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Motif Analysis of *Senga lucknowensis* (Cestoda: Bothriocephalidea) Infecting *Channa punctata*

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Abstract: Gene expression profiles often indicate gene regulatory patterns. The regulation of gene expression mechanisms is difficult to understand in helminth parasites. Finding regulatory elements, notably the DNA binding sites for transcription factors is crucial to this endeavour. These binding sites are Short DNA motifs or sequences. Technologies for high-throughput gene expression analysis and the availability of genomic sequences have recently advanced, enabling the creation of computerized algorithms for motif discovery. As a result, during the past ten years, numerous motif-finding tools have been developed and used with a wide range of motif models. All of the algorithms under consideration have been claimed to accurately uncover motifs that have already been discovered through laboratory experimental techniques. In the current communication, we evaluated the fitness of the discovered motifs for each *Senga* species using a standard data set, and our method provides precise motifs for each of them, which will be helpful to boost our knowledge of gene expression and fresh perspectives for future drug development studies of *Senga lucknowensis.* This is the first report of *in silico* identification of the position of DNA motifs in the 28S rDNA of *Senga lucknowensis* isolated from *Channa punctata* from India.

Keywords: *Senga lucknowensis*, Gene expression, *In silico*, Motif, Drug development

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Introduction

The study of motifs in particular species is important since it not only shows how particular genes function but also serves as a potential target for the treatment of diseases. Several parasites have been effectively exploited by *in silico* methods for the functional prediction of gene functioning. An arrangement of nucleic acids known as a DNA motif serves as DNA binding sites for a transcription factor or other regulatory protein. Transcription factors are proteins made up of a certain nucleotide sequence known as a "binding motif" that binds to DNA in a particular

Table 1: Depicting Comparison among various motifs of 28S rDNA Region of *Senga lucknowensis*

Sequence available of *Senga lucknowensis* available on NCBI database				
Accession number	**ON792316**	**ON778648**	**KR780891**	**OP358453 (Present Study)**
Country	India	India	Vietnam	India
1	ASN_GLYCOSYLATION N-glycosylation site.	-	ASN_GLYCOSYLATION *N-glycosylation site.*	ASN_GLYCOSYLATION *N-glycosylation site.*
2	CK2_PHOSPHO_SITE *Casein kinase II phosphorylation site.*	CK2_PHOSPHO_SITE Casein kinase II phosphorylation site.	CK2_PHOSPHO_SITE *Casein kinase II phosphorylation site.*	CK2_PHOSPHO_SITE *Casein kinase II phosphorylation site.*
3	LEUCINE_ZIPPER Leucine zipper pattern.	MYRISTYL N-myristoylation site.	MYRISTYL *N-myristoylation site.*	MYRISTYL *N-myristoylation site.*
4	MYRISTYL *N-myristoylation site.*	PKC_PHOSPHO_SITE Protein kinase C phosphorylation site.	PKC_PHOSPHO_SITE *Protein kinase C phosphorylation site.*	PKC_PHOSPHO_SITE *Protein kinase C phosphorylation site.*
5	PKC_PHOSPHO_SITE Protein kinase C phosphorylation site.	-	TYR_PHOSPHO_SITE *Tyrosine kinase phosphorylation site*	TYR_PHOSPHO_SITE *Tyrosine kinase phosphorylation site.*

way (Näär *et al*., 1991)

One of the initial tasks in a computational study of gene regulation is motif discovery. For instance, it is usual for researchers to look for over-represented motifs shared by groups of genes with comparable expression patterns. The output of motif-finders can be difficult to comprehend since it may contain a large number of different motifs with little to no information about their putative regulatory functions. Additionally, nothing is said about the transcription factor (TF) protein that might connect to them. Therefore, it is surprising that there are not many tools available right now that can compare novel, computationally identified motifs to those that are already recognized and kept in databases. The development of motif-finding tools has been approached in a variety of ways and the advancements made in this field of study are quite encouraging (Das and Dai, 2007).

Motif discovery is still a difficult task for biologists to complete, despite significant efforts to date because researchers are so confused about morphological identification yet and more of the previously reported *Senga* species have been synonymized (Kuchta and Scholz, 2007). Studies reported from outside on molecular analysis of Bothriocephalidea have shown (Brabec *et al*., 2015) that less data is available on molecular studies from India. The objective of the current study was to look into a motif in the 28S rDNA of *Senga lucknowensis* to understand the evolutionary relationship and drug discovery.

Materials and Methods

Retrieval of the Sequence: NCBI database was used to retrieve the 28S rDNA sequence of the *Senga lucknowensis* using accession number OP358453.

Motifs Analysis: Bioinformatics tools were used to analyze the motifs.

Results and Discussion

Motif analysis in our study uncovered five motifs. Among them, four were similar in all *Senga lucknowensis* (isolated from different regions) ASN_GLYCOSYLATION, CK2_PHOSPHO_SITE, MYRISTYL, PKC_PHOSPHO_SITE and TYR_PHOSPHO_SITE except LEUCINE_ZIPPER. The position of these motifs was from 46-49,105-108, 72-77, 65-67, 107-109 and 16-23, respectively (Tables 1, 2; Figs. 1a, b, c, d, e).

Table 2: Table showing match scores and motif information of 28S rDNA Region of *Senga lucknowensis*

Match scores		Motif information
46	49	ASN_GLYCOSYLATION N-glycosylation site.
105	108	CK2_PHOSPHO_SITE Casein kinase II phosphorylation site.
72	77	MYRISTYL N-myristoylation site.
65	67	PKC_PHOSPHO_SITE Protein kinase C phosphorylation site.
107	109	
16	23	TYR_PHOSPHO_SITE Tyrosine kinase phosphorylation site.

Fig. 1(a): ASN_GLYCOSYLATION.,

Fig. 1(b): CK2_PHOSPHO_SITE.

Fig. 1(c): MYRISTYLSITE.

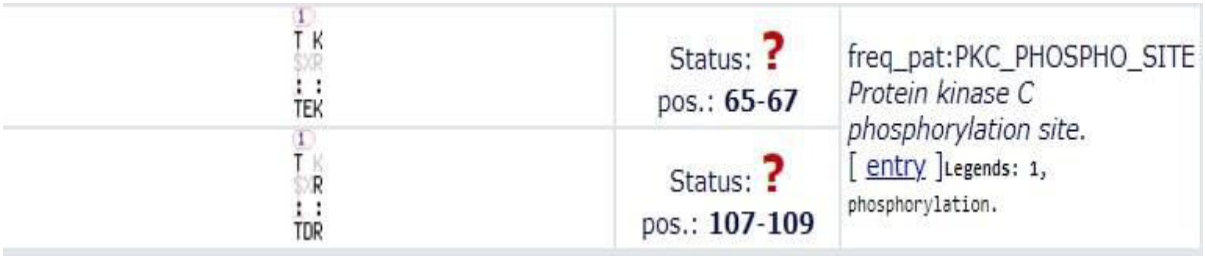

Fig. 1(d): PKC_PHOSPHO_SITE.

Fig. 1(e): TYR_PHOSPHO_SITE.

Aquaculture has a key role in India in terms of its contribution to the gross domestic product and economy. It generates 65% of all jobs, 21% of all exports, and 27% of India's GDP (Venkitaramanan, 2001). Because parasitic diseases, whether ecto- or endoparasites, cling to the host for their existence and cause mild to severe infections to the host body, mortality and infections in fish have been steadily rising. Genus *Senga* belongs to the order Bothriocephalidea having distinct morphological characters. Computational tools are an emerging technology that can successfully address problems (Azevedo *et al*., 2009) related to parasite management, which aims to provide effective parasite control through parasite monitoring, designing potent target drug molecules, genomic analysis, protein analysis, transcriptome analysis and metagenomics for figuring out the physiochemical characteristics of the host-parasite relationship.

Numerous biological processes are impacted by the crucial protein modification known as N-glycosylation. Membrane and secreted proteins, including GPCRs, require asparagine-linked glycosylation (N-glycosylation) motif in order to fold appropriately. Since novel medications with therapeutic and antilipolytic advantages are targeting GPCRs. N-glycosylation and its motif in GPCRs are involved in processes including receptor trafficking and intracellular signalling. A wide range of biological processes involving CK2 demonstrates a complex interaction between CK2 phosphorylation and other post-translational changes. The crucial fatty acylation known as protein N-myristoylation is brought out by N-myristoyltransferases, which are found in every eukaryote involved in a variety of biological steps, such as signal transduction, cellular localization and oncogenesis, a myristoyl group must be attached. Protein N-myristoylation is thought to have a role in host defense mechanisms, according to recent research that has uncovered unanticipated pathways.

The significance of PKC is proven in both health and disease. This family of kinases is still a desirable target for pharmaceutical research. As our understanding of PKC biology grows, it seems that even selectivity at the isozyme level might not be enough for a medicine to have the best possible efficacy and the fewest possible undesired "on-target" side effects. The capacity to track the pharmacodynamic activity of fresh PKC modulators would be significantly improved by biomarkers that are specific for PKC activity. The

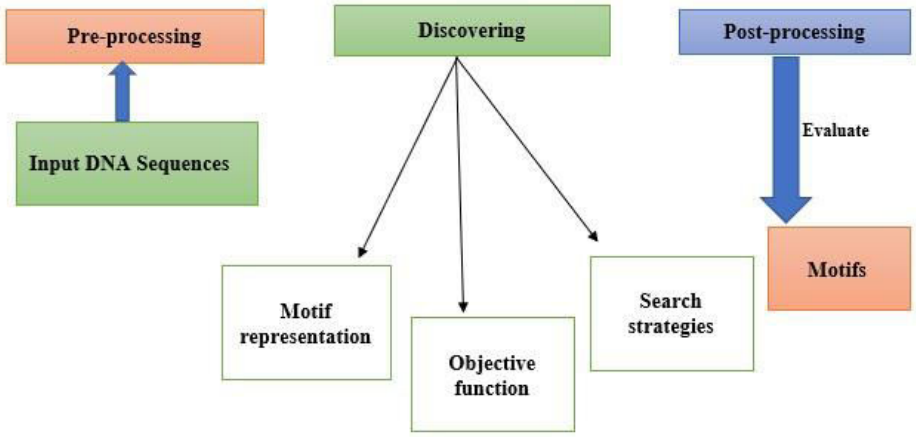

Fig. 2: Diagrammatic presentation of motif designing.

crucial posttranslational modification of protein tyrosine phosphorylation (PTP) regulates the cell signalling that play a role in cell growth, proliferation, differentiation, migration, survival and death. Numerous studies worldwide throw light on the significance of these motifs in coding DNA, however, no such studies have been reported from India.

Tyrosine phosphorylation (TYR PHOSPHO SITE), a key signal communication and controlling mechanism in every multicellular organism has emerged as a mechanism that regulates a number of procedures, including cell division, metabolic balance, transcriptional activation, neuronal transmission, differentiation and development, and ageing are all examples of procedures. Tyrosine kinase inhibitors were developed as a result of the discovery that many human diseases, including cancer, are triggered by alterations in tyrosine phosphorylation. these inhibitors have been approved for therapeutic purposes. The leucine zippers encourage the dimerization domains by placing a distinctive leucine at every seventh position among the five consecutive heptads of amino acid substances. Leucine zippers, structural motifs that typically consist of five successive heptads of amino acids with a distinct leucine at every 7th position, play an important role in the conformational changes of the bZIP family of transcription factors and their stabilization to the DNA promoter regions of target genes.

Information on parasite motifs may provide possibilities for *in silico* mining of innovative drug repurposing which will bring meaningful insights for vaccine development for several illnesses brought about by helminths for which the present work will serve as a foundation. The goal of the current study was to bring to light different DNA motifs in the 28S rDNA of *Senga lucknowensis* that have a role in preventing the parasite from synthesizing proteins and decreasing ribosome translocation activity, thus offering a special way to prevent the parasite from spreading. These motifs play a function in signal transduction, cellular localization, receptor trafficking, intracellular signaling, posttranslational modify-cation, immune response and phosphorylation sites etc. upon translation (Table 2; Figs. 1 a, b, c, d, e). This study will bring new insights into our understanding of the parasite genome and drug discovery which is essential for finding cost-effective drug candidates.

Conclusion

The first step towards understanding gene

function is the identification of DNA motifs. Finding transcription factor binding sites (TFBSs) is essential for understanding the mechanisms governing the control of gene expression. This process is aided by motif discovery. Drug development is a multi-step process that starts with target selection and screening continues with hit-to-lead creation, optimization, preclinical and clinical investigations, and ends with the official registration of a drug. Several techniques, including *in silico*, *in vitro* and *in vivo* studies, can be used to find drug-like compounds that can obstruct a protein's function (Azevedo *et al*., 2009). Understanding sequence patterns can help us to better understand processes including protein complex building, mRNA splicing, and transcription control. Motif designing process through online tools helps the biologist to find out protein motifs that can serve as the active sites of enzymes or other regions involved in the stability and structure of proteins (Fig. 2).

The ability to concentrate on drug-finding research on protein targets was made possible by the sequencing of the parasite genome. These studies typically entail high-level high-throughput molecular library screening using large structures to see if they block a certain protein target that was previously discovered through the sequencing of the parasite genome. Authors claim that identifying these motifs may be crucial for disease control by molecular docking since these motif locations may be appealing targets and also helpful in understanding the evolutionary relationship (Ashraf and Shafi, 2020).

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