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Integrative Genomic Analysis of rDNA from Zoonotic Gastrointestinal Parasite, *Fasciola gigantica* Parasitizing *Bubalus bubalis* from Bhojpur (Ghaziabad), Uttar Pradesh, India

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Abstract: *Fasciola gigantica* is a zoonotic, digenetic, gastrointestinal trematode causing infectious diseases in millions of ruminants worldwide leading to economic losses of livestock. Few human infections with *F. gigantica* reported from various parts of the world have shown severe clinical diseases like fever, abdominal pain and eosinophilia. Despite of its zoonotic significance and hazards, the gene functionality of this parasite remains unknown. The present study deals with the genomic study of rDNA of *F. gigantica* using *in silico* workflow. Primer designing, phylogenomic analysis and motif study estimated the set of primers for molecular studies, taxonomic validation of the worm and gene functioning, and cell signaling molecules involved in host parasite interactions. Analysis of the rDNA genome of *F. gigantica* identified two motifs- DNA Motif MYRISTYL (N-myristoylation site) which has a crucial role in regulating innate immune responses and PKC_PHOSPHO_SITE (Protein Kinase C phosphorylation site) which play important roles in several signal transduction cascades. This is the first report of integrative genomic analysis of rDNA from *F. gigantica* from the Indian sub-continent that aims to provide a better understanding of its genomics, gene functionality, host parasite interplay and also the identification of these sites will further help in drug design research that can prove as a vital tool for improving animal health. With the alarming threats of anti-fasciolid drug resistance reported from various parts of the world it is expected that this study will lead to new insights into the biology of this fluke and thus foster to identify potential anti-fasciolid compounds.

Keywords: Zoonotic, *Fasciola gigantica*, Motif, rDNA, Genomics, *In silico*

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Introduction

Parasitic infection is a serious constrain to health and productivity of the livestock in tropical and sub-tropical parts of the world including India. India being an agricultural country, its economy is

largely dependent on profitable agriculture production (Samanta and Santra, 2007; Jyoti *et al.*, 2012; Singh *et al.*, 2012; Rahman *et al.*, 2012; Krishna Murthy and D'souza, 2014). Animal

husbandry has been the most important component of the agricultural production system as it includes dairy, sheep, goat, poultry, piggery farming system. Livestock, goat and sheep are largely practiced by marginal farmers as their source of income and food. The productivity of dairy products, by-products from sheep and goat depends upon the health conditions and their management. Since decades, parasites have known to be the major cause of diseases that leads to significant loss in the productivity of livestock, frequently causing significant economic loss and various life-threatening impacts on animal welfare. Since then, a wide number of attempts have been made till date for developing resistance to helminth worms, lice, blowflies and various other microscopic parasitic infections. Bioinformatics has provided new insights for parasite management that aims to provide optimal parasite control by parasite monitoring, designing potent target drug molecules using docking, genomic analysis, understanding the proteins functionality, transcriptome analysis, meta-genomics, determining the physiochemical properties of the proteins which provide a scope for integrated analyses of the host-parasite relationship (Chauhan *et al.*, 2021).

Fasciolosis is a food and water-borne disease that has been widely caused by two digenetic trematode species, *F. hepatica* and *F. gigantica*. They have a wide range of distribution and cause enormous economic losses due to reduced productivity in domestic ruminants. Till date several cases of zoonosis of *F. gigantica* to humans has been reported from Geneva, Asia, Africa and Hawaii. The symptoms of the disease in humans caused by this zoonotic digenetic trematode includes fever, abdominal pain and eosinophilia.

The present study focuses on *in silico* genomic analysis of 5.8S rDNA of *Fasciola gigantica* that includes primer designing, phylogenomic analysis and motif studies. The designing of primer pairs will further help the researchers in molecular

studies of rDNA of *F. gigantica*. Phylogenetic analysis showed close similarity with different *F. gigantica* rDNA sequences submitted to NCBI under accession number MZ021344 that confirmed the taxonomic validity of the worm. The analysis further revealed the various positions at which the different types of motifs are present in the 5.8S rDNA of *F. gigantica*. The DNA motif analysis is crucial as it serves as the primary step in studying gene functionality and plays a vital role in identification of Transcription Factor Binding Sites that help in understanding the mechanisms for regulation of gene expression (Fatma *et al.*, 2019). The utilization of *in silico* approaches for the genomics has been successfully used in several helminthes like malaria parasite *Plasmodium falciparum* and *Plasmodium vivax*, *Toxoplasma gondii*, *Leishmania donovani*, *Explanatum explanatum* (Tyagi *et al.*, 2021), *Entamoeba nutalli* and *Schistosoma mansoni*. This study will further improve in elucidating the function of the motifs that plays a crucial role in cell signaling, gene functioning and how they infect and persist within their hosts. Thus, targeting the motif will serve as an important tool for controlling the disease caused by *F. gigantica*.

Materials and Methods

Retrieval of the sequence: In this work, we used rDNA of *F. gigantica* gene. The sequence was retrieved from NCBI (National Centre of Biotechnology Information) database (<http://www.ncbi.nlm.nih.gov>) under accession number MZ021344.

Primer designing: Primer designing was performed using computational tool of NCBI.

Phylogenomic analysis: For the taxonomic validity of the worm Phylo-genomic tree was constructed using neighbor joining method.

Motifs study of the zoonotic parasite: From the retrieved sequence, the motifs were studied using computational tools.

Results and Discussion

Analysis of primers of rDNA of zoonotic trematode: Ten pairs of primers were designed using *in silico* computational tools (Fig. 1).

Primer pair 1										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	CCAAAAAGTCGTGGCTTGGG	Plus	20	147	166	59.97	55.00	4.00	0.00	
Reverse primer	GTGACCGCCGTTTGTTGAA	Minus	20	477	458	59.90	50.00	4.00	3.00	
Product length	331									
Primer pair 2										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	CCCAAAAAGTCGTGGCTTGG	Plus	20	146	165	59.97	55.00	4.00	0.00	
Reverse primer	AACGCCATAGATCTGGCACC	Minus	20	228	209	60.18	55.00	6.00	2.00	
Product length	83									
Primer pair 3										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	GGTGCCAGATCTATGGCGTT	Plus	20	209	228	60.18	55.00	6.00	0.00	
Reverse primer	TCCATGCACCCACCATTTT	Minus	20	448	429	60.18	50.00	4.00	0.00	
Product length	240									
Primer pair 4										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TCTTGAACGCATATTGCGGC	Plus	20	68	87	59.62	50.00	6.00	3.00	
Reverse primer	ATTAAGCCACGATTGCGCCA	Minus	20	304	285	60.11	50.00	5.00	2.00	
Product length	237									
Primer pair 5										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	GTATCCGGATGCACCCTTGT	Plus	20	237	256	59.82	55.00	8.00	2.00	
Reverse primer	GAGTGACCGCCGTTTGTGTG	Minus	20	479	460	60.32	55.00	4.00	0.00	
Product length	243									
Primer pair 6										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	CTTGCTTTGGCAGAAAGCCG	Plus	20	252	271	59.76	55.00	5.00	2.00	
Reverse primer	CGGACACAGTGACATCGGAG	Minus	20	337	318	60.46	60.00	5.00	1.00	
Product length	86									
Primer pair 7										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	AACCGGTTGGTACTCCGATG	Plus	20	306	325	59.75	55.00	8.00	1.00	
Reverse primer	ATGAGTGACCGCCGTTTGT	Minus	20	481	462	60.53	50.00	4.00	0.00	
Product length	176									
Primer pair 8										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TATTGCGGCCATGGGCTTAG	Plus	20	79	98	60.54	55.00	6.00	3.00	
Reverse primer	CGCCATAGATCTGGCACCTC	Minus	20	226	207	60.32	60.00	6.00	3.00	
Product length	148									
Primer pair 9										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TTGTCTTTGGCAGAAAGCCGT	Plus	20	253	272	60.47	50.00	3.00	1.00	
Reverse primer	TGACATCGGAGTACCAACCG	Minus	20	328	309	59.47	55.00	4.00	2.00	
Product length	76									
Primer pair 10										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	CTCCGATGTCACTGTGTCCG	Plus	20	318	337	60.46	60.00	5.00	2.00	
Reverse primer	AATGAGTGACCGCCGTTTGT	Minus	20	482	463	59.41	50.00	4.00	0.00	
Product length	165									

Fig. 1: Ten pairs of designed primers for *F. gigantica*.

Phylogenomic analysis:

Phylogenomic analysis (Fig.2) revealed that the present rDNA of *F.gigantica* of interest showed close similarity with flatworms and different ITS sequence submitted on NCBI of *F.gigantica*, clustered out at a separate node in the form of separate branch that confirms the taxonomic validity of the worm.

Motif study of rDNA of *Fasciola gigantica*:

Two different motifs namely N-MYRISTOYLATION and PKC_PHOSPHORYLATION were found in the 5.8S rDNA of *F. gigantica*. The positions of these motifs were from position 60-65 (Fig. 3) and 95-97 (Fig. 4). The role of these motifs have been widely studied in coding DNA.

conducting molecular studies on this zoonotic parasite. Phylo-genomic analysis and DNA motif analysis within the sequence will serve as useful tool in reinforcing the understanding of particular characteristics of gene functionality annotated. The

study identifies the presence of two important motifs that upon translation in coding DNA has functionality role in protein folding and conformational stability, intracellular transport, antibiotic resistance and homeostasis processes, resistance to digestion by proteases, interaction with cell-surfaces, extracellular matrix proteins and other ligands, sexual commitment, inducing chemotaxis, DNA repair, cell cycle control, regulating the circadian rhythm and other cellular-processes, providing innate immune response, phosphorylation sites on human lamina and in nuclear lamina structural dynamics. The authors believe that the identification of these motifs might serve an important role in disease control by docking as these sites of motif could be an attractive target of intervention for the treatment of Amphistomiasis by blocking the gene functionality of the parasite and hindering its host-parasite interactions. The phylogenomic analysis showed similarities with flatworms and *F. gigantica* ITS regions from worldwide that further confirms the taxonomy of this digenetic trematode. Our findings open possibilities for more in depth analysis of this zoonotic parasite and to further our understanding of fluke biology and the search for new drug.

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