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In Silico Characterization of Glutathione-S-transferase from Genus *Trichinella*

Dr. Somnath Balbhim Waghmare*

Author's Affiliation:
Assistant Professor, Department of Zoology
Nowrosjee Wadia College, Pune, Maharashtra 411001, India

***Corresponding author:**
Dr. Somnath Balbhim Waghmare
Assistant Professor, Department of Zoology
Nowrosjee Wadia College, Pune, Maharashtra 411001, India

E-mail:
drsomnathwaghmare@gmail.com

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Abstract:
Glutathione-S-transferase is a major immunomodulator and belongs to the family of multifunctional enzymes which involves in the various detoxification processes in the nematodes. There are various kinds of membrane-bound glutathione-S-transferases are present and involved in xenobiotic metabolism processes. Glutathione-S-transferases have shown promising properties of a potential target molecule to control the population of Trichinella species which is the causative agent of trichinosis. In silico prediction of gene and proteins is of great importance for structural and functional characterization of genomic and proteomic sequences. In the present investigations, 07 sequences of Glutathione-S-transferase of nematodes from the various species of genus Trichinella were characterized. In silco analysis of Glutathione-S-transferase proteins like their amino acid compositions, atomic composition, the extinction coefficient of protein along with estimated half-life, instability index of protein, aliphatic index and grand average of hydropathicity were also analyzed. Physicochemical properties, secondary and tertiary structural details of proteins with their relationship with each other will help researchers to design a synthetic drug against Trichinellosis. These results may provide theoretical grounds for future studies of nematode vaccine development.

Keywords: *Trichinella*, Glutathione-S-transferase, Nematode, Physicochemical analysis, Structure prediction, Phylogeny.

INTRODUCTION

Nematodes from the Genus *Trichinella* is responsible for Trichinellosis (trichinosis). The main causative organism of trichinosis found worldwide in many carnivorous and omnivorous animals which is accountable for Trichinellosis is *T. spiralis*. In addition to the classical agent *T. spiralis* numerous other species of Trichinella are now recognized these are *T. pseudospiralis* which is present in mammals and birds worldwide, *T. nativa* occurred in Arctic bears, *T. nelsoni* present in African predators and scavengers, and *T. britovi* the parasites of carnivores from Europe and Western Asia. (https://www.cdc.gov/).

The most important and widespread foodborne endoparasitic disease is a Trichinellosis, which resulted after the consumption of the raw or semi-cooked meat infested with Trichinella infective larvae's (Murrell & Pozio, 2011). In animals and humans, Trichinella infestation has been documented in maximum provinces on the Chinese Mainland, and fifteen epidemics of trichinellosis consisting of nearly 1387 cases and four demises occurred from the year 2004-2009 (Wang et al., 2007; Cui et al., 2009; Cui et al., 2013). Consumption of the pork is the leading cause of infestation for trichinellosis outbreaks in China and South Asia. In pigs, Trichinella infestation is the most important problem which affects the meat products security and public health concerns (Jiang et al., 2016). So, an anti-Trichinella vaccine for domestic pigs has become an additional promising measure to avoid the transmission of trichinellosis from pigs to humans (Yang et al., 2015).

In the detoxification reactions, Glutathione-S-transferase (GST) acts as a catalyst and a major immunomodulator of the superfamily member of detoxification enzymes. The GST was expressed in all life-cycle stages of T. spiralis and distributed mainly in the cuticle of the parasitic nematode (Liu et al., 2017). GST is a group of enzymes that are present in various tissues from many different species of organisms. The GST enzyme is responsible for a variety of functions which include detoxification of lipid, nucleic acid hyperoxides and high affinity of many non-substrate hydrophobic ligands like heme and bilirubin also GST helps in the conjugation of the thiol group of glutathione to a variety of electrophiles. Due to the multiple functions of GST, it is the key enzyme of research interest for various researchers including the biochemists and parasitologists. As a main role of enzymes, they catalyze the reaction among the nucleophil reduced glutathione and a large number of electrophilic complexes. Along with this GST also binds to the number of amphipathic compounds which they do not metabolize and have been suggested to play a role as intracellular transport proteins for compounds that have limited solubility in water molecule (Boyer, 1989).

Hence the GST seems to be a significant and vital protein for growth and survival of the endoparasite and could be used as a potential target to design the vaccine which will be capable of preventing parasite infestation.

Currently, parasitologists are focusing on targeting GST as a potential and suitable target candidate for designing a synthetic drug against the disease Trichinosis caused by the nematodes. But in the diverse nematode populations, there is also variability between the species of Trichinella which includes variations in their molecular weight content, stability of protein, their amino acid compositions, family and domain to which they belong, their secondary and tertiary structure, (Prashant et al., 2010).

From various interdisciplinary fields, Bioinformatics is a widely used approach for structural and functional analysis of target proteins using several computations tools and databases. The biological data which has been retrieved and analyzed from various tools and databases of proteins might be important and useful for designing a synthetic drug against the *Trichinella*. By considering the GST from *Trichinella* as a potential drug candidate and applications of in silico approach in the field of molecular biology. The present study is aimed to explore the bioinformatics tools for the study and characterization of GST enzymes from different species of Trichinella for their physicochemical properties, their ancestral relationship and structural determination various levels.

MATERIAL AND METHODS

Sequence retrieval and alignment of the sequence

The Glutathione-S-transferase (GST) protein sequences from different *Trichinella* nematode species were retrieved from the Universal Protein Resources server (Uniprot) and for multiple sequence alignment of the protein sequences; the Clustal Omega (version 1.2.4) algorithm was used.

Physiochemical characterization of proteins

The targeted protein sequence of Glutathione-S-transferase from different *Trichinella* nematode species was analyzed using ProtParam tool for its different Physico-chemical properties like the molecular weight of protein, theoretical pI, the amino acid composition of protein, atomic

composition, the extinction coefficient of protein (Gill et al., 1989; Edelhoch, 1967; Nielsen et al., 2003) along with estimated half-life (Ciechanover and Schwartz, 1989; Varshavsky, 1997), instability index of protein (II) (Guruprasad et al., 1990), aliphatic index (AI) (Ikai et al., 1980) and grand average of hydropathicity of GST Protein (GRAVY) (Kyte and Doolittle, 1982) were analyzed by the ProtParam (Gasteiger et al., 2005).

During protein movement under denaturation environments, the isoelectric point (pI) is determined based on the pK value of protein (Bjellqvust et al., 1993). The purified protein concentration of the sample is assessed from the value of the extinction coefficient (Umang et al., 2012). Instability index (II) is the measure of the stability of protein and the stable proteins are predicted when their instability index is lesser than 40 and when the value of instability index is greater than 40, the protein is observed as unstable (Guruprasad et al., 1990). The thermostability of a globular protein is determined by the volume occupied by aliphatic amino acids side chain (alanine, valine, leucine, and isoleucine) relative to the total volume occupied is called aliphatic index (Walker, 2005). The grand average of hydropathicity (GRAVY) is the hydrophilicity or hydrophobicity of protein and it is the ratio of the sum of hydropathy values of all the amino acids to the total number of residues in the given sequence (Umang et al., 2012). The Kyte and Doolittle scale hydropathy based plots are developed for all the retrieved sequences of GST using Protscale tool (Kyte and Doolittle, 1982).

Phylogenetic analysis of GST from Trichinella species

MEGA X version 10.1 software is used to analyze and construct the common ancestral phylogenetic relationship between the retrieved GST protein sequences of different Trichinella species. The distance tree building and the bootstrap value was set at 1000 by using the Neighbor-joining (NJ) algorithm. The bootstrap value represents the generation of new data sets with substitutions (Kumar et al., 2018).

Secondary structure prediction

The SOPMA (Pradeep et al., 2012) and GOR IV (Ojeiru et al., 2010) tools a self-optimized prediction method with alignment were used for the analysis of secondary structure of GST proteins and results obtained from these two tools were also compared to determine α- helix, β- sheet, turns and loops from the above proteins.

Tertiary structure prediction

GST proteins tertiary structure was predicted and constructed by using "RaptorX" structure prediction server; this server provides high quality of a structural model by using the template of the primary protein sequence (http://raptorx.uchicago.edu/StructurePrediction/predict/).

RESULTS

ProtParam computes various Physico-chemical properties that can be deduced from a protein sequence, these predicted Physico-chemical properties plays a very crucial role and taken into consideration while drug designing or drug development process.

Sequence retrieval and GST protein sequence alignment

The protein sequences of glutathione-S-transferase from different endoparasitic species of nematodes were retrieved from the Uniprot database and the same protein sequences were analyzed by using various Uniprot Tools (Table 1). The molecular weight of GST proteins from Trichinella species varies between the 23,758 to 24,261 Da and all the GST proteins belong to the transferases. Clustal Omega multiple sequence alignment tool was used to align all the proteins for analysis after the multiple sequence alignment it was observed that all the GST protein sequences from the different Trichinella species diverge at the C terminus of the sequence. In the same alignment, some amino acid segments are aligned in the middle with good similarities. The amino acid positions with conservations are indicated with the asterisks (*) and amino acids of relative conservations in the compared sequences are represented by the (.) (Figure 1).

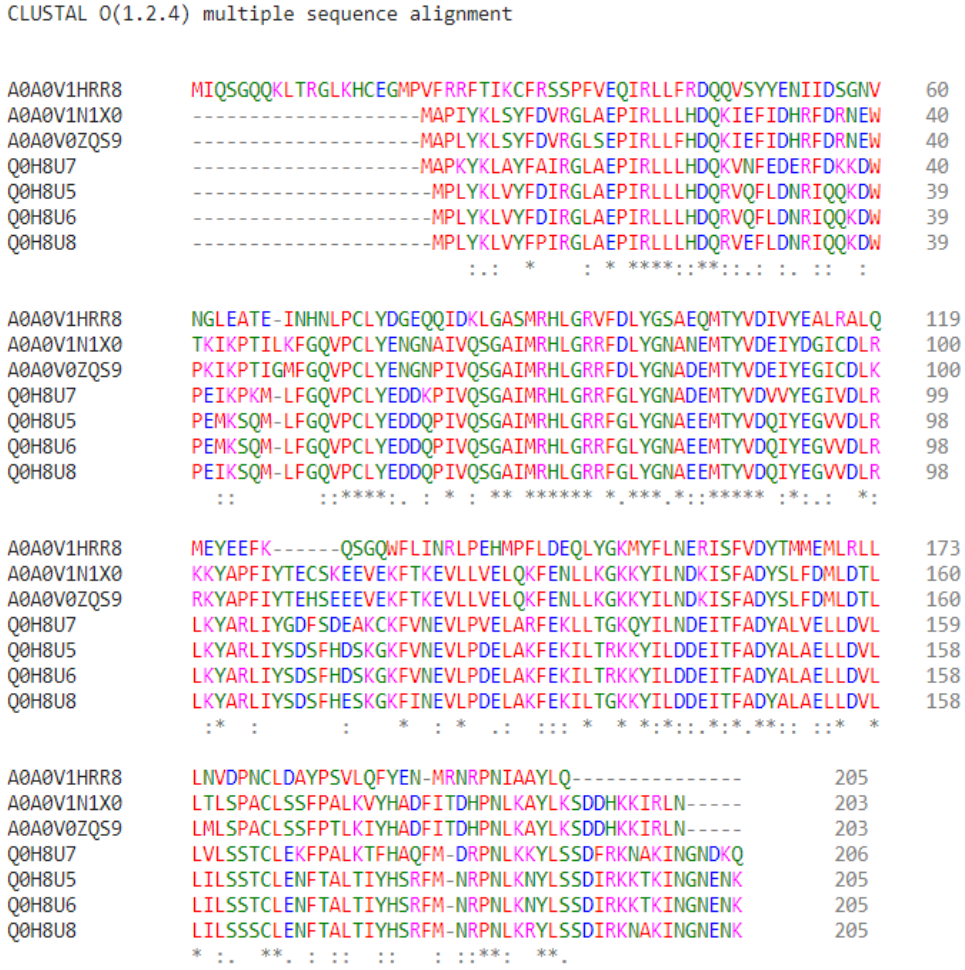


Figure 1: Multiple Sequence Alignment result of GST proteins from *Trichinella species.*

Table 1: Characterization of retrieved sequences of GST protein from *Trichinella species* by using UniProt tool

Sr. No	Nematode	Accession number	Protein	No of amino acids	Molecular weight in (Da)	Molecular function
1	*Trichinella nativa*	Q0H8U5	Transferase	205	24,099	glutathione transferase activity
2	*Trichinella britovi*	Q0H8U6	Transferase	205	24,099	glutathione transferase activity
3	*Trichinella pseudospiralis*	Q0H8U7	Transferase	206	23,960	glutathione transferase activity
4	*Trichinella spiralis*	Q0H8U8	Transferase	205	23,977	glutathione transferase activity
5	*Trichinella papuae*	A0A0V1N1X0	Transferase	203	23,758	glutathione transferase activity
6	*Trichinella zimbabwensis*	A0A0V1HRR8	Transferase	205	24,261	glutathione transferase activity
7	*Trichinella patagoniensis*	A0A0V0ZQS9	Transferase	203	23,901	glutathione transferase activity

Characterization of physicochemical properties of GST proteins

The analysis of physicochemical parameters of GST from different *Trichinella* species like the pI, number of positively and negatively charged amino acids of proteins, analysis of extinction coefficient, instability index of proteins, aliphatic indexes, and grand average of hydropathicity (GRAVY) along with the total number of atoms are shown in the table 2.

Table 2: GST proteins parameters computed using Expasy's Prot Param tool.

S. N.	Nematode	pI	+R	-R	EC (M^{-1} cm^{-1})	II	Stability	AI	GRAVY	Formula	TNA
1	*Trichinella nativa*	6.44	28	29	23505	32.66	Stable	98.44	-0.341	$C_{1091}H_{1713}N_{287}O_{312}S_8$	3411
2	*Trichinella britovi*	6.44	28	29	23505	32.66	Stable	98.44	-0.341	$C_{1091}H_{1713}N_{287}O_{312}S_8$	3411
3	*Trichinella pseudospiralis*	6.84	31	31	22015	24.34	Stable	95.95	-0.270	$C_{1097}H_{1712}N_{280}O_{305}S_8$	3402
4	*Trichinella spiralis*	6.12	27	29	23505	36.81	Stable	101.32	-0.289	$C_{1089}H_{1707}N_{285}O_{310}S_7$	3398
5	*Trichinella papuae*	7.00	29	29	23630	34.23	Stable	95.62	-0.291	$C_{1088}H_{1690}N_{276}O_{304}S_8$	3366
6	*Trichinella zimbabwensis*	5.27	20	26	25120	55.03	Stable	84.63	-0.340	$C_{1086}H_{1667}N_{289}O_{313}S_{15}$	3370
7	*Trichinella patagoniensis*	5.98	27	31	23505	32.97	Stable	90.79	-0.350	$C_{1095}H_{1683}N_{275}O_{307}S_9$	3369

where pI: Isoelectric point, +R: number of positive charged residues (Arg+ Lys), -R: number of negative charged residues (Asp+ Glu), EC: Extinction coefficient at 280 nm, II: Instability index, AI: Aliphatic index, GRAVY: Grand average of hydropathicity, TNA: Total number of atoms.

The Isoelectric Point (pI) is the pH rate at which the movement of protein becomes zero with extra compact and stable conformation. In the studied GST proteins of *Trichinella* species the pI value in *Trichinella papuae* is above 7, these values indicated that GST protein in *Trichinella papuae* contains a greater number of negatively charged amino acids. However, for the remaining species of *Trichinella*, the isoelectric point was found to be smaller than 7 and their GST proteins were acidic which contains a greater number of positive charge residues.

An inherent property of chemical species is the Extinction coefficient (EC) which is the extent of how powerfully a chemical molecule absorbs the light at a specific wavelength. It is depending on the chemical configuration and structure of the material and which is independent of concentration. To determine the extinction coefficient Expasy's Prot Param tool was used which compute the extinction coefficient for a variable range of 276, 278, 279, 280 and 282 nm, however, 280 nm is more ideal because proteins absorb 280nm wavelength more powerfully with the lowest interference from other ingredients. At 280nm *Trichinella zimbabwensis* shows the highest Extinction coefficient of 25120 and *Trichinella pseudospiralis* shows the lowest Extinction coefficient 22015. All the Glutathione-S-transferase from selected *Trichinella* species were observed to be stable with an instability index lower than 40.

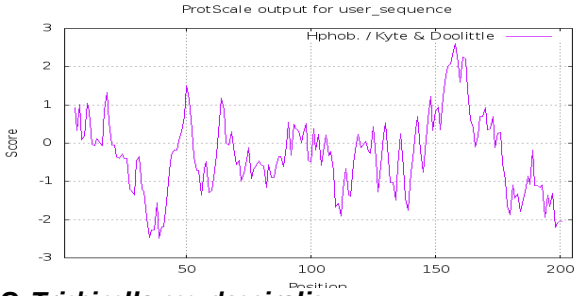
The Aliphatic Index (AI) of a GST protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). *Trichinella spiralis* exhibits more AI values i.e. 101.32 and *Trichinella zimbabwensis* shows the AI values 84.63 this AI values may be regarded as a positive factor for the thermostability of proteins. The computed GRAVY shows negative value for all the GST protein sequences and this has showed improved possibilities of aqueous interactions. The whole number of atoms in different GST proteins is fluctuating from 3366 to 3411. The comparison of GST proteins amino acid composition (%) in different sequences was also carried out (Table 3) and diverse amino acids were observed to be dominant in different sequences.

Table 3: Amino acid Composition of GST (%) predicted in different Trichinella using ProtParam tool.

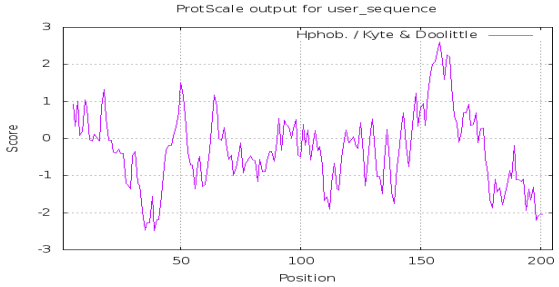
Sr. No	Amino Acid	*Trichinella nativa*	*Trichinella britovi*	*Trichinella pseudospiralis*	*Trichinella spiralis*	*Trichinella papuae*	*Trichinella zimbabwensis*	*Trichinella patagoniensis*
1	Ala (A)	4.40%	4.40%	6.80%	4.90%	5.40%	3.90%	3.90%
2	Arg (R)	6.30%	6.30%	5.30%	6.30%	4.40%	6.80%	4.40%
3	Asn (N)	4.90%	4.90%	3.90%	4.90%	4.40%	5.90%	3.90%
4	Asp (D)	7.80%	7.80%	8.30%	6.80%	7.90%	4.90%	7.90%
5	Cys (C)	1.00%	1.00%	1.50%	1.00%	2.00%	2.00%	1.50%
6	Gln (Q)	4.40%	4.40%	2.90%	3.90%	2.00%	7.30%	2.00%
7	Glu (E)	6.30%	6.30%	6.80%	7.30%	6.40%	7.80%	7.40%
8	Gly (G)	4.40%	4.40%	4.90%	4.90%	3.90%	5.40%	4.40%
9	His (H)	2.00%	2.00%	1.50%	2.00%	3.00%	2.00%	3.40%
10	Ile (I)	6.80%	6.80%	4.90%	7.80%	7.40%	5.40%	7.40%
11	Leu (L)	13.70%	13.70%	13.10%	13.70%	12.80%	11.70%	12.30%
12	Lys (K)	7.30%	7.30%	9.70%	6.80%	9.90%	2.90%	8.90%
13	Met (M)	2.90%	2.90%	2.40%	2.40%	2.00%	5.40%	3.00%
14	Phe (F)	4.90%	4.90%	6.30%	4.90%	5.90%	5.90%	6.40%
15	Pro (P)	3.40%	3.40%	4.40%	3.90%	3.90%	3.90%	4.90%
16	Ser (S)	4.90%	4.90%	2.90%	5.40%	4.40%	4.90%	4.90%
17	Thr (T)	3.40%	3.40%	2.40%	2.40%	3.90%	2.40%	3.40%
18	Trp (W)	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%
19	Tyr (Y)	5.90%	5.90%	5.30%	5.90%	5.90%	6.30%	5.90%
20	Val (V)	4.90%	4.90%	6.30%	4.40%	3.90%	4.90%	3.40%

Kyte and Doolittle hydropathy plots were constructed by using the Protscale tool (Figure 2) and as many of amino acids lie above the zero baseline, the transmembrane area of GST proteins from different *Trichinella* sp. was observed to be rich in hydrophobic amino acids. The minimum and maximum hydrophobic position and score for each GST sequence was also predicted (Table 4) with minimum and maximum score of -3.000 and 2.000 respectively.

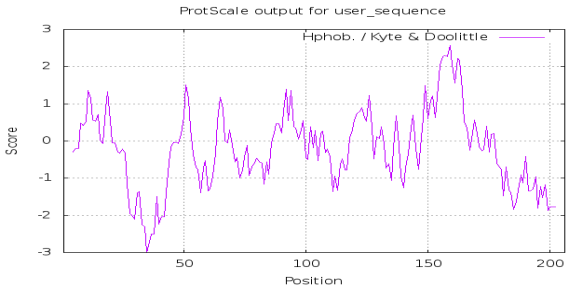
A. *Trichinella nativa*



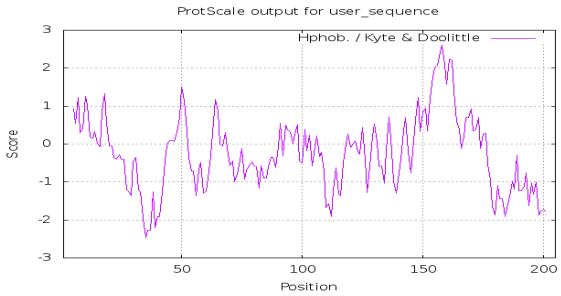
B. *Trichinella britovi*



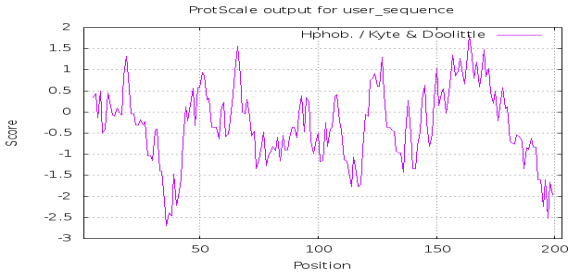
C. *Trichinella pseudospiralis*



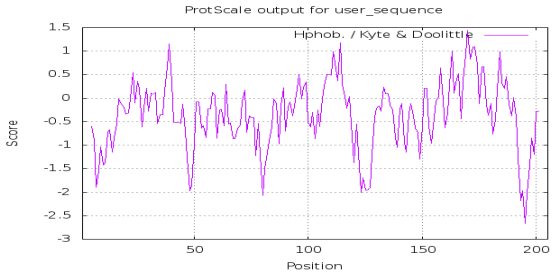
D. *Trichinella spiralis*



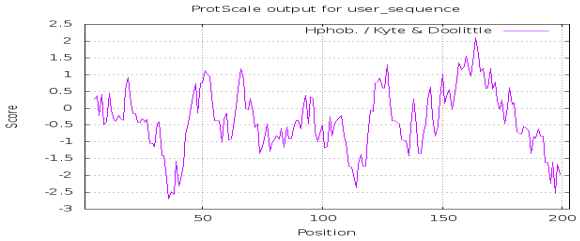
E. *Trichinella papuae*



F. *Trichinella zimbabwensis*



G. *Trichinella patagoniensis*



A. *Trichinella nativa*
B. *Trichinella britovi*
C. *Trichinella pseudospiralis*
D. *Trichinella spiralis*
E. *Trichinella papuae*
F. *Trichinella zimbabwensis*
G. *Trichinella patagoniensis*

Figure 2: Kyte and Doolittle Plots for GST proteins from different *Trichinella* species

Table 4: Computation analysis of Hydrobhobic score and position of GST using Kyte and Deolite ProtScale tool

Sr. No	Nematode	Accession number	Position		Score	
			Min	Max	Min	Max
1	*Trichinella nativa*	Q0H8U5	39	158	-2.489	2.600
2	*Trichinella britovi*	Q0H8U6	39	158	-2.489	2.600
3	*Trichinella pseudospiralis*	Q0H8U7	35	159	-3.000	2.567
4	*Trichinella spiralis*	Q0H8U8	35	158	-2.478	2.600
5	*Trichinella papuae*	A0A0V1N1X0	36	164	-2.700	1.822
6	*Trichinella zimbabwensis*	A0A0V1HRR8	195	170	-2.667	1.433
7	*Trichinella patagoniensis*	A0A0V0ZQS9	36	164	-2.700	2.111

Phylogenetic analysis

Phylogenetic analysis of GST proteins from different *Trichinella* species was carried out by using MEGA X version 10.1 software, which provides a subset of substitution model and neighbor-joining algorithm for distance tree building. Bootstrap values are depicted at the nodes with Bar value of 0.2. (Fig. 3). GST proteins from *Trichinella nativa* and *Trichinella britovi* were found to be closely related to each other while these two species slightly diverged from *Trichinella spiralis*. *Trichinella papuae* and *Trichinella patagoniensis* are closely related to each other while these two species slightly diverge from *Trichinella pseudospiralis* and *Trichinella zimbabwensis*. Secondary structure prediction of GST proteins from Trichinella sp.

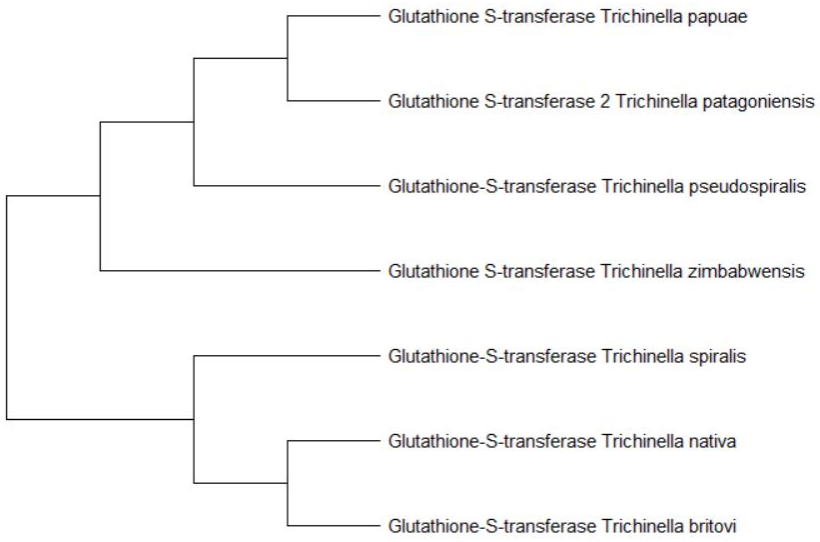


Figure 3: Phylogenetic tree constructed by MEGA X version 10.1 software (Molecular Evolutionary Genetics Analyses) with Neighbor joining method showing evolutionary relationship among *Trichinella* Species. Bootstrap values are depicted at the nodes with Bar value of 0.2.

SOPMA and GOR IV computational tools were used for the determination and prediction of the secondary structure of different GST sequences of T*richinella* species. The percent composition of α-helix, extended strand and random coils of GST proteins from different *Trichinella* species are shown in the table 5. The existence of GST proteins amino acid in the helix, strand or coil is represented from the secondary structure and the secondary structure of GST proteins was represented by secondary structure analysis tool SOPMA. After the computational analysis, it has been noticed that alpha helix was dominant in *Trichinella zimbabwensis* (54.63%), extended strands in *Trichinella papuae* (22.17%) is dominant over other species of *Trichinella*. In the same species, *Trichinella papuae* random coil (46.80) was observed to be dominant.

Table 5: Prediction of secondary structure of GST proteins from *Trichinella* by using Expasy's GOR IV and SOPMA tool.

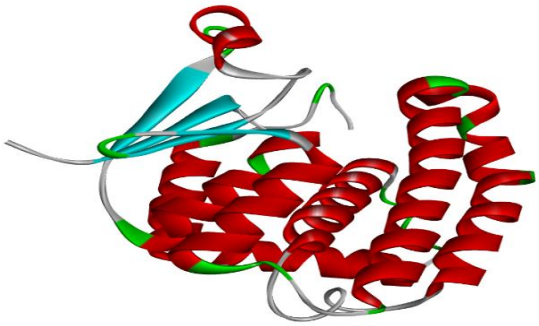
Sr. No	Nematode	SOPMA prediction			GOR analysis		
		α-helix (Hh) (%)	Extendedstrand (Ee) (%)	Random coil (Cc) %)	α- helix (Hh) (%)	Extended strand (Ee) (%)	Random coil (Cc) (%)
1	*Trichinella nativa*	50.73	10.73	31.22	51.71	12.20	36.10
2	*Trichinella britovi*	49.27	13.66	31.22	51.71	12.20	36.10
3	*Trichinella pseudospiralis*	50.97	11.65	30.10	46.12	10.19	43.69
4	*Trichinella spiralis*	48.78	11.22	33.66	50.24	08.78	40.98
5	*Trichinella papuae*	48.77	11.82	31.53	31.03	22.17	46.80
6	*Trichinella zimbabwensis*	54.63	08.78	30.24	39.51	18.54	41.95
7	*Trichinella patagoniensis*	48.28	10.84	34.98	30.05	25.62	44.33

Tertiary structure prediction of GST proteins from *Trichinella* sp.

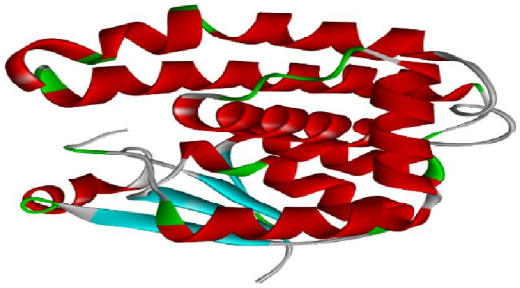
RaptorX structure prediction server was used to analyze the tertiary structures of different GST sequences from *Trichinella* species. Tertiary structure prediction server gives the results after

analyzing query sequence then template threading, assessment of alignment quality of query to the template and finally the multiple templates threading which results in the modeling of a GST proteins in a stepwise manner. Sequences of GST protein from various *Trichinella* species show the variability in the α-helix, in extended strand in β ladder, hydrogen-bonded turn, bend, and coil, etc. (Figure 4). The higher degree of interaction between side chains of amino acids and geometric shapes variations in proteins also occurred. These variations in numbers and shapes lead to differences in their functions.

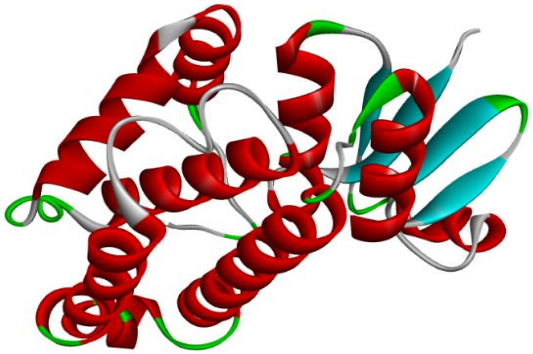
A. *Trichinella nativa*



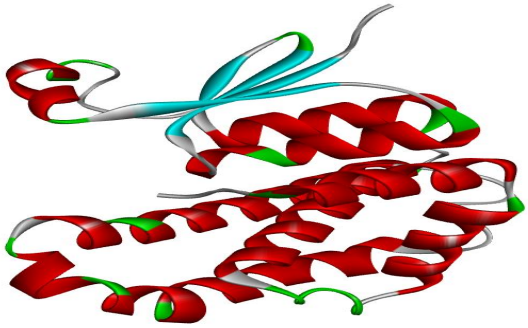
B. *Trichinella britovi*



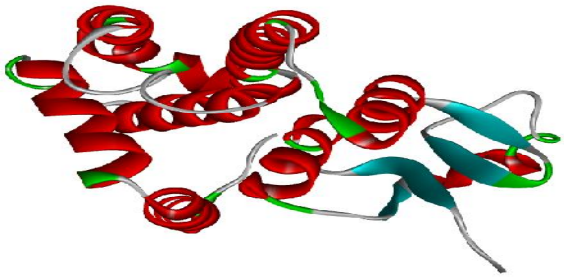
C. *Trichinella pseudospiralis*



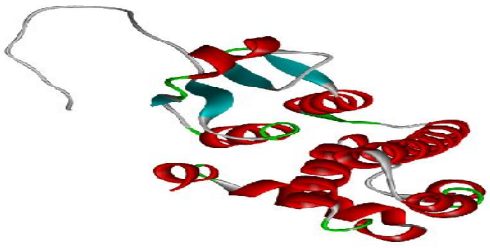
D. *Trichinella spiralis*



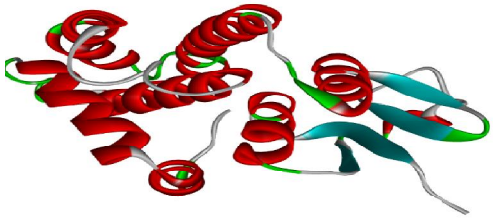
E. *Trichinella papuae*



F. *Trichinella zimbabwensis*



G. *Trichinella patagoniensis*



A. *Trichinella nativa*
B. *Trichinella britovi*
C. *Trichinella pseudospiralis*
D. *Trichinella spiralis*
E. *Trichinella papuae*
F. *Trichinella zimbabwensis*
G. *Trichinella patagoniensis*

Figure 4: Tertiary structure prediction of GST proteins from Trichinella sp

CONCLUSION

The role of GST proteins in the nematode species is very important as this protein involved in the various detoxification reactions of nematodes. In the current modern era in silico analysis of different potential drug, targets is very important as it saves the lots of time and money of researchers and these in silico tools are proved to be very promising tools in the pharma industries for the insight studies of target molecules.

To characterize the GST proteins, to compare and analyze the physicochemical characteristics, to understand the ancestral relationship among the species and structure prediction at different levels the different types of GST from *Trichinella* species. Various computational tools were used. Different GST proteins from various *Trichinella* species show variations in molecular weight, domain, number of amino acids, positive and negative charged residues, secondary and tertiary structure. Also, there is a great degree of diversity has been observed. *Trichinella* species from nematodes were also studies to find out their evolutionary pattern by considering the evolution in the GST proteins also. The phylogenetic analysis of proteins confirmed the ancestral divergence of different types of GST.

The present study concludes that GST proteins from the different *Trichinella* species are medicinally important as they are responsible for *Trichinellosis* (trichinosis) due to their endoparasitic nature and millions of peoples are get affected by these nematode parasites. In recent years many parasitologists and researchers have adapted the various approaches to control these parasites. However, these approaches are time-consuming and very tedious as there is a need for expertise and infrastructure. Which is the major obstacle for the designing new candidate for synthetically design the new drug candidates to control the nematode population? So, such limitations can be overcome by initial screening of some potential drug candidates through the in silco approach to understand the physicochemical properties, molecular structure, phylogenetic relationship of target molecule, etc. These computational approaches would be the promising tools to design the target drug molecule to control the *Trichinella* population. This information can also be useful for studying genomics, proteomics and system biology.

Conflicts of Interest: Nil

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