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Research Article

MOLECULAR CHARACTERIZATION OF MMP-9 GENE IN CYSTIC FLUID OF *CYSTICERCUS TENUICOLLIS* BY REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

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ABSTRACT
The present study was carried out to confirm the presence of MMP-9 gene in the cystic fluid of *Cysticercus tenuicollis.* Collection of cyst was made from goats slaughtered at local abattoirs and washed thoroughly with PBS (pH 7.4). The cystic fluid was aspirated, centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatants were used for further study. Total RNA was isolated from the cystic fluid of *Cysticercus tenuicollis*. The total cellular RNA was obtained from 400 µL of cystic fluid was 0.214 µg and the concentration of the RNA was 0.535 µg/mL. The RT-PCR product, 204 bp propeptide domain of MMP-9 was detected through agarose gel electrophoresis, which confirmed the presence of MMP-9 in the cystic fluid of *Cysticercus tenuicollis*

Keywords*: Cysticercus Tenuicollis, MMP-9 Gene, RT-PCR, Goats*

INTRODUCTION
Proteases have been considered as invasive arm for tissue penetration by parasitic helminths and many experiments revealed that characterization of protease enzyme activity has been evaluated from larval,young and adult crude extract as well as excretory and secretory (ES) products of many parasites (Knox *et al.*, 1990; Gamble *et al.*, 1996; Halfner *et al.*, 1998). Involvement of MMPs and, in particular, of gelatinases in both protozoan and helminth infections is well documented. According to their substrate specificity, MMPs can be categorized as collagenases, gelatinases, elastases, Stromelysins and membrane-type MMPs. Nematode MMPs generally include collagenases, gelatinases and elastases (Healer *et al.*, 1991).
Host invasion and tissue migration of several nematodes have been linked to the expression and release of parasite-derived proteases. MMPs are the proteases which are thought to play an important and essential role in these migratory and invasive phenomena (McKerrow *et al.*, 1990).
Expression and release of MMPs by various parasitic nematodes and trematodes have been associated with the pathology resulting from histolysis (Lai *et al.*, 2005). Most of these works evaluated MMPs from excretory/secretory (ES) products as well as the extracts of parasites (Lai *et al.*, 2005). But the work on MMPs in parasitic metacestodes is very limited. Hence, the present study was undertaken to characterize the MMP-9 gene in cystic fluid of *Cysticercus tenuicollis* by Reverse Transcription Polymerase Chain Reaction (RT-PCR).

MATERIALS AND METHODS
Collection of cysts was made from goats slaughtered at local abattoirs in Orathanadu, Thanjavur and Pattukottai areas The cysts collected were confirmed to be *Cysticercus tenuicollis* cysts using their predilection sites, size and morphology (FAO, 1995). After collection, the cysts were washed thoroughly with PBS (pH 7.4) and were subjected for RNA extraction (Skeurman and Hillard, 1966). Total RNA was isolated from the Cystic fluid samples by adopting the protocol given in the QIAGEN RNeasy Mini Handbook. Total RNA was isolated from the *Gastrothylax crumenifer* samples as per the protocol given in the QIAGEN RNeasy Mini Handbook. Primers targeting propeptide domain of the MMP-9 was

designed through the computer software "DNASTAR". The primers were supplied as desalted oligonucleotides by Imperial Biomed (USA).

The details of the primers used were as follows:

Target	Primer sequence	Product
Propeptide domain	Forward primer 5'-CGGCGGATCCCCCAGACCACACAAGCCCACC-3'	204 bp
	Reverse primer 5'- CGGCGTCGACTCTAGATTTGTCCAGCTCTCCAGTCTCAG-3'	

RE sites were incorporated viz. BamHI, XbaI and SalI along with appropriate overhanging sequences on forward and reverse primers respectively for directional cloning of the amplified product. After incorporation of the RE sites the expected size of the amplicon was at 204 bp. RT-PCR was carried out in 2 steps as and the first step was to synthesize cDNA and the second step to amplify the desired genes from cDNA by PCR. The first strand synthesis of cDNA was standardized in a 25 µL reaction mixture containing the following reagents Merck genei, Mumbai. The cDNA thus obtained was used for amplification by PCR in the next step. PCR was carried out to amplify the desired genes from the synthesized cDNA in a final volume of 25 µL. The thermocyclic conditions were set as follows

Reaction	PCR	No. of cycles
Initial denaturation	95ºC for 2 min	1
Denaturation	95ºC for 30 sec	32
Primer annealing	60ºC for 30 sec	32
Primer extension	72ºC for 90 sec	32
Final extension	72ºC for 10 min	1

The amplified PCR product was checked by submarine gel electrophoresis using 1.5% agarose mixed with ethidium bromide at 100 volts for 1 hour with 100 bp plus DNA ladder (MBI fermentas) ran simultaneously on a parallel well. Agarose gel electrophoresis of the PCR product was carried out.

RESULTS AND DISCUSSION

Total RNA was isolated from the cystic fluid of *Cysticercus tenuicollis* using Genei easy RNA isolation kit. The concentration and purity of RNA was determined at 260 and 280 nm, in a spectrophotometer. The total cellular RNA was obtained from 400 µL of cystic fluid was 0.214 µg and the concentration of the RNA was 0.535 µg/mL. The ratio of A 260/ A280 was 1.86 indicating that isolated RNA was reasonably pure. The integrity of RNA was assessed by agarose gel electrophoresis.

The RT-PCR products were subjected to 1% agarose gel electrophoresis and the results were shown. The expected sizes of 204 bp for propeptide domain, was observed. The products were very specific and devoid of any spurious amplification. The concentration of the amplicons appeared to be very high, as shown in figure. To purify the amplicons, products from 100 µL PCR reactions for each of the domains were run in LMP agarose. The bands of interest were cut and eluted with the QIAquick gel extraction kit. Two microliters of this gel eluted product was electrophoresed to check the presence of specific band. Specific discrete bands were observed and the gel- purified product was used for cloning.

Yokota *et al.*, (2001) reported the cDNA of MMP 9 isolated from canine adenocarcinoma 1080 bp and it was having 79.6 % homology with human 80.6 % with rabbit and 82.3% with bovine. Similar results were earlier reported by Lana *et al.*, 2000 and Tanaka *et al.*, (1993).

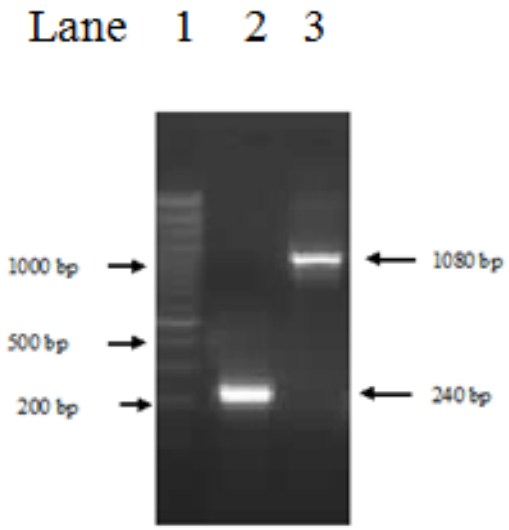

Figure 1: RT-PCR analysis for the presence of propeptide and catalytic domain of MMP-9 gene in the cystic fluid of Cysticercus tenuicollis
Lane 1- 100 bp DNA molecular marker
Lane 2- Propeptide domain of MMP-9 gene
Lane 3- Catalytic domain of MMP-9 gene

The PCR products revealed 75 % homology with human 92 KDa gelatinase activity (Tanaka *et al.,* 1993). The RT- PCR of catalytic domain of MMP 9 was not carried out in any of the domestic animals viz. cattle, buffaloes, sheep, goat and equines. This is the first report for confirmation of the presence of cDNA specific for MMP 9 gene in *Cysticercus tenuicollis*.

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