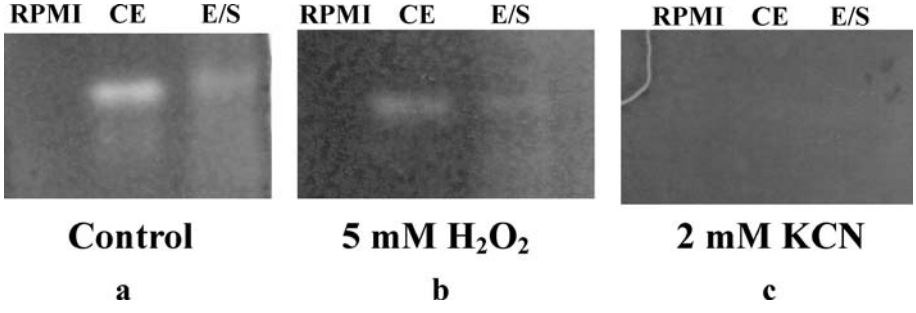

Fig. 1 Native gel electrophoresis showing SOD activity in CE and E/S products of the infective-stage larvae of *T. pseudospiralis*. Samples were separated in a 12.5% nondenaturing polyacrylamide gel. After electrophoresis, the gels were stained for SOD activity, with or without inhibitors. The RPMI medium served as negative control. **a** Without inhibitors. **b** Samples treated with 5 mM H_2O_2. **c** Samples treated with 2 mM KCN

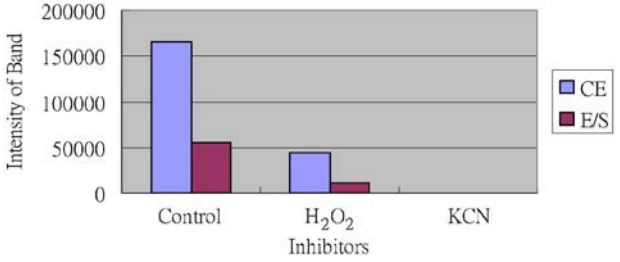

Fig. 2 Results (obtained by scanning the gels in Fig. 1 with a densitometer) showing a decrease in SOD activities after treatment with inhibitors

Cloning of cDNA by degenerative reverse transcription–polymerase chain reaction and rapid amplification of cDNA end

Larvae resuspended in 2 ml of Trizol reagent were homogenized using a polytron homogenizer. Total RNA was isolated following the protocols of the QuickPrep Total RNA Extraction Kit (Amersham). Single-stranded cDNA was synthesized by reverse transcription primed with an adaptor (5′-CTGCCAGAGATCTGATATCGTTTTTTTTT TTTTTTTTTT-3′).

Forward and reverse degenerative primers were designed based on the conserved regions of Cu/Zn SODs. They were designated as ZNSDF3 (5′-DSIGGISVICAYT WYAAYCC-3′) and ZNSDR4 (5′-ACGRSHWCCAGCR TTRCCRGT-3′). PCR was performed in the following conditions: 1 cycle of initial denaturation at 94°C for 1 min, 32 cycles of denaturation at 94°C for 45 s, annealing at 45°C for 60 s, extension at 68°C for 60 s, and an extra cycle of extension at 68°C for 5 min.

SMART PCR cDNA Synthesis Kit (Clontech) was used to synthesize single-stranded cDNA. Specific forward primer TpZnSDF5 (5′- CCATTTGGTAAAACTCACGGTGG) and reverse primer TpZnSDR6 (5′- TCCCAAATCATCTTCTTG AAT) were used for 3′ and 5′ rapid amplification of cDNA end (RACE), respectively. RACE reactions were performed in the following conditions: 1 cycle of initial denaturation at 94°C for 1 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 42°C for 60 s, extension at 68°C for 60 s, and an extra cycle of extension at 68°C for 2 min. RACE products were electrophoresed in a 1.2% agarose gel. They were cloned into pGEM-T Easy vector (Promega) and sequenced. Primers TpZnExS (5′-ATGCCGTTTAAAGCAATTTG) and TpZnExAS (5′-TCAAGCTGCCGGATTTGCAA) were used to amplify the full-length cDNA and the genomic DNA of Cu/Zn SOD by PCR.

Cloning of genes

Genomic DNA was isolated by standard method. PCR was performed in the following conditions: 1 cycle of initial denaturation at 94°C for 3 min 30 s, 30 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 60 s, extension at 68°C for 2 min, and an extra cycle of extension at 68°C for 5 min. Products of genomic PCR were cloned and sequenced. Known protein sequences of Cu/Zn SODs of various organisms were used to construct a phylogenetic tree using the CLUSTAL W program (www.ebi.ac.uk/clustalw/).

Semiquantitative reverse transcription–polymerase chain reaction

Single-stranded cDNA of infective-stage and adult worms were amplified by actin primers (as internal control) and Cu/Zn-specific primers (TpZnExS and TpZnExAS). PCR was performed in the following conditions: 29 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 1 min, and extension at 68°C for 45 s. PCR products were resolved in 1.2% agarose.

Fig. 3 Complete cDNA sequence and deduced amino acid sequence of the Cu/Zn SOD of *Trichinella pseudospiralis*. The putative stop codon is marked by an *asterisk*

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1   ATGCCGTTTAAAGCAATTTGTGTAATTCGTGGTGAAAATGTTACTGGTACCGTGATCTTC  60
    M  P  F  K  A  I  C  V  I  R  G  E  N  V  T  G  T  V  I  F

61  AAACAGAACACGGAAAATGATAAAACAACCATCACTGGAGAAATAAAAGGCTTGACTCCT 120
    K  Q  N  T  E  N  D  K  T  T  I  T  G  E  I  K  G  L  T  P

121 GGGAAACATGGATTCCACGTTCATGAATGGGGTGATAATAGCATGGGTTGCATTTCTGCT 180
    G  K  H  G  F  H  V  H  E  W  G  D  N  S  M  G  C  I  S  A

181 GGTGCTCACTACAATCCATTTGGTAAAACTCACGGTGGACCCACTGATACTGTACGTCAT 240
    G  A  H  Y  N  P  F  G  K  T  H  G  G  P  T  D  T  V  R  H

241 GTGGGAGATTTGGGTAATATCGTGGCCGGTTCAGATGGTGTTGCAAAAATTGATATTGTG 300
    V  G  D  L  G  N  I  V  A  G  S  D  G  V  A  K  I  D  I  V

301 GACGATCAGATCAAACTGACAGGTGAACATTCAATAATTGGTCGCACTATGGTAGTGCAT 360
    D  D  Q  I  K  L  T  G  E  H  S  I  I  G  R  T  M  V  V  H

361 ATTCAAGAAGATGATTTGGGAAAAGGAGGGTATGACGAAAGCCTGAAAACTGGAAATGCT 420
    I  Q  E  D  D  L  G  K  G  G  Y  D  E  S  L  K  T  G  N  A

421 GGAGCACGTGTTGGATGTGGAGTAATTGGCATTGCAAATCCGGCAGCTTGA 471
    G  A  R  V  G  C  G  V  I  G  I  A  N  P  A  A  *
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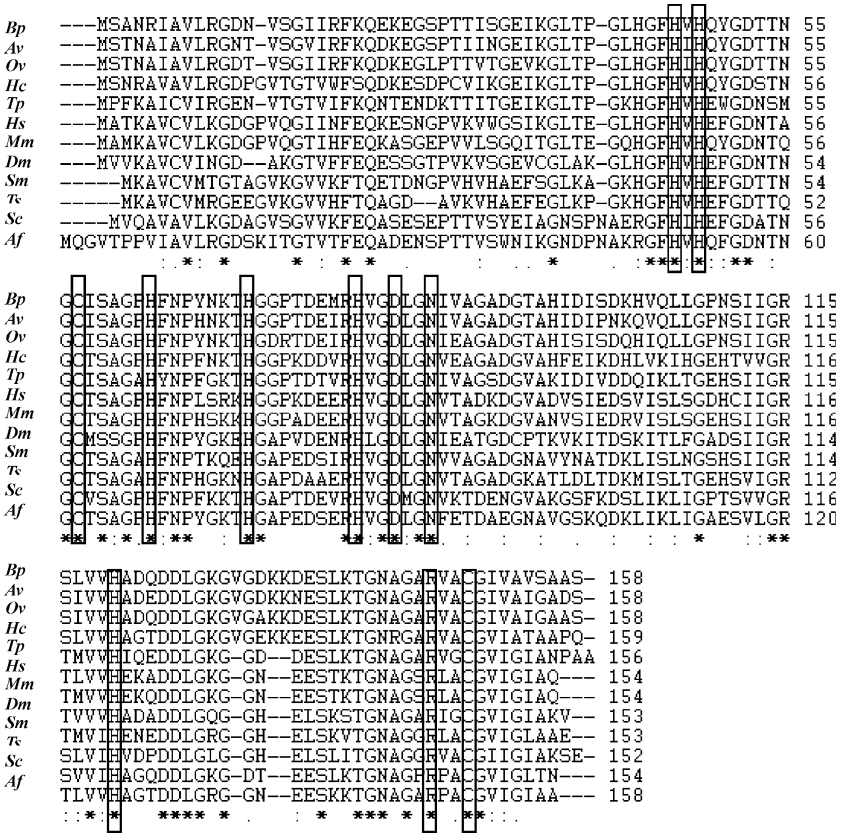

Fig. 4 Alignment of the amino acid sequence of the Cu/Zn SOD cytosolic gene of *T. pseudospiralis* with those of other species. *Bp Brugia pahangi* (GI:457482)**; *AvAcanthocheilonema viteae* (GI:5441514); *OvOnchocerca volvulus* (GI:134618); *HcHaemonchus contortus* (GI:1199519); *Tp Trichinella pseudospiralis* (GI:21702730); *HsHomo sapiens* (GI:4507149); *MmMus musculus* (GI:134614); *DmDrosophila melanogaster* (GI:8647); *SmSchistosoma mansoni* (GI:161121); *Ts Taenia solium* (GI:18252397); *ScSaccharomyces cerevisiae* (GI:6322564); *AfAspergillus fumigatus* (GI:66851351). Identical amino acid residues are indicated by (*) and similar amino acid residues are indicated by (:). Conserved amino acids are *boxed*. Histidine (*H*) at positions 43, 45, 60, 68, 77, and 117 and aspartate (*D*) at position 80 are involved in metal binding. Cysteine (*C*) residues at positions 54 and 143 are involved in disulphide bond formation. Arginine (*R*) at position 140 is involved in directing the superoxide anion to the copper atom. Asparagine (*N*) at position 83 is involved in N glycosylation. (**) GenBank accession number

Results

In vitro characterization of SOD activity

Superoxide dismutase activities in both CE and E/S products of infective-stage larvae were studied by native gel assay. After electrophoresis, the samples were immersed in an SOD assay solution containing flavin. Under illumination, the latter was reduced and generated O_2^-. O_2^- reduced NBT to blue formazan. When SOD activity occurred, an achromatic zone appeared on the gel. For both CE and E/S products, the experiments yielded a single band of similar molecular mass (Fig. 1a), indicating the presence of SOD activity. SOD activity was partially inhibited by 5 mM H_2O_2 (Fig. 1b) but was completely inhibited by 2 mM KCN (Fig. 1c). Similar results were obtained using 10 mM H_2O_2 and 4 mM KCN (data not shown). The results indicated that the detected SOD activities were those of Cu/Zn SOD. The intensity of the bands on the gels in different treatments was determined by a densitometer. A drastic drop in SOD activities occurred when inhibitors were used (Fig. 2).

Cloning and sequencing of Cu/Zn SOD

A 250-bp reverse transcription–polymerase chain reaction (RT-PCR) product was obtained using degenerate primers of Cu/Zn SODs (data not shown). Specific forward and reverse primers were designed for RACE-PCR. The complete

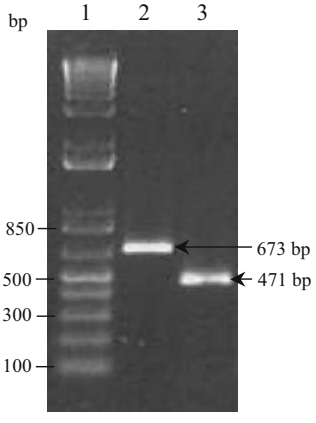

Fig. 5 PCR amplification of Cu/Zn SOD from *T. pseudospiralis* genomic DNA and cDNA with primers TpZnExS and TpZnExAS. Ten microliters of PCR products was resolved in a 1.2% agarose gel. *Lane 1*: 1-kb Plus DNA marker; *lane 2*: genomic Cu/Zn SOD; *lane3*: Cu/Zn SOD cDNA

Table 1 Exon–intron boundaries of the Cu/Zn cytosolic SOD of *Trichinella pseudospiralis*

Exon–Intron–exon–intron boundaries				Exon size (bp)	Intron size (bp)
1	…TTC AAA CAG	gtaatttc…gaatgcag	AAC ACG	66	53
2	…GGA CCC ACT	gtaagtgt…tattatag	GAT ACT	159	97
3	…GAT TTG G	gttagtat…tttaag	GA AAA	154	52
4	…GCA GCT TGA			92	

The 5′ donor (gt) and the 3′ acceptor (ag) are underlined

coding sequence of the Cu/Zn SOD of *T. pseudospiralis* contained 471 bp, encoding 157 amino acid residues (Fig. 3) (GenBank accession no. GI:21702730). Analysis of the protein sequence indicated that the Cu/Zn SOD contains conserved histidine and aspartate residues for dismutation and metal binding. It includes six histidine (H) residues at positions 43, 45, 60, 68, 77, and 117 and an aspartate (D) residue at position 80. It has a conserved arginine (R) residue located at position 140 to enhance the binding of O_2^- to the copper atom. The two-cysteine residues in positions C-54 and C-143 are involved in disulphide bond formation. This is essential to maintaining the functional structure of the enzyme. Asparagine N-83 is also conserved. It is believed to be involved in N glycosylation (Fig. 4).

Characterization of gene structure

The Cu/Zn SOD gene was amplified by genomic PCR and then sequenced. Compared to the cDNA sequence, the gene contained an extra 202 bp (Fig. 5). Sequence analysis indicated that the gene contained three introns. All introns were delineated by the conserved dinucleotide GT/AG boundary. The largest intron was 97 bp in length (Table 1).

Expression of SOD during worm development

The level of expression of the gene in infective-stage larvae and adult worms was compared by semiquantitative RT-PCR. Primers specific to actin served as an internal control to standardize the amount of cDNA used for PCR. The expression level of Cu/Zn SOD mRNA was significantly higher in infective-stage larvae than in adult worms (Fig. 6).

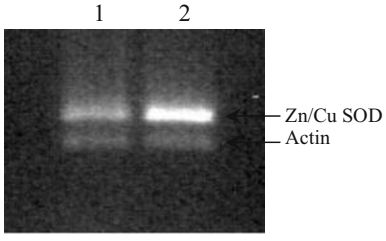

Fig. 6 Results of the semiquantitative RT-PCR of the Cu/Zn SOD of *T. pseudospiralis*. Actin primers served as internal control. *Lane 1*: result of adult-stage worms; *lane 2*: result of infective-stage larvae

Phylogenetic relationships of the Cu/Zn SOD gene

Using the CLUSTAL W program, a phylogenetic tree was constructed (Fig. 7). The result shows that the gene of *T. pseudospiralis* is more related to those of the trichostrongyle *H. contortus* (85%) and the filarioid nematodes *B. pahangi* (78%), *A. viteae* (76%), and *O. volvulus* (75%). It is, however, distinct from that of the trematode *Schistosoma mansoni* and the cestode *Taenia solium*.

Discussion

The present study reports, for the first time, the complete sequence of the cytosolic Cu/Zn SOD gene of an adenophorean nematode *T. pseudospiralis*. Prominent SOD activities in both the E/S products and the cytosolic site were of the Cu/Zn type. Basic information on the gene's structure and on its differential expression in the larval and adult stages was also elucidated. Such information may provide the basis for further studies on host–parasite interactions and on survival strategies between capsulated and noncapsulated trichinellids.

Using actin as an internal control in semiquantitative RT-PCR, we found that cytosolic Cu/Zn SOD was highly expressed in infective (first-stage) larvae, whereas in adult worms, the expression level was substantially low. The reason for such difference is not known. However, according to Ou et al. (1995), cytosolic SODs are supposed to be constitutively expressed housekeeping genes. Therefore, expression levels in infective-stage larvae and adult worms should be similar. However, Lattemann et al. (1999) observed an upregulation of one of the SODs of *A. viteae* after the transmission of third-stage larvae into the mammalian host. The authors suggested that an increase in SOD activity may be necessary for the filarioid to counteract oxidative stress in the vertebrate host. However, the situation is different in *Trichinella* because it is a one-host parasite.

One possible explanation for our observation is that the monoxenous *Trichinella* lives in two perilous niches (i.e., the adults live in the small intestine and the infective first-stage larvae live in skeletal muscles) (Wright 1989). This is especially true for *T. pseudospiralis*, whose larvae in skeletal muscles are unprotected by a collagenous capsule yet elicit few cellular infiltrations. In these niches, there are substantial differences in the exposure of worms to host immune response and physiological stress (Ko and Mak 1999). Immune cells release hydrogen peroxide and su-

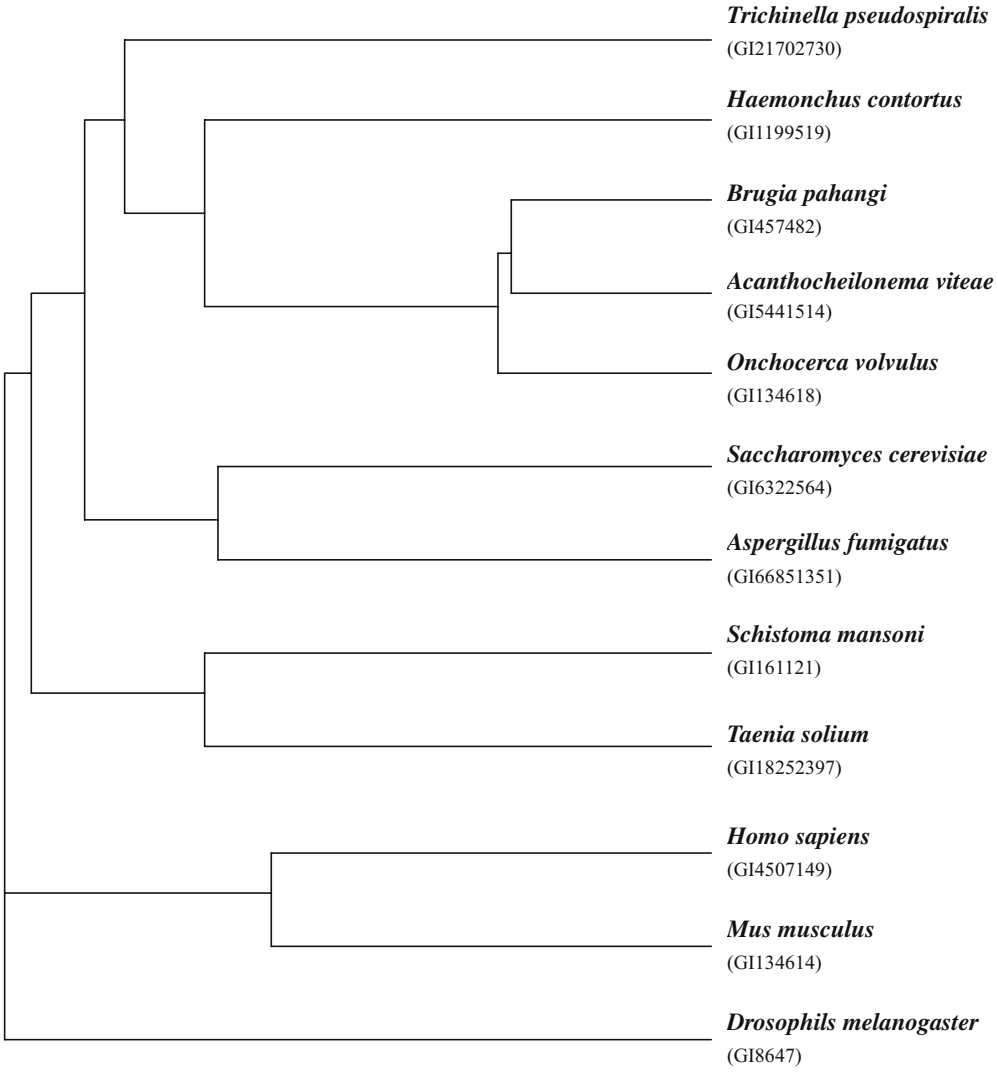

Fig. 7 Phylogenetic tree (constructed using the CLUSTAL W program) showing the relationship between the Cu/Zn SOD of *T. pseudospiralis* and the Cu/Zn SOD of other organisms. GenBank accession numbers are in *brackets*

peroxide anions, which may interact to form singlet oxygen and hydroxyl radicals through the Haber–Weiss reaction. According to Callahan et al. (1993), an upregulation of SOD can protect the parasite from host response by detoxifying host-generated reactive oxygen radicals. The exposure of worms to different oxidative stresses may lead to a different response by muscle-stage larvae. This may account for differences in the expression of SOD activities between stages. Nevertheless, this is only a speculation, which requires further study. However, Kazura and Meschnick (1984) reported a low activity of SOD in newborn larvae of *T. spiralis*.

The Cu/Zn SOD gene of *T. pseudospiralis* was found to contain an open reading frame of 471 bp, encoding 157 amino acid residues. The predicted size of this cloned Cu/Zn SOD protein is comparable to that reported by Rhoads (1983) for the infective-stage larvae of *T. spiralis*. The author used immunological methods to purify a metalloprotein with superoxide activity. He reported that the protein was a dimer consisting of two 16-kDa subunits.

Three introns and four exons were observed in the Cu/Zn SOD gene of *T. pseudospiralis*. This small number of introns may be a common feature. It is similar to that of *Caenorhabditis elegans*, which contained three introns, with 608 bp encoding a protein of 158 amino acids (Giglio et al. 1994).

Genomic PCR and sequencing analysis revealed that the deduced sequence of *T. pseudospiralis* Cu/Zn SOD possessed conserved residues for dismutation and metal binding. It was composed of several histidine and aspartate residues for Cu/Zn binding. Two cysteine residues were located near the 5′ and 3′ regions. No hydrophobic signal peptide was found in the 5′ region of the cloned sequence. This indicates that the cloned gene is cytosolic. However, we failed to clone extracellular SOD. This may be due to the fact that the level of mRNA expression can be six times higher in cytosolic SOD than in the extracellular type (Liddell and Knox 1998). The compliance of degenerative primers and the annealing temperature may also favor the amplification of the former. Nevertheless, as shown by the native gel experiment, extracellular SOD occurs in *T. pseudospiralis*. Such SOD was reported in *O. volvulus* and *A. viteae* (James et al. 1994; Lattemann et al. 1999).

The CLUSTAL W program indicates that the Cu/Zn SOD gene of *T. pseudospiralis* is more related to those of *Haemonchus* and filarioids than to those of Platyhelminthes, humans, and *Drosophila melanogaster*. This may be due to closer phylogenetic relationships between tissue-dwelling parasitic nematodes. The relatively high homology between the genes of the adenophorean and the sercenentean nematodes probably indicates that both groups had followed a similar path in the evolution of survival strategy.

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References

Al Karmi TO, Faubert GM (1981) Comparative analysis of mobility and ultrastructure of intramuscular larvae of *Trichinella spiralis* and *Trichinella pseudospiralis.* Int J Parasitol 67:685–691

Amemura-Maekawa J, Kura F, Watanabe H (1996) Cloning and nucleotide sequences of iron and copper–zinc superoxide dismutase genes of *Legionella pneumophila* and their distribution among *Legionella* species. Jpn J Med Sci Biol 49:167–186

Andrews JR, Bandi C, Pozio E, Gomez Morales MA, Ainsworth R, Abernethy D (1995) Identification of *Trichinella pseudospiralis* from a human case using random amplified polymorphic DNA. Am J Trop Med Hyg 53:185–188

Callahan HL, Hazen-Martin D, Crouch RK, James ER (1993) Immunolocalization of superoxide dismutase in *Dirofilaria immitis* adult worms. Infect Immun 61:1157–1163

Campbell WC (1983) *Trichinella* and trichinosis. Plenum, New York

Fukumoto S, Takechi M, Kamo H, Yamaguchi T (1987) Comparative studies on soluble protein profiles and isozyme patterns of seven *Trichinella* isolates. Parasitol Res 73:352–357

Garkavi (1972) The species of *Trichinella* isolated from wild carnivores. Veterinarija 10:90–91

Giglio AM, Hunter T, Bannister JV, Bannister WH, Hunter GJ (1994) The copper/zinc superoxide dismutase gene of *Caenorhabditis elegans*. Biochem Mol Biol Int 33:41–44

Hadas E, Rodriguez-Caabeiro F, Jimenez GA (1993) Superoxide dismutase of *Trichinella spiralis* and *Trichinella pseudospiralis* larvae. Trop Med Parasitol 44:195–196

Henkle-Duhrsen K, Kampkötter A (2001) Antioxidant enzyme families in parasitic nematodes. Mol Biochem Parasitol 114:129–142

Henkle-Duhrsen K, Tawe W, Warnecke C, Walter RD (1995) Characterization of the manganese superoxide dismutase cDNA and gene from the human parasite *Onchocerca volvulus*. Biochem J 308:441–446

James ER, McLean DC Jr, Perler F (1994) Molecular cloning of an *Onchocerca volvulus* extracellular Cu–Zn superoxide dismutase. Infect Immun 62:713–716

Kazura JW, Meshnick JR (1984) Scavenger enzymes and resistance to oxygen mediated damage in *Trichinella spiralis*. Mol Biochem Parasitol 10:1–10

Ko RC, Mak CH (1999) Trichinellosis as a model of new frontier research on parasitic infection. J Int Med Res 3:1–11

Lattemann CT, Matzen A, Apfel H (1999) Up-regulation of extracellular copper/zinc superoxide dismutase mRNA after transmission of the filarial parasite *Acanthocheilonema viteae* in the vertebrate host *Meriones unguiculatus*. Int J Parasitol 29:1437–1446

Li CK, Chung YY, Ko RC (1999) The distribution of excretory/secretory antigens during the muscle phase of *Trichinella spiralis* and *T. pseudospiralis* infections. Parasitol Res 85:993–998

Liddell S, Knox DP (1998) Extracellular and cytoplasmic Cu/Zn superoxide dismutases from *Haemonchus contortus*. Parasitology 116:383–394

Ou X, Tang L, McCrossan M, Henkle-Duhrsen K, Selkirk ME (1995) *Brugia malayi*: localisation and differential expression of extracellular and cytoplasmic Cu/Zn superoxide dismutases in adults and microfilariae. Exp Parasitol 80:515–529

Rhoads ML (1983) *Trichinella spiralis*: identification and purification of superoxide dismutase. Exp Parasitol 56:41–54

Sanchez-Moreno M, Leon P, Garcia-Ruiz MA, Monteoliva M (1987) Superoxide dismutase activity in nematodes. J Helminthol 61: 229–232

Sato K, Aoki T, Nakano M (1994) *Dirofilaria immitis*: a large-scale purification method and partial characteristics of a superoxide dismutase from adult worms. Exp Parasitol 78:210–216

Stewart GL, Niederkorn JY, Kennedy RR, Mayhew E (1991) Effect of acute versus chronic *Trichinella pseudospiralis* infections on systemic cell-mediated immunity. Int J Parasitol 21:935–940

Taiwo FA, Brophy PM, Pritchard DI, Brown A, Wardlaw A, Patterson LH (1999) Cu/Zn superoxide dismutase in excretory–secretory products of the human hookworm *Necator americanus*. An electron paramagnetic spectrometry study. Eur J Biochem 264:434–438

Tang L, Ou X, Henkle-Duhrsen K, Selkirk ME (1994) Extracellular and cytoplasmic Cu/Zn superoxide dismutases from *Brugia* lymphatic filarial nematode parasites. Infect Immun 62:961–967

Wright K (1989) Parasites in peril—the trichuroid nematodes. In: Ko RC (ed) Current concepts in parasitology. University of Hong Kong Press, Hong Kong, pp 65–80