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RESEARCH ARTICLE

ISOLATION, AMPLIFICATION AND SEQUENCE ANALYSIS OF THE GENE ENCODING ENOLASE FROM LYMPHATIC FILARIAL PARASITE *BRUGIA MALAYI*

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ABSTRACT

Lymphatic filariasis, caused by *Brugia malayi*, commonly known as elephantiasis, is a neglected tropical disease. No vaccines are available for the prevention of filarial infections. A number of pathogenic organisms including filarial parasites display specialized proteins on their cell surface to assist in invasion. One of the best characterized is the glycolytic enzyme enolase. Enolase represents a multifunctional protein involved in basic energy metabolism in pathogens. In the present study, gene encoding enolase of *B. malayi* was isolated, amplified and identified by sequencing. The amplification and sequencing was done using specific primers. The primers were designed based on the complete genome contig sequence of *B. malayi* to amplify the cDNA of enolase. The full length cDNA of this gene from *B. malayi* was obtained by overlapping the sequences of both amplification products using BioEdit version. The results showed that the full length cDNA comprised of 1314 bp. The gene encoding enolase from *B. malayi* (*BmEno*) was identified by BLAST result. The sequence of the *B. malayi* enolase was found to be identical to that of the *B. malayi* partial coding sequences. The complete coding sequence of *B. malayi* enolase was submitted to GenBank and accession number (KF830990.1) was obtained. Phylogenetic analysis of *B. malayi* enolase revealed the occurrence of homology with closely related filarial parasites. Further studies are being carried out to clone and express the enolase gene in the expression vector to study its enzyme activity for therapeutic potential.

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INTRODUCTION

Lymphatic filariasis caused by filarial nematode parasites *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori,* is estimated to infect over 129 million people in tropical and subtropical areas worldwide (WHO, 2012). Internationally, approximately 15 million people are affected by lymphatic filariasis related lymphoedema (or elephantiasis), which includes swelling of the limbs, breasts or genitals, and almost 25 million are affected by urogenital swelling, primarily scrotal hydrocele (Michael *et al*., 1996). Even though these clinical manifestations are not often fatal, they lead to lymphatic filariasis being ranked as one of the world's leading causes of permanent and long-term disability. In 1997, the World health Assembly resolved to eradicate lymphatic filariasis as a public health problem (WHO, 1997). A number of studies using single dose treatment of diethylcarbamazine, albendazole or ivermectin alone, or in various combinations have been carried out for the treatment of lymphatic filarial infection. However, there is a need for additional treatment strategies together with the identification of novel antifilarial (macrofilaricidal) drug targets and development of molecular vaccines since currently available drugs such as diethylcarbamazine, albendazole and ivermectin do not kill the adult parasites. Parasites living in their mammalian host are entirely dependent on glucose, abundantly available in the blood. Metabolic studies performed on bloodstream-form of parasites have shown that glycolysis represents the only process through which ATP is synthesized by the parasite. Inhibition of glycolysis, therefore, leads to rapid death of these parasites **(**Engel *et al.,* 1987). Glycolytic enzymes play an important role in parasites. Due to their importance in parasites for energy fabrication and further physiological functions, glycolytic enzymes can serve as important therapeutic targets (Vivas *et al*., 2005). Numerous pathogens have developed an approach to interact with host components for adherence, cell invasion, intracellular survival, persistence and tissue invasion (Chhatwal and Preissner, 2000).

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Enolase is a multifunctional enzyme after incorporated in a innovative cluster of proteins, called moonlighting proteins, that are present on the surface of several pathogens, although they lack a single peptide to be secreted or a transmembrane region to be anchored to the surface of cells (Pancholi, 2001; Jefferry, 2009). Enolase has been characterized in detail as a plasminogen receptor in dissimilar pathogens: bacteria (Bergmann *et al.,* 2001; Jones and Holt, 2007), fungi (Jong *et al*., 2003) and protozoa (Vanegas *et al*., 2007; Mundodi *et al*., 2008) and it has been found in the tissue of *Onchocerca volvulus* (Jolodar *et al*., 2003), *Fasciola hepatica* and in the secretions of *Echinostoma caproni* (Bernal *et al*., 2004; Marcilla *et al*., 2007). Therefore, enolase is an important protein in the energy metabolism and development of filarial nematodes, but relatively few studies of this molecule in *B. malayi* have been reported. In this study, we give an account of the isolation, amplification and sequence analysis of the gene encoding enolase from *B. malayi*. The results will increase our understanding of enolase in the filarial parasite and lead to the designing and development of new chemotherapeutic tools.

MATERIALS AND METHODS

Preparation of *B. malayi* L3 stage

Filarial parasite, *B. malayi* (sub-periodic) was maintained in the animal model *Mastomys coucha* in the animal house at Vector Control Research Centre (VCRC), Pondicherry (India) for coding institutional reference. Laboratory reared *Ae. aegypti* Liverpool strain susceptible to *B. malayi* infection maintained in our laboratory was used as mosquito host for the development of the arthropod stages (mf, L_1, L_2 and L_3) of the parasites. L_3 stage of *B. malayi* was responsible for the amplification of enolase gene. *M. coucha* was infected by inoculating L_3 stage *B. malayi* subcutaneously or intra-peritoneally. L_3s migrated to different organs, reproduced and mf released in the circulating blood. Infected animal was used for feeding *Ae.* aegypti (Liverpool strain) mosquitoes for infection and development of *B. malayi* L_3 stage (Paily *et al.,* 1995). Institutional Animal ethical clearance was obtained from the committee for the use of laboratory animals in the above experiments. Initially, the eggs of *Ae. aegypti* Liverpool strain were floated and grown up to fourth instar by feeding larval food and it was maintained carefully until the emergence of pupae. The pupae that emerged were collected and kept in the paper cup containing water inside a cage for adult mosquitoes. The adult mosquitoes were allowed to feed on *B. malayi* infected *M. coucha.* These mosquitoes were separately maintained (at 25^{o}C, 70-80% RH) on raisins till the development of the infective (L_3). L_3 stage parasites were harvested on day 12post feeding. Harvested *B. malayi* L_3s were stored in trizol reagent for RNA extraction.

RNA extraction and conversion to cDNA by RT-PCR

Total RNA from L_3 parasites stored in Trizol was extracted using the total RNA Mini prep (Axygen, Scientific Inc, USA) kit according to the manufacturer's instructions and quantified using a spectrophotometer (Genequant, Amersham Biosciences, USA). The total RNA was converted into first strand cDNA by using reverse transcriptase. The RT reaction mixture contained 7.0 µl of water, 2.0 µl of buffer, 2.0 µl of dNTP's, 1.0 µl of RNase inhibitor, 2.0µl of Oligo dT, 1.0 µl of Sensiscript RT and 5.0 µl of total RNA. RT reaction was performed at 37ºC for 5 min, 65°C for 10 sec. The RT product was confirmed with L3 specific primers (L_3F and L_3R) by PCR. The reaction mixture for the confirmation of the RT product contained 12 µl of Go Taq Green Master mix, 2.0 µl of L_3F, 2.0 µl of L_3 R, 3.0 µl of RT product and 5.5 µl of water. PCR was performed at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, 72 °C for 1min and final extension was carried out at 72°C for 10 minutes. The amplified product was run on 1% (wt/v) agarose gel electrophoresis, followed by ethidium bromide staining and visualized under UV transillumination. The confirmed first strand cDNA was used as template for the amplification of *BmEno*.

Amplification of Enolase Gene by PCR

First-strand cDNA was synthesized from *B. malayi* L_3 RNA using the Sensiscript (Qiagen, Germany). The cDNA of the *B. malayi* enolase (*BmEno*) was amplified using two sets of degenerate primers designed using *B. malayi* full genome contig sequence (GenBank accession number gi/170582776). Extensive optimizations were carried out using different concentrations of the constituents of reaction mixture and the PCR amplification protocols. The reaction mixture (25 µl) for the amplification of PCR contained 12.5 µl of Go Taq Green Master Mix, 10-20 pmol of each primer, 3-5 µl of RT product and made up to 25 ul with Milli Q water. PCR was performed at 94°C for 3-5 min, followed by 35 cycles at 94°C for 30 sec-1 min, 52-56°C for 30 sec-1.5 min, 72 °C for 0.5-1 min and a final extension step at 72°C for 5-10 min. All reactions were performed in a thermal cycler (Eppendorf, Germany). The PCR product was electrophoresed in an agarose gel (1.0%) and a band of the expected size (1.3Kb) was observed.

Sequence analysis

The PCR product of *B. malayi* enolase was purified using Nucleospin® Gel purification kit (Macherey-Nagel, Germany) as recommended by the manufacturers. Sequencing reactions were carried out in both directions using same forward and reverse primers in an automated DNA sequencer (3130Xl Genetic analyser, Applied bio-systems/HITACHI), in both forward and reverse direction. The contig sequences were assembled with Bio-Edit (Version 7.0). The sequence of the amplified product for identification was made using the BLAST engine (NCBI).

Phylogenetic analysis of *B. malayi* enolase

Phylogenetic analysis of the *B. malayi* enolase was performed with molecular evolutionary genetic analysis software (Mega4) (Tamura *et al*., 2007). The evolutionary relationship of the newly isolated gene encoding enolase of *B. malayi* was compared with closely related filarial parasites and a phylogenetic tree was constructed using amino acid sequence of *B. malayi* enolase with other filarial parasites. The evolutionary history was inferred using the Neighbor-joining method (Saitou and Nei, 1987). The percentage of replicate

trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Poisson Correction Method (Zuckerkandl and Pauling, 1965) and are in the units of the number of amino acid substitutions per site.

RESULTS AND DISCUSSION

Preparation of sample and RNA extraction

Infected animal model *M. coucha* which showed 100 mf/20µl of peripheral blood was used for feeding *Ae. aegypti* (Liverpool strain) mosquitoes for infection and development of *B. malayi* L_3s. Infective (L_3) stage parasites of 40-50 numbers were harvested from infected animals. Harvested L_3s stored in Trizol was used as the parasite source for the extraction of total RNA. Total RNA (~ 50 ng/ul) was extracted from the parasite and utilized for the conversion to cDNA using RT-PCR. Converted cDNA was confirmed with L_3 specific primers by PCR. The confirmed cDNA was used as template for the amplification of *B. malayi* enolase.

Amplification of 1.3 kb enolase by PCR

Out of two sets of primers designed and used for amplification of cDNA of the *B. malayi*, second set of primers with the following sequences:

*Bm*EnoF:
(5'CGCGGATCCGATGCCGATCACACGTGTTCACG-3')
and
*Bm*EnoR:
(5'AAACTGCAGTTACTATGCTTGAGGATTTCGGAACT T-3'), resulted in successful amplification of the *BmEno* specific band of 1.3 kb length. The optimized reaction mixture (25 ul) for the amplification of PCR contained 12.5 µl of Go Taq Green Master Mix, 20 pmol of each primer, 3 µl of RT product, 5.5 µl of water. The optimum annealing temperature was found to be 55°C. Standardized PCR protocol was performed at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, 72 °C for 1 min and a final extension step at 72°C for 10 min. An intense single band of size 1.3 kb was visible on 1% agarose gel stained with ethidium bromide (Fig.1). No bands were visible in negative control, indicating that the amplified DNA was a copy of the specific gene of the template and the primers were highly specific for the enolase gene. The use of specific primers coupled with the size of 1.3 kb of the amplified product indicated that the amplified product was enolase gene. When the size of the *BmEno* was compared with the earlier amplified enolase genes reported from other sources, *O. volvulus* (1615 bp) (Jolodar *et al*., 2003) and *Haemonchus contortus* (1583bp) (Kaikai Han *et al*., 2012) were both bigger than the *B. malayi* enolase gene. But the gene encoding enolase of *B. malayi* was longer than *Wuchereria bancrofti* (624bp) (EJW79927. 1:Vasuki and Hoti, 2008). cDNA length of *B. malayi* enolase was similar to *Loa loa enolase* (1314bp) (EU370162.1: Nutman *et al*., 2010).

Sequence analysis

Sequencing reactions carried out in both directions using the same forward and reverse primers in an automated DNA sequencer revealed a sequence of 1314 bp which could be read from Chroma software (Goodstadt and Ponting, 2001). As shown in the figure 2, the sequence has an ATG codon at nucleotide position 1-3 and a termination codon at 1312-1314. The sequence was compared with the published sequence (Fig.3) which showed homology of 99% (*B. malayi* partial cds, XM 001896246.1), 99% (*Onchocerca volvulus* complete cds, AF532606.1) and 89% (*Loa loa* complete cds, EU370162.1). The nucleotide sequence of *B. malayi* enolase was submitted to GenBank and accession number KF830990.1 obtained. The GC content of the amplified gene was 40% and AT content was 60%. Our study forms the first report on the amplification of the complete coding sequence of enolase gene of *B. malayi* infective (L_3) stage.

Phylogenetic analysis

While analysing the phylogenetic tree, *B. malayi* enolase showed high evolutionary relationship with other filarial parasites (Fig.4 and Table 1). The evolutionary relationship of *BmEno* with other filarial parasites was inferred using NJ method. A total of 500 replicates were analysed using bootstrap test. When the evolutionary distances were computed using Poisson correction method, *B. malayi* and *Wuchereria bancrofti* occupied in the same cluster in the phylogenetic tree, indicating high evolutionary relationship with *W. bancrofti* than other related filarial parasites. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

Table 1. Gene, amino acid, GenBank accession number of organisms which were used for the construction of phylogenetic tree

Organisms	Gene	Amino acid	GenBank Acc. No
Wuchereria bancrofti	Enolase	253	EJW79927.1
Onchocerca volvulus	Enolase	435	AAP81756.1
Loa loa	Enolase	437	EFO27563.1
Ascaris suum	Enolase	436	ADQ00605.1
Haemonchus contortus	Enolase	433	AIZ75644.1
Caenorhabditis elegans	Enolase	434	NP495900.1

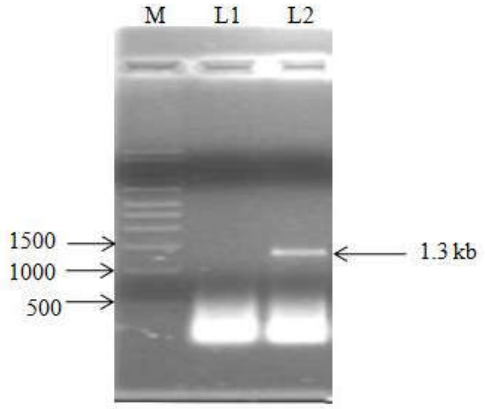

Fig. 1. Agarose gel electrophoresis of PCR product of *Brugia malayi* enolase Lane M, 1 kb ladder, Lane 1, Negative ;Lane 2, PCR amplified enolase gene (1.3 kb)

```
ORIGIN
        1 atgccgatca cacgtgttca cgcccgtcct atttatgatt cacgtggtaa tccaaccgtc
       61 gaagttgatt tgaccaccga caaaggtatt ttccgtgcgg ctgtaccaag tggtgcttca
      121 actggtgtac atgaagcact tgaacttcgg gacaatgata aagctgtgaa tcatggcaaa
      181 ggtgttttga aagctgtaag aaatgtcaac gaacatattg gacctgctct agttgctaag
      241 aatttttgtc caactcaaca acgtgaaatc gaccatttta tgctagaact cgatggaacc
      301 gaaaataaag caaaactggg tgccaatgca attttgggtg tttcattggc ggtttgcaag
      361 gctggtgcag tgcataaagg tatgccgttg tataagtata tagcagaatt ggctggtacc
      421 aaacagattg ttctgccagt tcctgctatg aatgttatca acggtggttc tcatgctggt
      481 aataaactgg caatgcagga atttatgatc atgcctattg gagctagttc attcagtgaa
      541 gcaatgcgca tgggttctga aatttaccat tacttgaagg cagaaatcaa aaaacgatac
      601 ggtctcgatg caacagcagt gggtgatgaa ggtggtttcg ctcctaatat tcaggataac
      661 agggaaggtc ttgatttgtt gaatacagca attgcaacag ctggatacac gggaaaagta
      721 gcaattgcta tggattgtgc cgcatcagaa tattatatgg aatcagctaa gctgtacgat
      781 ttagacttca aaaatccaaa ctcggataaa gcccagtgga aaaactggtg atcaaatgat
      841 gaaatctatc aatccttcat taaggaatat ccagttgtat cgatttgagg attggtttgc
      901 caggatgact gggaaaattg gaccaaagca ttggctaata cgcatattca aattgttggc
      961 gatgacttaa ctgttacgaa tcctaagaga attgctatgg ctgctgagaa gaaagcttgc
     1021 aactgcctgt tactcaaggt taatcaaatt ggctcagtga ctgaatcaat tgatgcggct
     1081 aacttagcac gtaaaaatgg atgggggtga atggtatcgc atcgttcagg tgaaacggaa
     1141 gatacattta tcgctgatct cgtcgttgga cttgctaccg gacagatcaa aactggagca
     1201 ccatgtcgtt cggagcgtct cgccaaatac aatcagatac ttcgtattga agaagaactt
     1261 ggatcagctg ccatttacgc tggtcaaaag ttccgaaatc ctcaagcata gtaa
```

Fig.2. Complete coding sequence of *Brugia malayi* enolase gene [initiation (ATG) and termination sites (TAA) underlined]

Brugia malayi enolase partial mRNA Sequence ID: ref|XM_001896246.1| Length: 1377 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
2412 bits(1306)	0.0	1309/1311(99%)	0/1311(0%)	Plus/Plus

```
Query  1    ATGCCGATCACACGTGTTCACGCCCGTCCTATTTATGATTCACGTGGTAATCCAACCGTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  45   ATGCCGATCACACGTGTTCACGCCCGTCCTATTTATGATTCACGTGGTAATCCAACCGTC  104

Query  61   GAAGTTGATTTGACCACCGACAAAGGTATTTTCCGTGCGGCTGTACCAAGTGGTGCTTCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  105  GAAGTTGATTTGACCACCGACAAAGGTATTTTCCGTGCGGCTGTACCAAGTGGTGCTTCA  164

Query  121  ACTGGTGTACATGAAGCACTTGAACTTCGGGACAATGATAAAGCTGTGAATCATGGCAAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  165  ACTGGTGTACATGAAGCACTTGAACTTCGGGACAATGATAAAGCTGTGAATCATGGCAAA  224

Query  181  GGTGTTTTGAAAGCTGTAAGAAATGTCAACGAACATATTGGACCTGCTCTAGTTGCTAAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  225  GGTGTTTTGAAAGCTGTAAGAAATGTCAACGAACATATTGGACCTGCTCTAGTTGCTAAG  284

Query  241  AATTTTTGTCCAACTCAACAACGTGAAATCGACCATTTTATGCTAGAACTCGATGGAACC  300
            ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  285  AATTTTTGTCCAACTCAACAACGTGAAATCGACCATTTTATGCTRGAACTCGATGGAACC  344

Query  301  GAAAATAAAGCAAAACTGGGTGCCAATGCAATTTTGGGTGTTTCATTGGCGGTTTGCAAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  345  GAAAATAAAGCAAAACTGGGTGCCAATGCAATTTTGGGTGTTTCATTGGCGGTTTGCAAG  404

Query  361  GCTGGTGCAGTGCATAAAGGTATGCCGTTGTATAAGTATATAGCAGAATTGGCTGGTACC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  405  GCTGGTGCAGTGCATAAAGGTATGCCGTTGTATAAGTATATAGCAGAATTGGCTGGTACC  464

Query  421  AAACAGATTGTTCTGCCAGTTCCTGCTATGAATGTTATCAACGGTGGTTCTCATGCTGGT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  465  AAACAGATTGTTCTGCCAGTTCCTGCTATGAATGTTATCAACGGTGGTTCTCATGCTGGT  524

Query  481  AATAAACTGGCAATGCAGGAATTTATGATCATGCCTATTGGAGCTAGTTCATTCAGTGAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  525  AATAAACTGGCAATGCAGGAATTTATGATCATGCCTATTGGAGCTAGTTCATTCAGTGAA  484

Query  541  GCAATGCGCATGGGTTCTGAAATTTACCATTACTTGAAGGCAGAAATCAAAAAACGATAC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  585  GCAATGCGCATGGGTTCTGAAATTTACCATTACTTGAAGGCAGAAATCAAAAAACGATAC  644

Query  601  GGTCTCGATGCAACAGCAGTGGGTGATGAAGGTGGTTTCGCTCCTAATATTCAGGATAAC  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  645  GGTCTCGATGCAACAGCAGTGGGTGATGAAGGTGGTTTCGCTCCTAATATTCAGGATAAC  704

Query  661  AGGGAAGGTCTTGATTTGTTGAATACAGCAATTGCAACAGCTGGATACACGGGAAAAGTA  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  705  AGGGAAGGTCTTGATTTGTTGAATACAGCAATTGCAACAGCTGGATACACGGGAAAAGTA  764

Query  721  GCAATTGCTATGGATTGTGCCGCATCAGAATATTATATGGAATCAGCTAAGCTGTACGAT  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  765  GCAATTGCTATGGATTGTGCCGCATCAGAATATTATATGGAATCAGCTAAGCTGTACGAT  824

Query  781  TTAGACTTCAAAAATCCAAACTCGGATAAAGCCCAGTGGAAAACTGGTGATCAAATGATG  840
            |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  825  TTAGACTTCAAAAATCCAAACCTCGGATAAAGCCCAGTGGAAAACTGGTGATCAAATGATG  884

Query  841  GAAATCTATCAATCCTTCATTAAGGAATATCCAGTTGTATCGATTGAGGATTGGTTTGAC  900
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  885  GAAATCTATCAATCCTTCATTAAGGAATATCCAGTTGTATCGATTGAGGATTGGTTTGAC  944

Query  901  CAGGATGACTGGGAAAATTGGACCAAAGCATTGGCTAATACGCATATTCAAATTGTTGGC  960
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  945  CAGGATGACTGGGAAAATTGGACCAAAGCATTGGCTAATACGCATATTCAAATTGTTGGC  1004

Query  961  GATGACTTAACTGTTACGAATCCTAAGAGAATTGCTATGGCTGCTGAGAAGAAAGCTTGC  1020
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1005 GATGACTTAACTGTTACGAATCCTAAGAGAATTGCTATGGCTGCTGAGAAGAAAGCTTGC  1064

Query  1021 AACTGCCTGTTACTCAAGGTTAATCAAATTGGCTCAGTGACTGAATCAATTGATGCGGCT  1080
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1065 AACTGCCTGTTACTCAAGGTTAATCAAATTGGCTCAGTGACTGAATCAATTGATGCGGCT  1124

Query  1081 AACTTAGCACGTAAAAATGGATGGGGTGTAATGGTATCGCATCGTTCAGGTGAAACGGAA  1140
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1125 AACTTAGCACGTAAAAATGGATGGGGTGTAATGGTATCGCATCGTTCAGGTGAAACGGAA  1184

Query  1141 GATACATTTATCGCTGATCTCGTCGTTGGACTTGCTACCGGACAGATCAAAACTGGAGCA  1200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1185 GATACATTTATCGCTGATCTCGTCGTTGGACTTGCTACCGGACAGATCAAAACTGGAGCA  1244

Query  1201 CCATGTCGTTCGGAGCGTCTCGCCAAATACAATCAGATACTTCGTATTGAAGAAGAACTT  1260
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Sbjct  1245 CCATGTCGTTCGGAGCGTCTCGCCAAATACAATCAGATACTTCGTATTGAAGAAGAACTT  1304

Query  1261 GGATCAGCTGCCATTTACGCTGGTCAAAAGTTCCGAAATCCTCAAGCATAG  1311
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Sbjct  1305 GGATCAGCTGCCATTTACGCTGGTCAAAAGTTCCGAAATCCTCAAGCATAG  1355
```

Fig 3. Sequence alignment showing homology between query sequence with *B. malayi* partial sequence (gi|001896246.1)

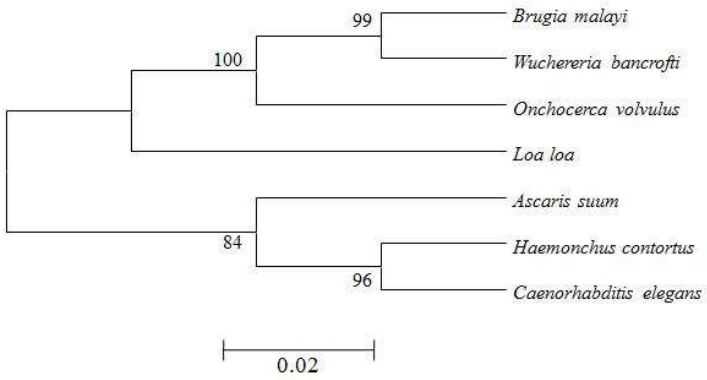

Fig.4. Dendrogram showing the relatedness between *Brugia malayi* enolase and other filarial sequence derived from Genbank database.

Conclusion

In the present study, isolation, amplification and sequence analysis of the gene encoding enolase from lymphatic filarial parasite, *B. malayi* have been accomplished. Our study forms the first report on the amplification of the complete coding sequence of enolase from infective stage *B. malayi* and the results will enhance the understanding of enolase in the filarial parasite and lead to the designing and development of new chemotherapeutic tools. Parasites living in their mammalian host are entirely dependent on glucose, abundantly available in the blood. Metabolic studies performed on bloodstream-form parasites have shown that glycolysis represents the only process through which ATP is synthesized by the parasite. Inhibition of glycolysis, therefore, leads to rapid death of these parasites (Engel *et al.,* 1987). Glycolytic enzymes play an important role in parasites for energy production and other physiological functions and hence termed as important therapeutic targets (Vivas *et al.,* 2005). Enolase (2-phospho-D-glycerate hydrolase) is a ubiquitous dimeric glycolytic enzyme that catalyzes the dehydration of 2-phophoglycerate (2-PGE) to phosphoenolpyruvate (PEP) (Lebioda *et al*., 1989), an important metabolic intermediate. Enolase has been characterized in detail as a plasminogen receptor in different pathogens-bacteria (Bergmann *et al*., 2001; Jones and Holt, 2007), fungi (Jong *et al.,* 2003) and protozoa (Vanegas *et al,* 2007) and it has been found in *Onchocerca volvulus* tissues (Jolodar *et al*., 2003), and in *Fasciola hepatica* and *Echinostoma caproni* secretions (Bernal *et al.,* 2004). Therefore, enolase is an important protein in the energy metabolism and development of filarial nematodes, but relatively very few studies of this molecule in filarial nematodes have been reported. Further knowledge on the molecular and functional characterization of this enzyme *BmEno* and its pathogenic mechanisms are very essential, as it not only helps to understand parasite's evolution but also leads to design new potential therapeutic molecules.

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