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In silico modeling and characterization of phytoparasitic nematodes translationally-controlled tumor proteins

R.M. Moraes Filho[1,3], A.F. Menezes[1] and L.S.S. Martins[2,3]

[1]Departamento de Agronomia da Universidade Federal Rural de Pernambuco, Recife, PE, Brasil
[2]Departamento de Biologia, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil
[3]Programa de Pós-Graduação em Agronomia/Melhoramento Genético de Plantas, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil

Corresponding author: R.M. Moraes Filho
E-mail: romulommfilho@yahoo.com.br

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ABSTRACT. Plant parasitic nematodes infect a wide range of hosts representing the largest source of biotic stress experienced by plants. *Meloidogyne* genus comprises the most important parasitic nematodes, also known as root-knot nematodes. These parasitic organisms obtain nutrients to support their development through complex interactions with their hosts. The translationally-controlled tumor protein (TCTP) is widely expressed in eukaryotic organisms, and is related to a great diversity of biological processes such as calcium binding, cell proliferation and growth, pluripotency, regulation of apoptosis, microtubules stabilization, and histamine release. TCTP has been identified in the secretions of plant-parasitic nematodes, and may play a role in suppressing the plant immunity and programmed cell, hence promoting nematode parasitism. Our results revealed a high conservation

of the evaluated protein sequences and little variation in their physico-chemical characteristics, such as isoelectric points and hidropathicity. Phylogenetic analysis also revealed the presence of three main groups of TCTPs, corresponding to plant parasitic, animal parasitic and free-living nematodes. Six plant parasitic TCTPs tertiary structure models were generated by homology modeling. The constructed models were highly similar and most of the structural variations occurred outside the characterized functional domains. To our knowledge, these are the first theoretical models of plant parasitic nematodes TCTPs and these results may provide a theoretical basis for future studies of host plant resistance to nematode infection.

Key words: Computational analysis; Parasitism resistance; Homology modeling

INTRODUCTION

The nematodes, or roundworms, are organisms adapted to a wide range of environmental conditions. Most nematodes are free-living, but a great number of them are parasites of animals and plants. Plant parasitic nematodes, or phytonematodes, represent the largest source of biotic stress experienced by plants and can cause stunting, early senescence, or even total crop loss. Almost all crop plants are parasited by at least one nematode species and the devastating root-knot nematodes (*Meloidogyne* spp) and cyst nematodes (*Globodera* spp, *Heterodera* spp) are the most important parasitic nematode group, causing billions of dollars of agricultural losses each year (Bird, 2004; Haegeman et al., 2009; Perry and Moens, 2011).

Nematode infection triggers complex changes in plant gene expression as they penetrate the roots of the host. They induce dramatic changes in the selected root vascular cells, forming elaborate feeding cells to permanently supply nutrients needed to their development to reproductive adults (Hussey et al., 2002). Phytonematodes all share a hollow needle-like structure that is used to withdraw nutrients of plant cells on which they feed. In addition to this, they secrete cell-wall degrading enzymes (CWDE) to allow migration through the plant root, and secretory effector proteins (SEP) to suppress host defense responses (Williamson and Gleason, 2003; Davis et al., 2004; Baum et al., 2007; Davis et al., 2008; Perry and Moens, 2011; Haegeman et al., 2012; Rai et al., 2015). In response to pathogen infection, plants can induce a hypersensitive reaction that causes a rapid death of cells surrounding the site of infection;thus, restricting the growth and spread of pathogens (Heath, 2000; Jones and Dangl, 2006; Chisholm et al., 2006; Zhuo et al., 2016). Ability to suppress this programmed cell death could be preponderant to successful parasitism in plant parasitic nematodes.

The translationally-controlled tumor protein (TCTP) is a highly conserved protein widely expressed in eukaryotic organisms. It is a multifunctional protein related to biological processes such as calcium binding, cell proliferation and growth, pluripotency, regulation of apoptosis, microtubules stabilization, and histamine release (Bommer and Thiele, 2004; Arcuri et al., 2004; Koziol and Gurdon, 2012). TCTP is related to interact with a large number of different proteins, however, its binding partners are not well conserved in eukaryotes, and therefore its alternative functions may differ from an organism to another (Wu et al., 2015). It was first identified in mice (Yenofsky et al., 1982) and humans (Gross et al., 1989), and then in

other organisms such as yeasts, invertebrates and plants (Bonnet et al., 2000; Gnanasekar et al., 2002; Rao et al., 2002; Hoepflinger et al., 2013). TCTP has been identified in the secretions of animal-parasitic nematodes (Gnanasekar et al., 2002), and of *Meloidogyne enterolobii*, a plant parasitic nematode (Zhuo et al., 2016). Expressed specifically within the dorsal oesophageal gland, which produces SEPs, *M. enterolobii* TCTP may play a role in suppressing the plant immunity and programmed cell, hence promoting nematode parasitism (Zhuo et al., 2016).

Little is known about biological functions, structure and conservation of TCTP plant parasitic nematodes. Therefore, the aim of the present study was to characterize structurally and functionally, TCTP sequences from *M. enterolobii* and other phytoparasitic nematodes with bioinformatic methodologies, and develop tertiary structure models of these proteins. This study will provide valuable theoretical insights for TCTP functions and structure in plant parasitic nematodes that can be useful for future studies involving these proteins and host plant resistance to nematode infection.

MATERIAL AND METHODS

Data retrieval from plant pathogenic, animal pathogenic, and free living nematodes

MeTCTP sequence described by Zhuo et al. (2016) was used as query in the tool BLASTp from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) database. Sequences of animal and free-living nematodes with high similarity to the query were selected and their FASTA protein sequences were retrieved (Table 1). Additionally, we ran the BLASTp tool to search for protein homologues from the plant parasitic nematodes genomes in the wormbase database (parasite.wormbase.org). This database contains five publically available genomes of completely sequenced plant parasitic nematodes, *Meloidogyne floridensis*, *Meloidogyne incognita*, *Meloidogyne hapla*, *Bursaphelenchus xylophilus*, and *Globodera pallida*.

Sequence analysis

Physico-chemical parameters of phytonematodes TCTP sequences were analyzed by ProtParam (http://web.expasy.org/protparam) (Gasteiger et al., 2005). Subcellular localizations were predicted by the CELLO2GO server (Yu et al., 2014). The presence of signal peptide cleavage sites were investigated using the TOPCONS server (http://topcons.cbr.su.se/) (Tsirigos et al., 2015).

Phylogenetic analysis

Sequence alignment of TCTPs was performed with ClustalW algorithm implemented in Molecular Evolutionary Genetic Analysis (MEGA 6.06) (Tamura et al., 2011), with default parameters. The phylogenetic tree was constructed using the neighbor-joining method for 2000 bootstrap replicates.

Protein-protein interactions

STRING 9.1 database (Franceschini et al., 2013) (http://string-db.org/) was used

to predict potential interacting proteins. The database contains information from numerous sources, including experimental repositories, computational prediction methods, and public text collections.

Tertiary structure prediction, evaluation, and validation of the model

3-D models of TCTPs were generated using the Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2) (Kelley et al., 2015) in multi-template intensive mode and visualized by UCSF Chimera package (Pettersen et al., 2004). Model quality was evaluated using the Molprobity server (http://molprobity.biochem.duke.edu/) (Chen et al., 2010) by Ramachandran plot analysis. Z-score was calculated using interactive ProSA-web server (https://prosa.services.came.sbg.ac.at/prosa.php) to recognize errors in 3-D structures (Wiederstein and Sippl, 2007).

RESULTS AND DISCUSSION

In this study, we analyzed TCTP sequences from plant parasitic nematodes from the order Tylenchida and homologue sequences from animal parasitic and free-living nematodes. The protein sequences were retrieved in FASTA format from the NCBI database and from complete genomes of plant parasitic nematodes (Table 1).

Table 1. Fourteen evaluated TCTP sequences identifications and their species of origin.

Species	Identification	Family	Common name	Mode of life
Bursaphelenchus xylophilus	BXY_0592900.1	Aphelenchoididae	Pine wilt nematode	Plant parasitic
Globodera pallida	GPLIN_000622600	Heteroderidae	Cyst nematode	Plant parasitic
Meloidogyne enterolobii	JN968577.1	Meloidogynidae	Root-knot nematode	Plant parasitic
Meloidogyne incognita	Minc02150	Meloidogynidae	Southern Root-knot nematode	Plant parasitic
Meloidogyne hapla	MhA1_Contig1983.frz3.gene9	Meloidogynidae	Northern Root-knot nematode	Plant parasitic
Meloidogyne floridensis	Scaf00174-processed-gene-0.5-mRNA-1	Meloidogynidae	Root-knot nematode	Plant parasitic
Brugia malayi	XP_001897741.1	Onchocercidae	Agent of lymphatic filariasis	Animal parasitic
Dirofilaria immitis	AGI74995.1	Onchocercidae	Canine heartworm nematode	Animal parasitic
Loa loa	EFO28099.2	Onchocercidae	Eye worm	Animal parasitic
Toxocara. canis	KHN75998.1	Toxocaridae	Dog roundworm	Animal parasitic
Caenorhabditis briggsae	XP_002639808.1	Rhabditidae	Roundworm	Free living
Caenorhabditis elegans	NP_492767.1	Rhabditidae	Roundworm	Free living
Caenorhabditis remanei	XP_003112086.1	Rhabditidae	Roundworm	Free living
Pristionchus pacificus	ABF69523.1	Neodiplogasteridae	Roundworm	Free living

Physico-chemical properties of the TCTP protein sequences were deduced by the ProtParam server. Table 2 shows the molecular weight, theoretical isoelectric point (pI), and grand average of hydropathicity (GRAVY) of the evaluated protein sequences.

As shown in Table 2, plant parasitic TCTP sequences varied in size from 156 (*M. hapla*) to 186 (*M. enterolobii*) amino acids. The molecular weight varied from 17.78 kDa (*M. hapla*) to 21.56 kDa (*M. enterolobii*). The pI was between 4.67 (*G. pallida*) and 5.48 (*M. hapla*). The GRAVY was between -0.572 (*M. enterolobii*) and 0.402 (*G. pallida*). ProtParam analysis revealed proteins with very similar physico-chemical properties. All TCTP sequences were predicted to be hydrophilic. As seen in Table 2, the pI of the evaluated proteins was between 4.67 and 5.48 indicating their acidic character. The pI of a protein indicates the pH at which the protein is most unstable and is least soluble (Shaw et al., 2001).

TOPCONS server was used to predict post-translational modifications. This tool predicts the presence and location of signal peptide cleavage sites in protein sequences incorporating a prediction of cleavage sites and a signal peptide/non-signal peptide prediction

based on a combination of several artificial neural networks. TOPCONS predicted that none of the evaluated sequences had an N-terminal signal peptide, this fact was expected as demonstrated by other studies that TCTP is secreted by a non-classical pathway without the aid of signal peptides (Amzallag et al., 2004, Bommer and Thiele, 2004; Zhuo et al., 2016).

The analysis of subcellular location with the CELLO2GO server predicted that the evaluated TCTPs are intracellular proteins, being classified as cytoplasmatic or nuclear.

Table 2. Primary structure analisys and subcellular locations of the evaluated TCTP sequences.

Origin species	Protein size	MW (kDa)	pI	GRAVY	SPCS	ScL
B. malayi	181	20.766	4.62	-0.485	-	Cy, Nc
B. xylophilus	181	20.622	4.71	-0.501	-	Cy, Nc
C. briggsae	181	20.626	4.78	-0.360	-	Cy
C. elegans	181	20.542	4.78	0.370		Cy
C. remanei	181	20.598	4.78	-0.386	-	Cy
D. immitis	181	20.739	4.62	-0.425	-	Cy, Nc
G. pallida	180	20.391	4.67	-0.402	-	Cy
L. loa	187	21.398	4.69	-0.452	-	Cy, Nc
M. enterolobii	186	21.536	4.72	-0.572	-	Cy, Nc
M. floridensis	173	19.780	4.74	-0.498	-	Cy
M. hapla	156	17.779	5.48	-0.505	-	Cy, Nc
M. incognita	179	20.584	4.75	-0.445	-	Cy
P. pacificus	170	19.315	4.75	-0.402	-	Cy, Nc
T. canis	181	20.880	4.72	-0.482	-	Cy

Multiple alignment and phylogenetic analysis

To examine the phylogenetic relationships of the evaluated TCTP sequences, a multiple alignment was performed by ClustalW algorithm implemented in the MEGA 6.06 software (Figure 1). The signature regions of TCTPs described by Bommer and Thiele (2004) and Microtubule binding sites (Gachet et al., 1999) are highlighted in the multiple alignment. All three regions are present in the 14 evaluated sequences and are highly conserved. A phylogenetic tree was constructed using the neighbor-joining (NJ) method and the bootstrap test carried out with 2000 replicates. For this analysis, animal parasitic and free-living nematodes were included in the alignment to evaluate the conservation of TCTPs in the phylum Nematoda. In the NJ tree, the evaluated TCTP sequences from 14 nematode species were divided into three groups by similarity (Figure 2). Plant parasitic and animal parasitic TCTPs showed more similarity to each other than to TCTPs from free living nematodes.

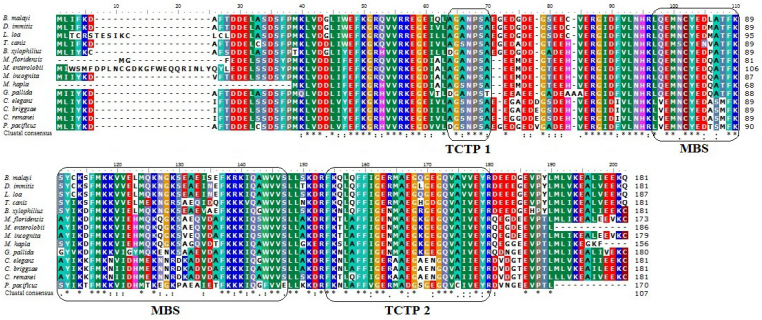

Figure 1. Sequence alignment nematodes TCTP proteins. Sequences were aligned by ClustalW, and identical and similar residues are displayed in the same color. Microtubule-binding sites (MBS) (Gachet et al., 1999) and TCTP 1 and TCTP2 signature sequences (Bommer and Thiele, 2004) are indicated below the sequences.

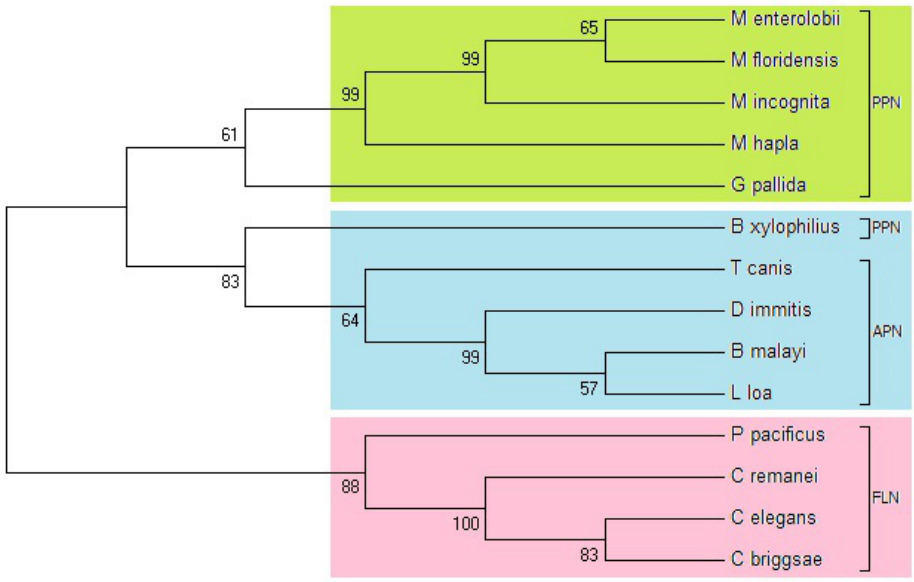

Figure 2. Neighbor-joining phylogenetic tree of plant parasitic (PPN), animal parasitic (APN), and free-living (FLN) nematodes TCTPs. The percentage of 2000 bootstrap replicate is given at each node. Based on the phylogenetic tree result, TCTP sequences were divided into three groups with different colors.

Functional interaction network analysis

In order to predict protein interactions of TCTPs, *Caenorhabditis elegans* TCTP was analyzed by the STRING 9.1 tool as the most similar representative of the MeTCTP in the software database. STRING is a database of known and predicted protein interactions.

STRING 9.1 revealed 20 putative interaction partners with score >0.9 for *C. elegans* TCTP (Figure 3a). Seventeen of these identified partners were ribosomal proteins, the other three were an elongation factor (eef-2) that catalyzes the GTP-dependent ribosomal translocation step during translation elongation, an ubiquitin (Ubq-2) and an hypothetical protein (Y37E3.8). STRING also showed TCTP co-expression patterns with these proteins (Figure 3b). Experimental evidences points that TCTP is co-regulated with ribosomal proteins (Brown et al., 2000; Langdon et al., 2004). TCTP was also described to bind an elongation factor, which is responsible for the transport of the aminoacyl tRNA to the ribosome during protein synthesis (Le Sourd et al., 2006; Sasikumar et al., 2012; Wu et al., 2015). STRING results suggest that TCTP and the identified functional partners are strongly correlated and likely in the same biological pathway.

Tertiary structure prediction, evaluation and validation of the model

The six plant parasitic TCTPs were selected for tertiary structure prediction by homology modeling. The model was constructed using the Phyre2 server. To produce a three dimensional model, Phyre2 uses the alignment of hidden Markov models via HHsearch (Söding, 2005) to improve the accuracy of alignment and detection rate, and incorporates

an *ab initio* folding simulation to model regions in the proteins with no detectable homology to known structures, the Poing tool (Jefferys et al., 2010). The methodology also combines multiple templates to improve model accuracy if necessary.

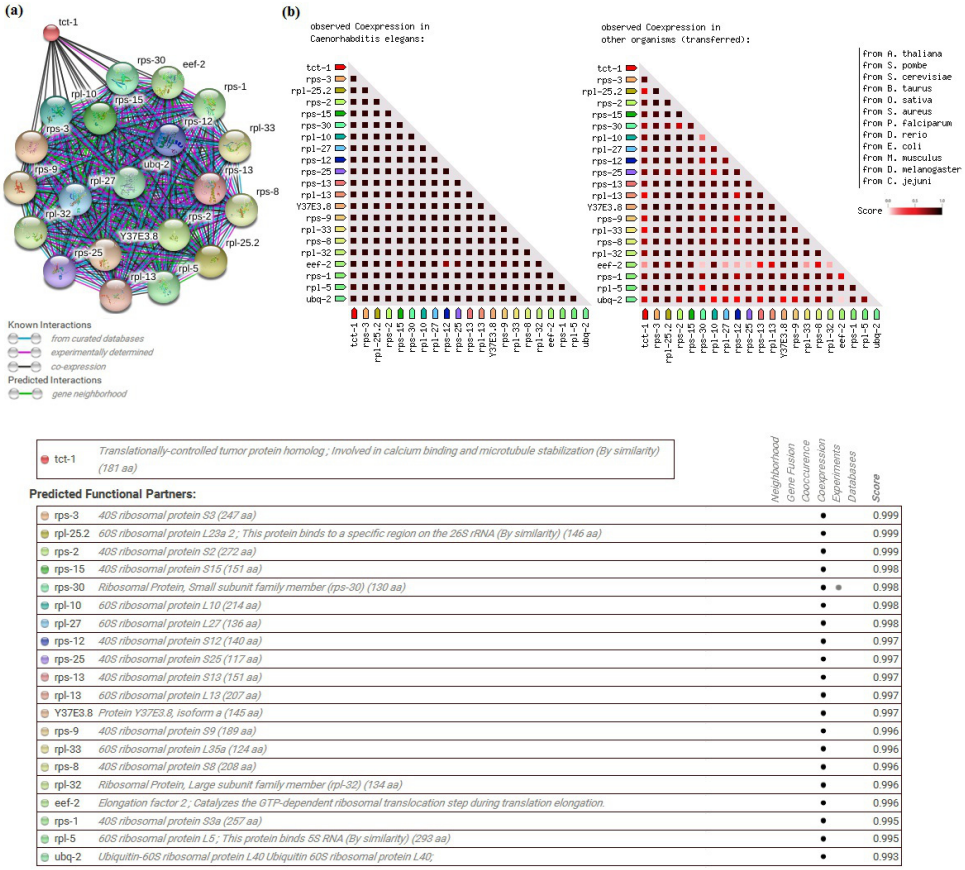

Figure 3. Protein-protein interactions predicted by the STRING 9.1 tool. **a.** Predicted Interactive network of *Caenorhabditis elegans* TCTP. **b.** Coexpression of *C. elegans* TCTP and other eukaryotic species: the intensity of color indicates the level of confidence that two proteins are functionally associated, given the overall expression data in the organism.

Figure 4 shows the six models of plant parasitic TCTPs generated. Colored regions are correspondent to the TCTP1, TCTP2 and MBS signature regions. Verification of stereochemical quality of the models using Ramachandran plot analysis was performed by the Molprobity server. ProSA-web (Protein Structure Analysis web) was used for error recognition in the tertiary structure prediction of the models. The Z-score was used to measure energy, as it indicated overall quality of the model. Positive Z-score values show that the structure is not stabilized while zero and negative scores represent one of the ideal structures. The generated models had 88.59% (*M. enterolobii*) to 94.16% (*M. hapla*) of amino acid residues were in Ramachandran plot analysis favored regions and Z-scores of -5.06 (*B. xylophilius*) to -3.90 (*M. enterolobii*) (Table 3). Due to the presence of Ramachandran outliers, model refinement

was carried out with the KiNG software (Chen et al., 2009). MeTCTP validation results are showed in Figure 5. The plot of residue scores shows local model quality by plotting energies as a function of amino acid sequence position (Figure 5b). Positive values correspond to problematic or erroneous parts of the input structure. As was demonstrated in the graph of Figure 5c, most of amino acid residues of MeTCTP are below zero on x-axis.

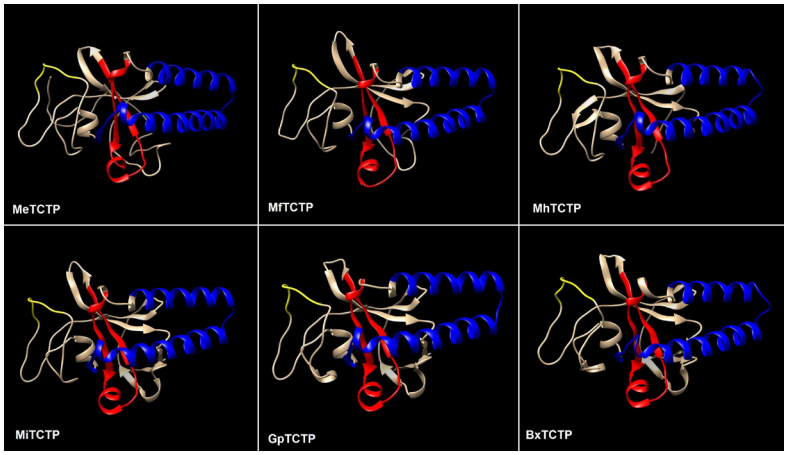

Figure 4. Tertiary structure prediction of phytonematodes TCTPs. Colored regions are correspondent to TCTP1 (yellow), MBS (blue) and TCTP2 (red).

Table 3. Modeling and validation results of phytonematodes TCTPs.

Origin Species	PDB Models	*Ab initio* residues	Identity	Query coverage	Z-score	%RP	Outliers
M. enterolobii	c2kwbA	20	70%	89%	-3.90 (-3.84)	88.59% (96.74%)	2.17% (0)
M. floridensis	c2kwbA	2	72%	90%	-4.56 (-4.61)	92.40% (97.66%)	0.58% (0)
M. hapla	c2kwbA	1	71%	99%	-4.99 (-4.91)	94.16% (98.70%)	1.30% (0)
M incognita	c2kwbA	0	73%	100%	-5.01(-4.92)	93.22% (98.87%)	1.69% (0)
B. xylophilius	c2kwbA	0	72%	100%	-5.06 (-4.91)	89.39% (98.32%)	2.79% (0)
G pallida	c2kwbA	0	72%	100%	-4.68 (-4.67)	89.33% (97.75%)	2.81% (0)

The six models have highly similar structures and variations occurred mostly outside the TCTP1, TCTP2 and MBS regions. Figure 6a shows the surface area of the MeTCTP and the location of the signature regions. Hydrophobicity generated by the UCSF Chimera software as can be seen in Figure 6b, confirms the hydrophilic character of TCTP. The electrostatic surface (Figure 6c) shows a mostly negatively charged surface for MeTCTP.

Knowledge on protein three-dimensional structures is of great importance to understand its functions and molecular interactions. Homology modeling methodologies compares a given sequence with proteins with known tertiary structure to construct a theoretical model. Bioinformatics analysis, can play a vital role in the interpretation of proteomic data. These methodologies have been extensively used for predicting function and structure of proteins from its amino acid sequences (Darabi and Seddigh, 2015; Vatansever et al., 2015; Moraes Filho and Martins, 2016). In this study, we present the first tertiary models of TCTPs from *Meloidogyne enterolobii* and correlated plant parasitic species obtained from public databases. These findings can provide useful information on the molecular basis of the functions of these proteins and the understanding of nematode infection processes.

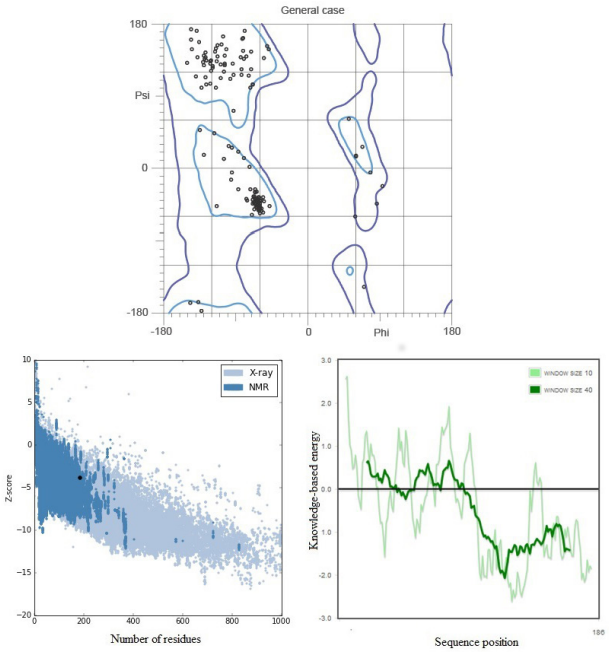

Figure 5. a. Ramachandran plot of MeTCTP generated by the MOLPROBITY server. **b.** ProSA-web Z-score plot of MeTCTP showing the Z value (black dot) and **c.** ProSA-web plot of MeTCTP showing the energy graph of residue scores of a native protein structure.

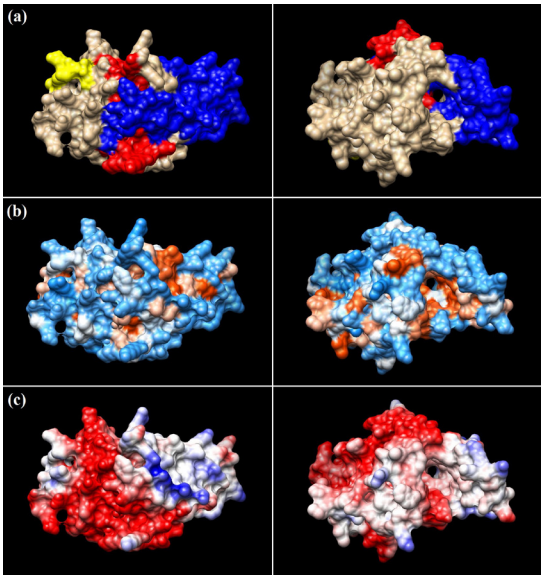

Figure 6. Tertiary structure prediction of MeTCTP. **a.** MBS, TCTP1 and TCTP2 regions are highlighted in blue, yellow and red respectively. **b.** Hydrophobicity represented as a color gradient, with blue being the most hydrophilic, to white, to orange red for the most hydrophobic. **c.** Electrostatic surface represented as a color gradient, from the most negatively charged (red) to the most positively charged (blue).

Conflicts of interest

The authors declare no conflict of interest.

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