The Pharma Innovation Journal 2018; 7(8): 442-444

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(8): 442-444

www.thepharmajournal.com
Received: 01-06-2018
Accepted: 03-07-2018

Prasanta Kumar Koustasa Mishra
Ph.D. Scholar, Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Indhu MS
Ph.D. Scholar, Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Correspondence
Prasanta Kumar Koustasa Mishra
Ph.D. Scholar, Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Molecular amplification and phylogenetic analysis of coding sequences of goat complement component 9 (C9)

Prasanta Kumar Koustasa Mishra and Indhu MS

Abstract
The complement mediated lysis is one of the few combating responses from host defence system. It acts coherently with innate and adaptive immune system. The complement system contains many glycoproteins which are normally present in inactive state. Upon activation, they initiate cascade of reactions leading to formation of a cytolytic complex otherwise known as membrane attack complex (MAC). C9 is the terminal component of such complexes. More than about 15, C9 molecules aggregates in this process. Goats are considered as hardy animals, resistant to various diseases. Very few literatures are there describing the goat immune system components. In the present study, an attempt was made to amplify the C9 coding sequence of goat which came to be around 2 kb. A phylogenetic analysis of the C9 sequence was also done to examine the distribution pattern across other ruminants and carnivores

Keywords: Complement, C9, immune system, amplification, goats, phylogeny

1. Introduction
The complement system plays important role in pathogen recognition, lysis of pathogen, removal of allergen and solubilisation of immune complexes. Formation of membrane attack complex is the crucial step for complement mediated lysis [1]. The association of C5b, C6, C7, C8 into an activated complex, invites C9 polymerization and deposition at the target surface. The polymerized pricking complement complex creates pore on the surface of the target membrane. The resulting osmotic unbalance leads to lysis of the target cells [2]. The multi domain protein C9 can be designated as a key player in the lysis processes [3]. In case of goat, isolation of C9 from goat serum is recently reported [4]. In the present study, attempt was made to amplify C9 encoding nucleotide sequences of goat.

2. Methods
2.1. Collection of goat liver from local abattoir
Goat liver (~50 Gms) was collected local abattoir in a sterile test tube with *RNAlater* ™ stabilisation solution. The tissue sample was kept on ice and immediately transported to laboratory. The consumables for the experiment were cleaned with DEPC (0.01%) treated water. With a sterile needle the tissue was minced to smaller pieces.

2.2 RNA isolation and cDNA synthesis
TRIzol™ Plus RNA Purification Kit (Invitrogen) was used for isolation of liver tissue RNA. 100 mg of minced liver tissue was taken in a DEPC treated 2 ml centrifuge tube and 1 ml of TRIzol reagent was added to it followed by grinding with the help of a micro-pestle. RNA was essentially isolated following the manufacturers protocol. The quantity and quality of isolated RNA was checked by spectrophotometric observation at 260 nm and agarose gel electrophoresis. The first strand cDNA synthesis was done by using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit. The components were mixed in the order as described in table. 1. Oligo $(dT)_{18}$ primer was used for amplification at 42° C for 60 minutes. The reaction was terminated by heating the reaction mixture at 70° C for 5 minutes

2.3 Amplification of C9 encoding sequences of goat by polymerase chain reaction
Gene specific primers were (table. 2) designed from the coding C9 sequences of goat, retrieved from gene bank with the accession number is JO420209.2 (reference ASM170441v1). Primers were designed manually with the help of online tool reverse complement finder. The primers were checked in Oligo analyzer software for any hybrid formation. A polymerase chain reaction was performed to amplify the desired coding regions

of C9. The reaction components and reaction mixtures are given in table. 3 and 4 respectively. The amplified product was analysed in a 1% agarose gel.

2.4 Construction of phylogenetic tree

A phylogenetic tree was constructed by the available open access MEGA software.C9 coding sequences of seventeen different species were retrieved from gene bank and were aligned followed by construction of a dendrogram.

2.5 Molecular model prediction

The FASTA format of the goat C9 amino acid sequence was retrieved from uniprot site. The sequence was used for prediction of the 3D protein structure by using Swiss model (https://swissmodel.expasy.org/) module.

3. Results

The isolated RNA showed 2 distinct bands upon gel electrophoresis. These bands correspond to the 18s rRNA and 28s rRNA (fig 1). The amplified coding sequences for goat C9 was found to be near 2.1 kb (fig 2). The phylogenetic tree was constructed with 1000 boot strap value with neighbour joining method (fig 3). The output of swiss model is being presented in fig 4.

4. Discussion

The C9 polymerization on surface of target cell is the most important event for complements system [4]. Pathogens targets to evade the immune system by modulating the polymerization process of C9 [5]. In the present study the C9 coding sequence of goat was amplified from liver tissue cDNA. From the phylogenetic it was found that C9 shares sequence homology with the Bovidae family but are distantly related to Canidae. The 3D structure of the C9 molecule showed a mixture of helix and strands. Additionally, two curved free ends are seen which might be responsible for anchoring to the cell surface (fig 4).

Table 1:

Reaction components	Volume
Template RNA	5 µl
Oligo $d(T)_{18}$ primers	1 µl
NFW	6 µl
5X reaction buffer	4 µl
Ribo lock RNA se inhibitor	1 µl
10 m M dNTP mix	2 µl
Revert Aid M-Mu LV RT	1 µl
Total	20 µl

Table 2:

Primers	5'-3'
FP C9	AT**GAATTC**TTTGCAAATAAGTGTCTTCCAGGAAC
RP C9	AT**AAGCTT**TCTTTTTAACCAATATTCTCTTTTTAATTC

Table 3:

Reaction components	Volume
5X Phusion HF buffer	4 µl
10 mM dNTP	0.4 µl
FP C9	1 µl
RP C9	1 µl
DMSO	0.6 µl
Template (cDNA)	2 µl
Phusion DNA Polymerase	0.2 µl
NFW	10.8 µl
Total	20 µl

Table 4:

Cyclic conditions and temperature	Time	
Initial Denaturation at 95 °C	4 minutes	
Denaturation at 95 °C	30 seconds	32 cycles
Annealing at 67 °C	30 seconds	
Extension at 72 °C	2 minutes	
Final extension 72 °C	10 minutes	

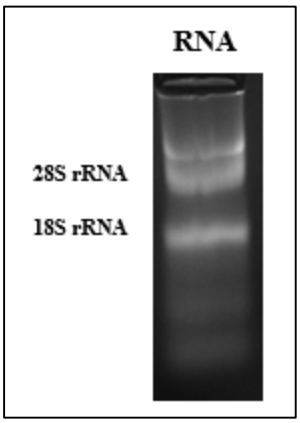

Fig 1: Isolated RNA was analysed on a 1% agarose gel

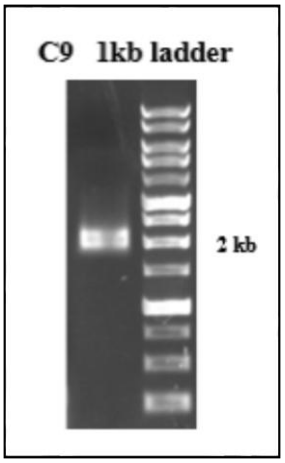

Fig 2: PCR amplified C9 cds analysed on a 1% agarose gel

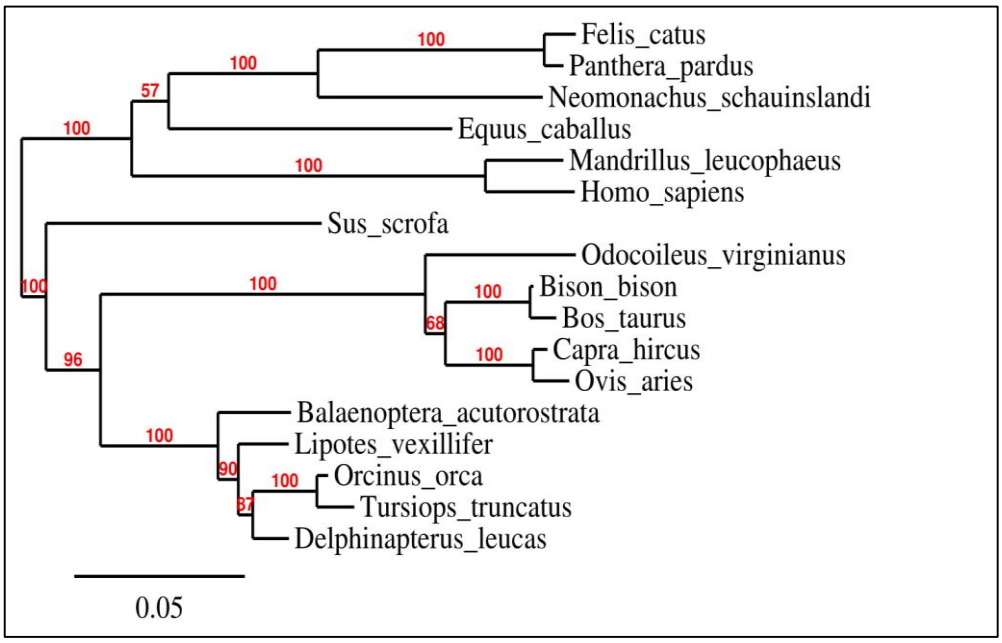

Fig 3: Phylogenetic analysis of 17 different cds of C9 from different species.

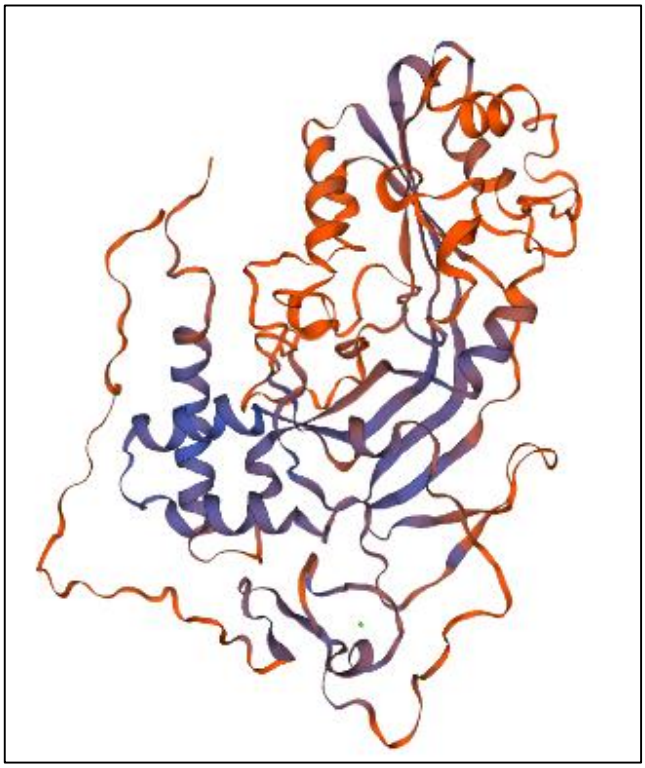

Fig 4: 3D structure of goat C9 predicted *in silico*. Arrow marks indicate the overhangs, probable sites for membrane attachment.

5. Acknowledgment
The authors are thankful to Dr Paritosh Joshi and Director, IVRI for providing necessary funds and facilities. PKKM was supported by ICMR-SRF and Indhu M.S. by IVRI fellowship.

6. References
1. Hänsch GM. The complement attack phase: control of lysis and non-lethal effects of C5b-9. Immuno pharmacology. 1992; 24(2):107-17.
2. Setién F, Alvarez V, Coto E, Di Scipio RG, López-Larrea C. A physical map of the human complement component C6, C7, and C9 genes. Immuno genetics. 1993; 38(5):341-4.
3. Podack ER, Tschopp J. Polymerization of the ninth component of complement (C9): formation of poly (C9) with a tubular ultrastructure resembling the membrane attack complex of complement. Proceedings of the National Academy of Sciences. 1982; 79(2):574-8.
4. Prasada RT, Lakshmi PT, Parvathy R, Murugavel S, Karuna D, Paritosh J. Identification of second arginine-glycine-aspartic acid motif of ovine vitronectin as the complement C9 binding site and its implication in bacterial infection. Microbiology and immunology. 2017; 61(2):75-84.
5. Singh B, Jalalvand F, Mörgelin M, Zipfel P, Blom AM, Riesbeck K. Haemophilus influenzae protein E recognizes the C-terminal domain of vitronectin and modulates the membrane attack complex. Molecular microbiology. 2011; 81(1):80-98.