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CLONING AND CHARACTERISATION OF ALKALI MYOSIN LIGHT CHAIN GENE (MLC-3) OF CATTLE FILARIAL PARASITE SETARIA DIGITATA

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ABSTRACT

Lymphatic filariasis is a tropical disease caused by filarial parasites including Wuchereria bancrofti. Although bancroftian filariasis causes severe disabling and debilitating clinical conditions in human, very little is known about the molecular biology of the parasite. The paucity of parasitic material is the main reason for this lack of knowledge. Setaria digitata is a cattle filarial parasite, closely resembling W. bancrofti in many aspects. Therefore it can be used as a model organism to study W. bancrofti. In the present study, the genomic library of S. digitata adult parasites was constructed and probed with a ^{32}P labeled partial mRNA sequence PCR amplified from a previously isolated cDNA clone containing a 661 bp mRNA transcript of S. digitata alkali myosin light chain gene. Isolated positive clones were sequenced and edited by using bioinformatics tools. Though the 5´ flanking region did not reveal any consensus TATA box sequences, a potential CAAT box like sequence, CCAAT and seven possible transcription factor elements were identified. The entire gene had four exons encoding 149 amino acids interrupted by three introns of varying lengths of 87, 295 and 69 bp respectively. Sequences around the splice junctions were fairly conserved and agreed with the general GT-AG splicing rule. The 3´ flanking region consists of three putative polyadenylation signals with the sequence AATAAA. The gene was AT rich with a GC content of 35%. Southern hybridisation studies suggested that this gene is likely to be a single-copy gene. Homology search of amino acid sequences showed more than 80% similarity with Caenorhabditis species and 40-50% with other vertebrate and invertebrate myosin light chains. Analysis of the amino acid sequence with the NCBI conserved domain database for interactive domain family identified the protein as a member of calcium binding protein family as it comprised of two highly conserved EF hand motifs, and may suggest a possible function in Ca^{2+} binding.

Keywords: *Myosin; Setaria digitata; Genomic library; Filarial parasite; EF hand*

[I] INTRODUCTION

Setaria digitata is a common filarial nematode that parasitises in the peritoneal cavity of cattle and buffalo. Though the infections in natural host are usually non-pathogenic, accidental transmission of the infective larva to aberrant hosts such as sheep and goat by mosquitoes results in cerebrospinal nematodiasis [1]. In Sri Lanka cerebrospinal nematodiasis is a major setback in goat and sheep husbandry that leads to severe economic losses especially in the dry zone of the country [2]. One of the constraints in the treatment and eradication of filariasis is that little is known about the biology of these parasites at molecular level. Though a number of protein coding genes have been characterised from other parasitic nematodes only a few of these are from filariids and only two from *S. digitata* [3].

Lymphatic filariasis, a disabling and disfiguring tropical disease is caused by the tissue dwelling filaroids species *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. Around 120 million people living in 83 countries have already been infected and 1.307 billion people (20% of the population) are at risk of acquiring the infection in the tropics including Sri Lanka and in some sub tropical areas worldwide [4, 5]. Approximately 90% of the cases are caused by *W. bancrofti* and the majority of the remainder by *B. malayi* [6].

Human filariasis in Sri Lanka is caused by *W. bancrofti*, but the parasite material is not easy to obtain. Adult parasites that live

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in lymphatics are not accessible and nocturnally periodic microfilariae cannot be cultured in the laboratory. Presence of adult *S. digitata* worms in the peritoneal cavity of cattle provides readily available material for investigations. It closely resembles the *W. bancrofti* not only in morphological and histological aspects [7] but also in antigenic properties [8]. *S. digitata* thus become a useful model organism to study the molecular biology of *W. bancrofti*.

Myosin is an ubiquitous actin based motor molecule present in both muscle and non-muscle cells of eukaryotes. The conventional myosin of vertebrate skeletal muscle is a hexamer composed of two heavy chains and two pairs of light chains. Based on the solubility, myosin light chains (MLCs) can be further divided in to alkali and regulatory myosin light chains. The former class can be dissociated from the myosin heavy chain in high pH conditions whereas the other one can be extracted with 5-5´-dithiobis (2-nitrobenzoic acid) (DTNB). The alkali myosin light chain of skeletal muscle is further divided into two types MLC1 and MLC3. These two types differ from each other due to the divergence of the amino acid sequences in their amino terminus and they are identical in sequence over their C-terminal [9]. Myosin like proteins have been identified as potential antigens from various filarial parasites [10 – 13]. In our previous studies two antigens of *S.digitata* with molecular weights of 52 and 130 kD showed strong cross reactivity with the serum of *W. bancrofti* infected individuals [14]. When a cDNA library of adult *S. digitata* was immunoscreened with the sera of *W. bancrofti* infected individuals, a clone designated PCSA1 showed very strong reactivity [15]. Bioinformatics analysis confirmed that the mRNA sequence coded for a protein 83% homologous with the alkali (essential) myosin light chain gene of the free living nematode *Caenorhabditis* species. It was also homologous with a 343 bp partial mRNA of the filarial parasite *Brugia malayi* [Genbank: XM_001894833] encoding a hypothetical protein. Similar nucleotide sequences have not been reported for other parasitic nematode. Thus by considering the veterinary importance, immunogenic potential and the lack of information about this gene in filarial nematodes, the objective of the present investigation was to clone and characterize the whole gene at the molecular level.

[II] MATERIALS AND METHODS

2.1. Parasites

Adult *S. digitata* worms were collected from the peritoneal cavity of cattle slaughtered at Kandy abattoir, Sri Lanka. The motile worms were immediately transported to the laboratory and repeatedly washed with phosphate buffered saline to avoid contamination by any cattle tissue or blood and stored at -70^{o}C until use.

2.2. DNA Extraction

Genomic DNA of adult *S.digitata* was extracted, ethanol precipitated and reconstituted in TE (pH 8) buffer by the method described previously [16] and stored at -20^{o}C until use.

2.3. Construction of genomic Library

Genomic DNA of *S. digitata* was partially digested with the restriction enzyme *Sau*3A1 to yield fragments of 5 to 12 kb in length. The fragments were separated by electrophoresis on low melting agarose and suitable fragments (5 to 12 kb) were eluted using illustra GFX PCR DNA and gel band purification kit (Catalogue no. 28-9034-70, GE Healthcare Biosciences, USA).

Genomic library was constructed by using the ZAP Express Predigested Gigapack Cloning Kit (Catalog no. 239615, Stratagene, Switzerland). Briefly, the purified genomic fragments were ligated to Zap express vector pre-digested with *Bam*H1 and dephosphorylated. Ligated products were packaged in vitro by using the Gigapack Gold111 packaging extract. The primary library was amplified by using the *E. coli* strain XL1Blue MRF (Catalog no. 200301, Stratagene, Switzerland) and stored at 4^{o}C in 0.7% chloroform.

2.4. Preparation of the probe

A previously isolated cDNA clone of *S.digitata* [15] carrying a length of 661bp mRNA transcript consisting of the 450 bp full open reading frame of myosin light chain gene was used to generate the PCR primers (sense primer 5'-ACTATACGACGAGGAATTGG-3' ; anti-sense primer 5'-CGAAAGAAAGCAGAAGGAGTATG-3') to amplify 510 bp middle region of the insert. The amplified PCR product was purified from low melting agarose gel and radio labeled with ^{32}P (Catalog no. NEG013H, Perkin Elmer, USA) by random priming method [17].

2.5 Screening the genomic library

A total of 20 plates (132 mm) each containing approximately 2000 plaque forming units were plaque lifted and screened by in situ plaque hybridization on duplicate colony/plaque screen hybridization membrane (Catalogue no. NEF978Y, Perkin Elmer, USA) as previously described with few modifications [18]. Briefly the filters were prehybridized for two hours at 65^{o}C in the 100 µlcm^{-2} prehybridization solution of 20×SSC (1×SSC= 0.15 M NaCl, 0.015 M trisodium citrate), 5× Denhardt's and 100 µg ml^{-1} of denatured salmon sperm DNA. After discarding the prehybridization solution, hybridization was performed overnight at 65^{o}C with the hybridization solution (50 µlcm^{-2}). This contained 0.5% SDS and ^{32}P labeled probe in addition to the constituents of the prehybridization solution. Post hybridization washing was carried out at 65^{o}C with the preheated (65^{o}C) 2×SSC and 0.5% SDS for one hour with three changes. Autoradiography was performed by exposing the filters to Amersham Hyperfilm MP (Catalogue no. 28-9068-45, GE Healthcare Biosciences, USA) at -70^{o}C with intensifying screen. Secondary and tertiary screening was performed as described above and the well isolated positive plaques were picked and stored at 4^{o}C with 0.7% chloroform.

2.6. Southern blot analysis

Aliquots of *S. digitata* genomic DNA (3 µg) was cleaved individually with five fold excess of four different restriction enzymes *Eco*R1, *Bam*H1, *Hind*111 and *Sal*1. DNA fragments were separated by electrophoresis on an agarose gel with a 1kb ladder. A photograph was taken after placing a fluorescent gel ruler alongside the ladder lane and then visualizing the bands under UV. Separated fragments were partially depurinated by incubating in 0.25 M HCl (15 min at room temperature) and transferred to nitrocellulose membrane [17]. The blotted membrane was hybridized with the same probe used to screen the genomic library

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as described earlier. The distance of migrated ladder bands were measured by comparing with the ruler scale using the above photograph and the locations were depicted schematically in the autoradiogram.

2.7. In-vivo excising and Restriction analysis

Isolated clones were in-vivo excised by using the Bacterial strains XL1Blue MRF (Catalog no. 200301, Stratagene, Switzerland), XLOLR (Catalog no. 200304, Stratagene, Switzerland) and the ExAssist Interference-Resistant Helper Phage (Catalog no. 200253, Stratagene, Switzerland) according to the single clone excision procedure recommended by the manufacturer to obtain the Kanamycine resistant PBK-CMV phagemid form. Candidate clones were screened for the insert size by restriction digestion with two enzymes, *Xba*1 and *Sac*1.

2.8. Sequencing

One clone designated pSMC-3 carrying an insert size of ~2kb was fully sequenced using MegaBACE 1000 automated sequencing system (GE Healthcare Biosciences, USA) using universal T3, T7 primers and the gaps were filled with synthetic oligonucleotides. Resulted sequences were deposited in the GenBank data bank at the National Center for Biotechnology Information (NCBI) under the accession number of GQ227356.1.

2.9. Bioiformatic analysis

The open reading frame (ORF) of the MLC-3 mRNA was predicted using the ORF finder program at NCBI by submitting the complete cDNA sequence as query sequence [19]. Amino acid sequences of *Brugia malayi* (Hypothetical protein), *C. briggsae* CBR-MLC-3, *C. elegans* mlc-3, *C. brenneri* mlc-3 were acquired by executing an interactive protein-protein BLAST at NCBI [20] using the amino acid sequence of *S.digitata* alkali myosin light chain as the query sequence. Multiple alignment of nucleotide sequences was carried out using ClustalW of the BioEdit software program. Analysis of amino acid sequence for interactive domain family was done with the NCBI conserved domain database [21]. PSORT II program was used to predict the sub cellular localization of the characterized protein from the amino acid sequence [22]. WWWSIGNAL SCAN transcription factor database, which predict the common eukaryotic transcriptional elements was used to analyse the sequence of the 5' UFR [23].

[III] RESULTS AND DISCUSSION

The genomic clone designated pSMC-3 isolated from the genomic library of *S. digitata* contained the complete gene including the 5´ and 3´flanking region of which 580 bp and 541 bp were sequenced respectively. Comparative analysis of the genomic and cDNA sequences showed that the 450 bp long open reading frame was interrupted by three introns in positions 76-3, 98-3 and 138-1. The introns positions were numbered by considering the initiator methionine as codon 1 and the codon split after the first or second nucleotides were given the phase number -1 and -2 respectively, while -3 indicates that the intron directly follows the codon. As observed from many other parasitic and non-parasitic nematodes [18, 24, 25], the introns were relatively short with varying lengths of 87 bp, 295 bp and 69 bp respectively **[Figure-1]**.

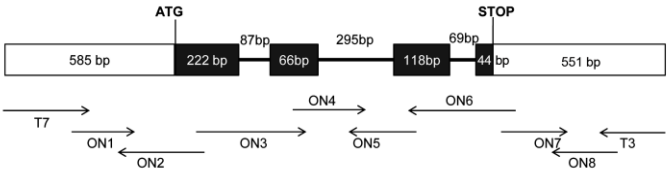

Fig: 1. Schematic representation of the alkali myosin light chain gene of *S. digitata*: Boxed regions are exons. Protein coding portion of the genes are shaded. 5' and 3' untranslated regions are open. Arrows indicate sequencing strategy used. ON=synthetic oligonucleotides.

Alignment of sequence around the 5´ (donor) and 3´ (acceptor) splice junctions of *S. digitata* alkali myosin light chain gene with the consensus sequences for eukaryotes [26] identified that the sequences around the splice junctions are fairly conserved **[Figure-2]** and agreed with the general GT-AG splicing rule of the eukaryotes and also with those of parasitic nematodes [24]. Moreover from this alignment it was possible to develop a consensus for the sequence around the splice sites of *S. digitata*. This consensus would be useful to identify exon-intron junctions of other genes that may be cloned from this parasite in the future.

Analysis of the 5´ flanking region did not reveal any consensus TATA box sequences but, the potential CAAT box like sequence, CCAAT was identified at nucleotide position -449. Though there is no data available on the 5' flanking region of similar myosin light chain gene of other filarial nematodes to compare possible common regulatory elements, analysis of the sequence with the WWWSIGNAL SCAN transcription factor database hypothetically identified seven possible regulatory elements. Details of the identified elements are summarized in **Table-1**. It has been reported that there are some myosin light chain genes using atypical sequences instead of consensus TATA sequence [27].

The analysis of the 3´ UTR revealed three polyadenylation signals 143 bp, 168 bp and 410 bp downstream from the stop codon respectively **[Figure-3]**. Comparative analysis of the cDNA clone revealed that the first polyadenylation signal located 143 bp downstream from the stop codon was utilized. The molar ratio of A: T and G: C generally known as GC content is an important parameter of genome in the analysis of phylogenetic relationship. Filariids are reported to have some of the most AT rich genome [28]. The G+C content of the entire gene including the 3´ and 5´ UTR was 35% while the coding region had a G+C content of 44%. The introns were A+T rich (69%) while the 5´ and 3´ UTR have an AT content of 68%.

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Regulatory element	Signal Sequence	Location	Number of copies
CBF	ATTGG	-454, -451	2
CDF	ATTGG	-454, -451	2
GATA-1	WGATAMS	-543	1
H1_ conserved_US	AAACACA	-417	1
H2A_ conserved_U	YCATTC	-321	1
Myb	YAACKG	-461,-374,-299	3
PEA3	AGGAAR	-245	1

Table: 1. Regulatory elements in the 5′ flanking region of the alkali myosin light chain gene of *S.digitata* identified by the WWWSIGNAL SCAN transcription factor database.

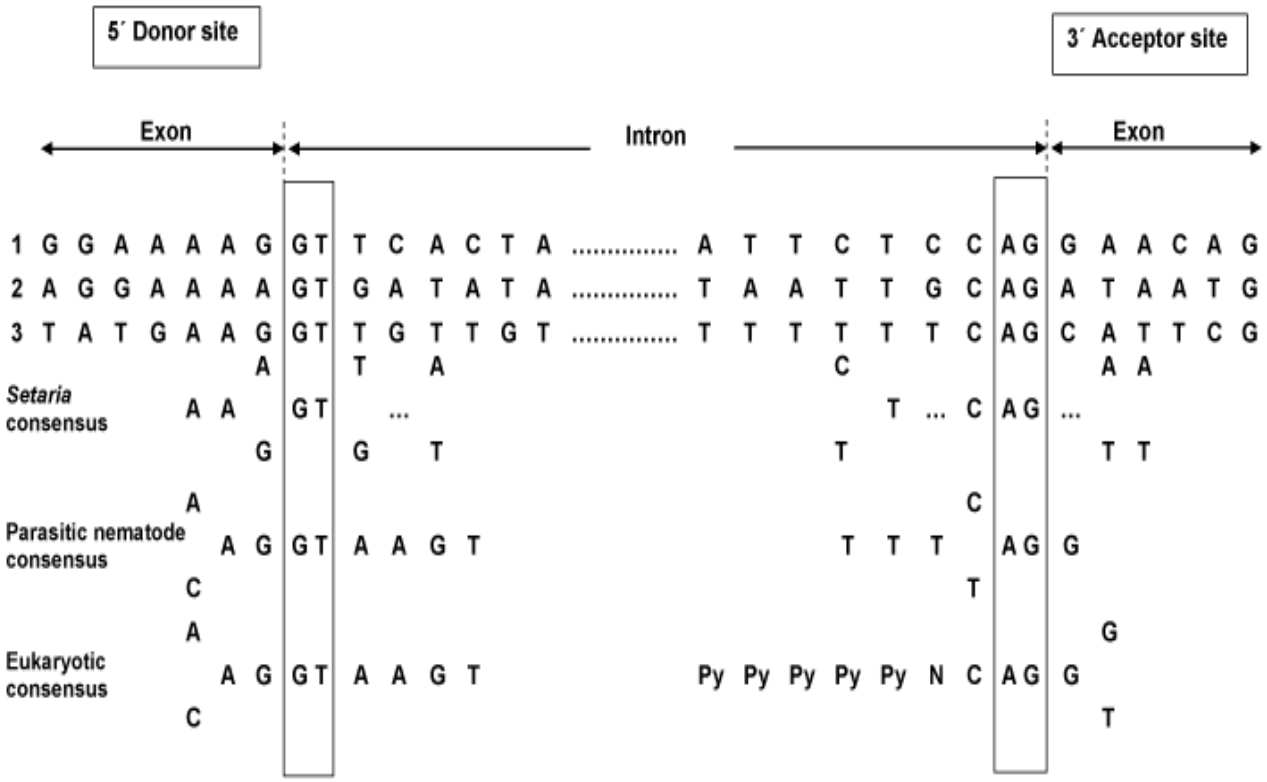

Fig: 2. Alignment of the sequence around 5′ and 3′ splice junctions. Comparison of sequence around 5′ and 3′ splice junctions of *S. digitata* alkali myosin light chain gene with consensus sequences for parasitic nematodes and Eukaryotes. Py = pyrimidine

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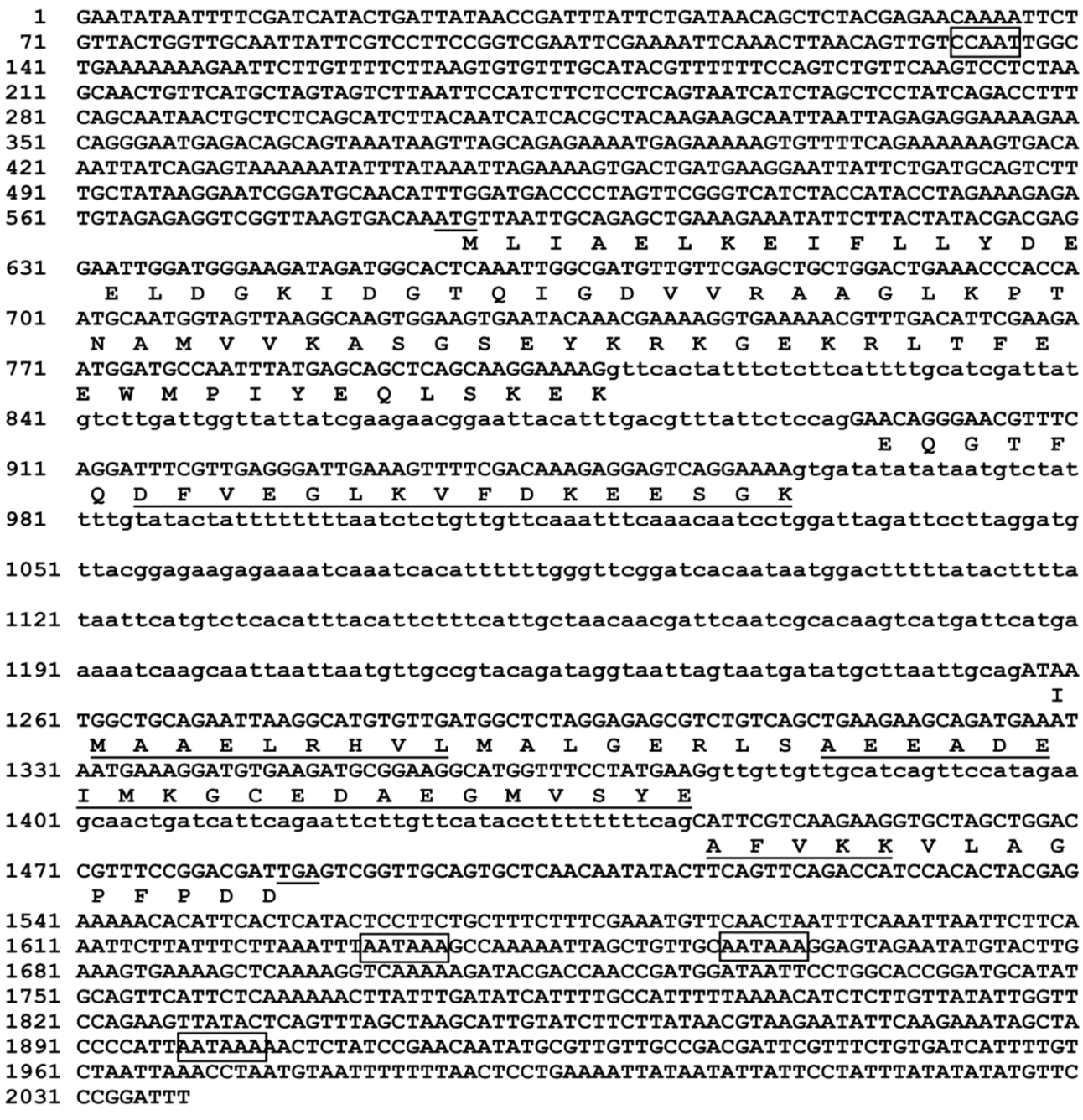

Fig: 3. Sequence of alkali myosin light chain gene of *S. digitata*. The nucleotide sequence of exons and the 5´ and 3´ flanking sequences are presented in capital letters while the intron sequences are in lower case. The predicted amino acid sequence, in single-letter terminology, is indicated below the nucleotide sequences. The potential CAAT box like sequence, CCAAT in the 5´ flanking region and the polyadenylation signal AATAA in the 3´ flanking region are boxed. Two EF hand domains are underlined with a single line.

The Southern blot analysis of the *S. digitata* genomic DNA revealed a single hybridization band in the lanes cleaved with *Bam*H1, *Hind*111 and *Sal* 1 while the lane cleaved with *Eco*R1 resulted in two hybridization fragments as the alkali myosin light chain gene of *S. digitata* had one restriction site for *Eco*R1and none for other enzymes used. The banding pattern of the Southern blot analysis remained same even

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under low stringency conditions **[Figure-4]** suggesting that the alkali myosin light chain gene of *S. digitata* is likely to be a single copy gene. However, alternative splicing resulting in several isoforms cannot be excluded.

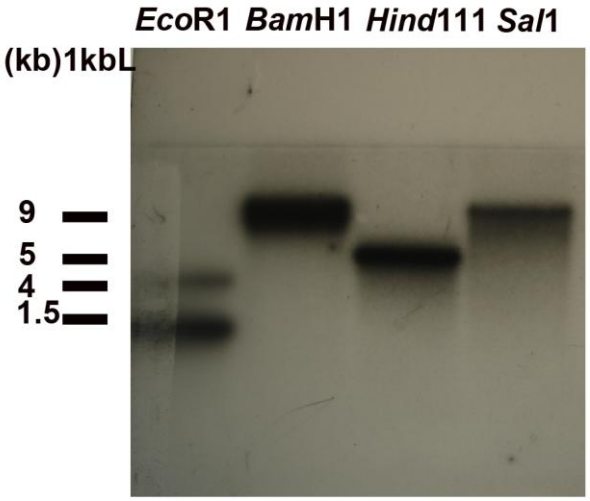

Fig: 4. Southern blot of *S. digitata* genomic DNA. *S. digitata* genomic DNA cleaved with *Eco*R1, *Bam*H1, *Hind*111 and *Sal*1 and probed with ^{32}P labeled *S. digitata* alkali myosin light chain cDNA sequences. I kb Ladder (1kb L) was used as the size marker

Analysis of the nucleic acid sequence of the entire coding region revealed a full open reading frame of 450 nucleotides. Translation of this full open reading frame encoded a putative 149 amino acid protein with the predicted molecular weight of 17 kD. Based on the results obtained from the PSORTII program, which predict the intracellular location of the proteins by known sequence fingerprints, the characterized alkali myosin light chain appears to be of cytoplasmic origin.
The codon usage pattern of alkali myosin light chain gene of *S. digitata* revealed that only 50 codons were utilized out of the 61 codons. Certain amino acids showed a strong preference for a specific codon, GTT was used 60% in valine, TCA was used 43% in serine, GGA was used 61% in glycine.

In order to identify similar genes of other taxonomic groups a homology sequence search was done with NCBI database. A partial mRNA fragment of the human filarial parasite *B. malayi* [Acc No. XM_001894833] showed a high degree of homology of 90% at protein level. But this protein was denoted as one of the hypothetical protein of *B. malayi* because neither the complete mRNA nor the gene of the relevant protein was fully characterised. Next to that, a high degree homology of more than 80% was observed with free-living nematode *Caenorhabditis briggsae.*

The alignment of the homology sequences in order of decreasing homology is shown in **[Figure-5].** The homology was around 48-50% when compared with higher eukaryotes including the avian and mammalian species.

Thus, the alkali myosin light chain gene appears to be highly specific for nematodes and less conserved across the taxonomic groups unlike other genes like actin. Analysis of sequenced ESTs from 30 different nematode species across the phylum has shown that only about 15% of the genes common to all four clades of nematodes have sequence matches outside the phylum. In addition, they identified ~1300 genes that are nematode-specific found only in most of the nematodes [29]. Myosin light chain genes are reported to show a vast diversity not only between different genetic groups but also in different tissues within the same organism [30].

Many of these isoforms especially from the lower eukaryotes are not characterized yet. In some cases, the transcript of a single gene is alternatively spliced resulting in different isoforms [31]. The analysis of amino acid sequence with the NCBI conserved domain database [32] for interactive domain family revealed that the protein belongs to the calcium binding protein which contain one or several of EF hand domains.

Though the Ca^{2+} binding proteins such as calmodulin, troponin C, myosin light chains and parvalbumin have evolved from a common ancestor with four Ca^{2+} binding domains, the NCBI conserved domain database identified only two domains in *S. digitata* alkali myosin light chain gene **[Figure-3]**. Alkali myosin light chain genes from other eukaryotes also contain only two Ca^{2+} binding domains [33, 34]. The loss of other two domains is perhaps due to some evolutionary substitution of amino acids in the Ca^{2+} loop, which makes them functionally inactive. Detail analysis of this domain revealed that there were four acidic residues among its six ligating groups in the Ca^{2+} loop to make it functionally active. Ca^{2+} binding EF hand conserved domain region of *S. digitata* alkali myosin light chain extending from amino acids 80 to 112 is highly conserved with the ancestral Ca^{2+} binding domain of higher eukaryotes although only around 48-50% amino acid homology was seen when the whole protein was compared **[Figure-6]**.

[IV] CONCLUSION

In the present study, we have characterised the alkali myosin light chain gene of the cattle filarial *parasite S. digitata.* Since this is the first alkali myosin light chain gene characterised from filarial parasites, this will probably be helpful in the study of the same gene in related human filarial parasite *W. bancrofti* specially in designing PCR primers and DNA probes targeting this gene. Moreover, being one of the structural components of the cuticle, this myosin and myosin related proteins could be specifically focused as targets for novel vaccines and therapeutic agents. Further studies to express the protein for investigating the immunogenic potential would be desirable.

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                                10        20        30        40        50        60        70
                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Setaria digitata                ---------- ---------- ---------- ---------- ---------M LIAELKEIFL LYDEELDGKI
B.malayi(Hypothetical protein)  ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. briggsae CBR-MLC-3           ---------- ---------- ---------- ---------- ------MPAP SQDV.....N ..........
C.elegans mlc-3                 ---------- ---------- ---------- ---------- ------MAPP SQDV.....N ..........
C.brenneri mlc3                 ---------- ---------- ---------- ---------- ------MPVN -PDV.....N ..........
Taeniopygia guttata mlc         MAPKKDVKKP --AAAAAPAP APAPAPAPA- -KPKEPAIDL KSIKIEFSKE QQDDF..A.. .F.RTG.A..
Haemaphysalis qinghaiensis mlc  ---------- ---------- ---------- ---------- ---MADLKPN EVEQAR.H.E I..MCAE...
Rabbit mlc3                     ---------- ---------- ---------- ---------- ----MSFSAD Q...F..A.. ...RTG.S..
Chick mlc1                      MAPKKDVKKP --AAAAAPAP APAPAPAPAP AKPKEPAIDL KSIKIEFSKE QQDDF..A.. .F.RTG.A..
zebrafish mlc6                  ---------- ---------- ---------- ---------- ---MSDFTED Q.C.F..A.. .F.RTG....
Chick mlc3Sk.mus.isoform        ---------- ---------- ---------- ---------- ----MSFSPD E.NDF..A.. .F.RTG.A..
Canis familiaris mlc1           MAPKKDVKKP AAAAAAAPAP APAPAPAPAP AKPKEEKIDL SAIKIEFSKE QQD.F..A.. .F.RTG.S..
Salmo salar m.l polypeptide     ---------- ---------- ---------- ---------- ---MSDFSED Q.I.F..A.. .F.RTG....
H.sapiens fast sk.alkali mlc1   ---------- ---------- ---------- ---------- ----MSFSAD Q...F..A.. .F.RTG.S..

                                80        90        100       110       120       130       140
                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Setaria digitata                DGTQIGDVVR AAGLKPTNAM VVKASGSEYK RKG-EKRLTF EEWMPIYEQL SKEKEQGTFQ DFVEGLKVFD
B.malayi(Hypothetical protein)  ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. briggsae CBR-MLC-3           ........A. .......Q.. ......Q.F. ...-...I.. ...L...... ........YA ..F.......
C.elegans mlc-3                 ....V...A. .......Q.. .T..A.Q.F. ...-...... ...L.M.... A.......YA ..Y.......
C.brenneri mlc3                 ........A. .......Q.. ......Q.F. ...-...I.. ...P...... ........YA ..F.......
Taeniopygia guttata mlc         TLS.V..II. .L.QN....E .N.IL.NPS. EEMNA.KI.. ..FL.MLQAA ANN......E ......R...
Haemaphysalis qinghaiensis mlc  .CADL.SLL. SLD.R..K.L .E.NG.--CA K..-..KM.L ..FL...S.I K.D.D...HA ..M.....Y.
Rabbit mlc3                     TLS.V...L. .L.TN....E .K.VL.NPSN EEMNA.KIE. .QFL.MLQAI .NN.D...YE ......R...
Chick mlc1                      TLS.V..I.. .L.QN....E IN.IL.NPS. EEMNA.KI.. ..FL.MLQAA ANN.D....E ......R...
zebrafish mlc6                  MYN.C...M. .L.QN.V..E .L.VL.NPSN EDMNM.M.D. .QFL.MLQAI A.N.D..S.E ......R...
Chick mlc3Sk.mus.isoform        TLS.V..I.. .L.QN....E IN.IL.NPS. EEMNA.KI.. ..FL.MLQAA ANN.D....E ......R...
Canis familiaris mlc1           TLS.V...L. .L.TN....E .K.VL.NPSN EEMNA.KIE. .QFL.MMQAI .NN.D...YE ......R...
Salmo salar m.l polypeptide     SYS.C...M. .L.QN.V..E .L.VL.NPKS EEMNH.M.D. .QFL.MLQAI A.N.D..S.E ...G..R...
H.sapiens fast sk.alkali mlc1   TLS.V...L. .L.TN....E .R.VL.NPSN EELNA.KIE. .QFL.MMQAI .NN.D.A.YE ......R...

                                150       160       170       180       190       200
                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|.
Setaria digitata                KEESGKIMAA ELRHVLMALG ERLSAEEADE IMKGCE---D AEGMVSYEAF VKKVLAGPFP DD----
B.malayi(Hypothetical protein)  -------... K......... ......X.N. ....T.---N .K...A.... .......... ..----
C. briggsae CBR-MLC-3           ...T...L.. ....I.L... .....D.... LL..V.---. G....K..D. I......... .QD---
C.elegans mlc-3                 ...T...L.. ....I.L... .....D.... LL..V.---. G....K..D. I......... .QD---
C.brenneri mlc3                 ...T...L.. ....I.L... .....D.... LL..I.---. G....K..D. I......... .QD---
Taeniopygia guttata mlc         ..GN.TV.G. ......AT.. .KMTE..V.. L...Q.---. SN.CIN.... ..HI.SV--- ------
Haemaphysalis qinghaiensis mlc  .A.N.QM.E. ..A...LS.. ...TDA.V.. ..HD.AGQV. ED.FIK..M. I.N..... EEQKDK
Rabbit mlc3                     ..GN.TV.G. ......AT.. .KMKE..VEA L.A.Q.---. SN.CIN.... ..HIMSI--- ------
Chick mlc1                      ..GN.TV.G. ......AT.. .KMTE..VE. L...Q.---. SN.CIN.... ..HIMSV--- ------
zebrafish mlc6                  ..GN.TV.G. ......TT.. .KMTE..VET LLA.H.---. .N.CIN.... .RHIM..--- ------
Chick mlc3Sk.mus.isoform        ..GN.TV.G. ......AT.. .KMTE..VE. L...Q.---. SN.CIN.... ..HIMSV--- ------
Canis familiaris mlc1           ..GN.TV.G. ......AT.. .KMKE..VEA L.A.Q.---. SN.CIN.... ..HIMSN--- ------
Salmo salar m.l polypeptide     ..GN.TV.G. ......TT.. .KMTE..VET LLA.H.---. .N.CIN..V. .RHIMS.--- ------
H.sapiens fast sk.alkali mlc1   ..GN.TV.G. ......AT.. .KMKE..VEA L.A.Q.---. SN.CIN.... ..HIMSI--- ------
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Fig: 5. Alignment of the highly similar deduced amino acid sequences of nematode alkali myosin light chain mlc-3 proteins with *S. digitata* in order of decreasing homology. The sequences were obtained from the protein database at NCBI. Residues identical to the consensus are indicated by dots. Gaps are indicated by hyphen.

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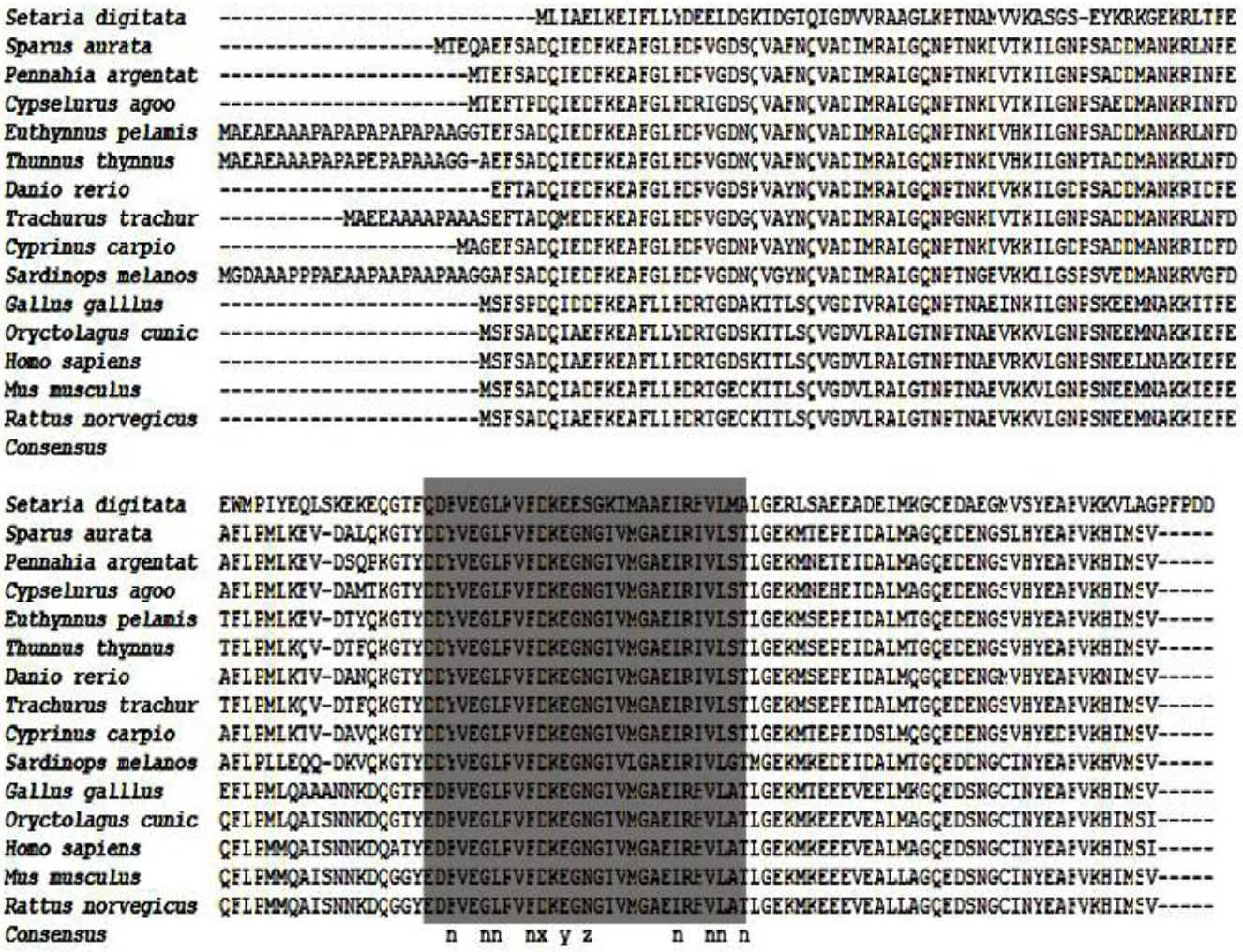

Fig: 6. Alignment of avian and mammalian myosin light chain (MLC3) amino acid sequences with *S. digitata*. The MLC3 specific domain of avian and mammalian sequences and the ancestral Ca^{2+} binding domain are indicated in shaded box. n indicates those residues which form the core of the E and F α helices. X, Y, Z, -X,-Y and -Z are residues which might be involved in the binding of divalent metal ions in EF hand domain.

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CONFLICT OF INTERESTS

There is no financial or personal interest that might pose a conflict.

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