International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(7): 767-770
www.biochemjournal.com
Received: 15-05-2025
Accepted: 19-06-2025

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Sequencing and bioinformatic analysis of goat β-defensin-1 like cDNA from Osmanabadi goat

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i7Sj.4984

Abstract
Antimicrobial peptides (AMPs), especially β-defensins, serve as critical innate immune components in various organisms, including goats. The present study aimed to sequence and analyze the coding region of a β-defensin-1 like (GBD-1 Like) gene from the Osmanabadi goat, a breed known for its disease resistance. Tongue epithelial tissue from an Osmanabadi goat was collected aseptically, and total RNA was extracted. Complementary DNA (cDNA) synthesis was performed using standard protocols, and PCR amplification was conducted with primers specific to the GBD-1 Like gene. The PCR product of 275 bp was sequenced, and bioinformatic analysis was performed using DNASTAR and Phyre2 tools.
The open reading frame (ORF) comprised 195 nucleotides, encoding a 64-amino acid peptide. Sequence alignment at the nucleotide level showed up to 100% identity with other goat defensins and high similarity (89-99.6%) with defensins from other ruminants such as sheep, cattle, and buffalo. At the amino acid level, identity ranged from 71.9% to 100% with corresponding peptides from various species. Phylogenetic analysis confirmed that Osmanabadi GBD-1 Like clustered closely with goat defensins, forming a distinct group from those of other ruminants. Notably, an amino acid substitution (Valine replacing Leucine) was observed at position 13, which may affect the structural and functional properties of the peptide. Structural prediction using Phyre2 indicated this mutation's location and potential impact on peptide conformation. Further studies are warranted to explore the biological significance of this variation in immune response and disease resistance.

Keywords: Osmanabadi goat, β-defensin-1 like gene, antimicrobial peptides, sequence analysis

Introduction
Antimicrobial peptides (AMPs) are a diverse class of short, cationic, amphipathic molecules that serve as a critical component of the innate immune system across virtually all forms of life, from bacteria to humans [1, 2]. These peptides, typically comprising fewer than 100 amino acids, act as one of the first lines of defense against invading pathogens, including bacteria, viruses, fungi, and even some parasites [1-3]. AMPs are synthesized by a wide array of host cells. These include epithelial cells located at common points of microbial entry such as the skin, gastrointestinal tract, and respiratory epithelium, as well as various immune cells including neutrophils, macrophages, and lymphocytes. Additionally, mucosal surfaces of internal organs—such as the intestines, lungs, and urinary tract—are important sites of AMP production. The primary antimicrobial mechanism of AMPs involves disruption of microbial membranes, either by forming pores or by permeabilizing the lipid bilayer, leading to cell lysis and death. Some AMPs go beyond membrane interactions and exert intracellular effects by inhibiting essential processes such as DNA, RNA, and protein synthesis [4, 5]. Beyond their direct antimicrobial activity, AMPs also possess significant immunomodulatory properties, making them important mediators of immune homeostasis. They can recruit immune cells like neutrophils and monocytes to sites of infection, stimulate the production of cytokines and chemokines, and enhance antigen presentation by dendritic cells and macrophages. Furthermore, AMPs play a dual role in modulating inflammation—they can exhibit either pro-inflammatory or anti-inflammatory functions depending on the cellular context and the stage of immune response [6, 7]. In goats (Capra hircus), the defensin gene family is represented exclusively by β-defensins. A comprehensive genomic analysis identified 50 β-defensin genes in the goat genome, comprising 48 functional genes and 2 pseudogenes [8].

Among the various β-defensins identified in Capra hircus, GBD-1 and GBD-2 are the most extensively studied. GBD-1 (Goat β-Defensin 1) was the first caprine β-defensin to be cloned and characterized, providing foundational insights into its amino acid sequence, structural features, and tissue-specific expression—particularly in the tongue, respiratory tract, and gastrointestinal epithelium. In parallel, GBD-2, a closely related defensin with high sequence similarity to GBD-1, has been functionally explored in greater detail. The remaining genes from this species are yet to be studied in detail. In the present investigation, sequence analysis of coding sequence of β-defensin-1 Like (GBD-1 Like) from Osmanabadi goat was carried out. Osmanabadi goat is an important goat breed from Maharashtra. This goat breed is known to have high disease power against diseases [9]

In the present study, goat tongue tissue was aseptically collected from a local abattoir on ice. The epithelial layer was carefully scraped and processed for total RNA extraction using RNAiso Plus reagent (Takara, India). Complementary DNA (cDNA) was synthesized from the isolated RNA using the PrimeScript™ 1st strand cDNA Synthesis Kit (Takara, India), following the manufacturer's protocol. For amplification of the GBD-1 Like gene, PCR was performed using cDNA as the template and a specific primer pair designed based on the *Capra hircus* mRNA sequence for GBD-1 Like (Accession No. XM_018042143). The primer sequences used were: Forward-5′ CCTGGGACCTTTATAAAGCG 3′ and Reverse-5′ GCGATCTGTCTAAGGGC 3′. PCR reactions were carried out in a total volume of 50 µl, comprising 38 µl nuclease-free water, 5 µl 10× PCR buffer, 2.5 µl $MgCl_2$, 1 µl dNTP mix, 0.5 µl of each primer, 0.5 µl Taq DNA polymerase (Invitrogen, India), and 2 µl of cDNA template. The thermal cycling conditions included an initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of denaturation at 94 °C for 40 seconds, annealing at 58 °C for 1 minute, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. Amplified PCR products were purified, quantified, and submitted to GeneOmbio Technologies Pvt. Ltd., India for sequencing. The resulting sequences were analyzed by aligning them with published GBD-1 Like and defensin sequences available in the NCBI database using DNASTAR software (USA). The three-dimensional structure of the deduced peptide was predicted and analyzed using Phyre2 [10].

The 275 bp PCR product of GBD-1 Like is shown in Fig. 1.

ORF of Osmanabadi GBD-1 Like consists of 195 nucleotides and encodes a peptide of 64 amino acids (The sequences XM_018042143 and XM_018041797 did not consider the stop codon at positon 193-195, thus they have longer protein products). This results are in agreement with the previous report by zhao *et al.* [11]. Multiple alignment at nucleotide level revealed that Osmanabadi GBD-1 Like had 100, 99.6, 99.6, 95.2, 93.2, 92.8, 92.8, 90.6, 90.3, 89.1, and 89.4 percent homology with goat Assam Hill def KY971640, goat XM_018042143, Goat Def1 Y17679, Goat XM_018041797, Ovis canadensis Def2 XM_069572894, Ovis aries Def2 XM_042241482, Ovis aries Def2 XM_042241483, Bubalus bubalis Def AY301005, Rangifer tarandus Def1DQ861296, Bos indicus XR_011564477, and Bos taurus LAP AY911374 respectively (Fig2a). Furthermore multiple alignment at the amino acid level revealed that Osmanabadi GBD-1 Like had 100, 100, 98.4, 85.9, 81.2, 71.9, 71.9, 78.1, 78.1, 78.1 and 76.6 percent identity with Goat Pred XM_018042143, Goat Assam Hill def KY971640, Goat Def1 Y17679, Goat XM_018041797, Ovis canadensis Def2 XM_069572894, Bos indicus XR_011564477, Bos taurus LAP AY911374, Bubalus bubalis Def AY301005, Ovis aries Def2 XM_042241482, Ovis aries Def2 XM_042241483, and Rangifer tarandus Def1DQ861296 peptides respectively (Fig 2b).

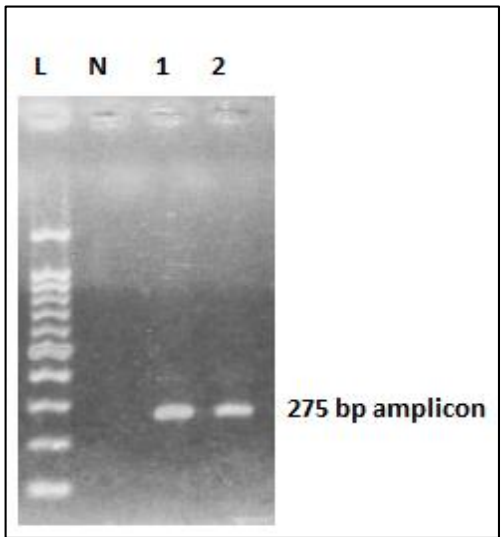

Fig 1: Osmanabadi GBD-1 Like amplification: L-DNA ladder, 1 & 2 Osmanabadi GBD-1 Like, N-negative control

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12		
1		100.0	99.6	99.6	95.2	93.2	92.8	92.8	90.6	90.3	89.1	89.4	1	Goat Def1 Like cds.xdna.seq
2	0.0		100.0	99.5	94.4	92.3	91.8	91.8	91.3	91.3	88.7	88.7	2	Goat Assam Hill def KY971640.seq
3	0.4	0.0		99.2	94.8	92.8	92.4	92.4	91.0	89.9	89.5	89.8	3	Goad Pred XM_018042143.seq
4	0.4	0.5	0.8		95.6	92.8	92.4	92.4	91.0	89.9	89.5	89.8	4	Goat Def1 Y17679.seq
5	5.0	5.9	5.4	4.6		90.4	90.0	90.0	90.6	90.3	89.1	89.4	5	Goat XM_018041797.seq
6	7.2	8.1	7.6	7.6	10.3		99.2	99.2	88.6	87.9	88.3	88.6	6	Ovis canadensis Def2 XM_069572894.s
7	7.6	8.7	8.1	8.1	10.8	0.8		100.0	88.6	87.4	88.3	88.6	7	Ovis aries Def2 XM_042241482.seq
8	7.6	8.7	8.1	8.1	10.8	0.8	0.0		88.6	87.4	88.3	88.6	8	Ovis aries Def2 XM_042241483.seq
9	10.2	9.4	9.7	9.7	10.1	12.4	12.4	12.4		91.4	94.3	94.3	9	Bubalus bubalis Def AY301005.seq
10	10.6	9.4	11.1	11.0	10.4	13.3	13.8	13.8	9.2		91.9	91.5	10	Rangifer tarandus Def1DQ861296.seq
11	12.1	12.5	11.6	11.6	12.0	12.8	12.8	12.8	6.0	8.7		99.6	11	Bos indicus XR_011564477.seq
12	11.6	12.6	11.1	11.2	11.5	12.4	12.4	12.4	6.0	9.2	0.4		12	Bos taurus LAP AY911374.seq
	1	2	3	4	5	6	7	8	9	10	11	12		

Fig 2a: Sequence distance of Osmanabadi GBD-1 Like at nucleotide level

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12		
1		100.0	100.0	98.4	85.9	81.2	71.9	71.9	78.1	78.1	78.1	76.6	1	Goat Def1 Like cds.pro
2	0.0		100.0	98.4	87.8	81.2	71.9	71.9	78.1	78.1	78.1	76.6	2	Goad Pred XM_018042143.pro
3	0.0	0.0		98.4	85.9	81.2	71.9	71.9	78.1	78.1	78.1	76.6	3	Goat Assam Hill def KY971640.pro
4	1.6	1.6	1.6		87.5	79.7	73.4	73.4	79.7	76.6	76.6	75.0	4	Goat Def1 Y17679.pro
5	15.6	13.3	15.6	13.7		73.4	75.0	75.0	81.2	70.3	70.3	76.6	5	Goat XM_018041797.pro
6	21.6	21.6	21.6	23.7	32.8		75.0	75.0	75.0	96.9	96.9	73.4	6	Ovis canadensis Def2 XM_069572894.pro
7	35.2	35.2	35.2	32.8	30.4	30.4		100.0	92.2	75.0	75.0	85.9	7	Bos indicus XR_011564477.pro
8	35.2	35.2	35.2	32.8	30.4	30.4	0.0		92.2	75.0	75.0	85.9	8	Bos taurus LAP AY911374.pro
9	25.9	25.9	25.9	23.7	21.6	30.4	8.3	8.3		75.0	75.0	87.5	9	Bubalus bubalis Def AY301005.pro
10	25.9	25.9	25.9	28.2	37.8	3.2	30.4	30.4	30.4		100.0	70.3	10	Ovis aries Def2 XM_042241482.pro
11	25.9	25.9	25.9	28.2	37.8	3.2	30.4	30.4	30.4	0.0		70.3	11	Ovis aries Def2 XM_042241483.pro
12	28.2	28.2	28.2	30.4	28.2	32.8	15.6	15.6	13.7	37.8	37.8		12	Rangifer tarandus Def1DQ861296.pro

Fig 2b: Sequence distance of Osmanabadi GBD-1 Like at amino acid level

Phylogenetic tree analysis revealed that Osmanabadi GBD-1 Like is closely related to goat sequences (both at nucleotide and amino acid level) (Fig 3a & 3b). The Osmanabadi GBD-1 Like formed separate cluster with other goat sequences at nucleotide level and amino acid level. At both nucleotide and amino acid level Cattle, buffalo and reindeer sequences formed separate cluster, whereas sheep sequences also formed a separate cluster.

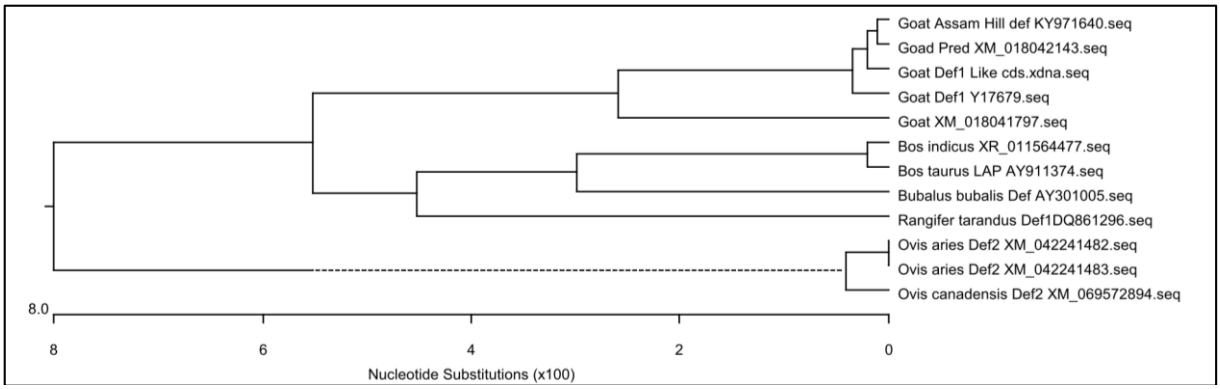

Fig 3a: Phylogeny of Osmanabadi GBD-1 Like at nucleotide level

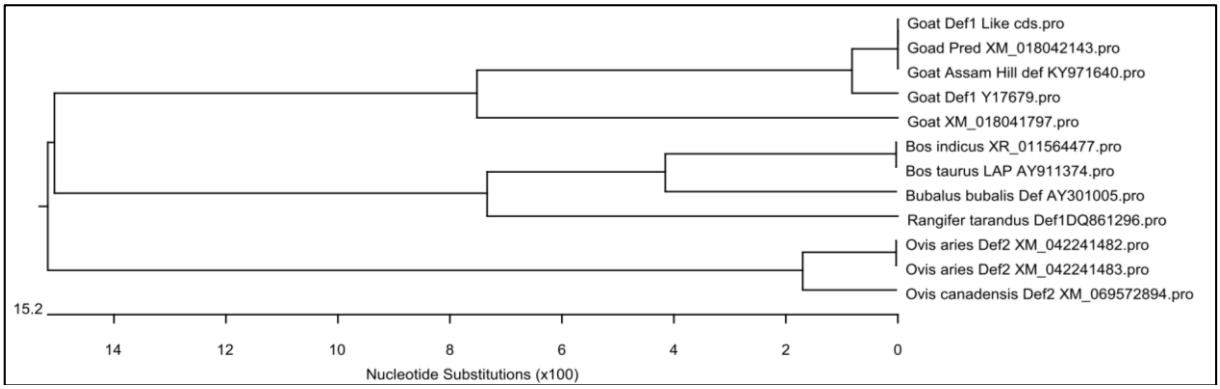

Fig 3b: Phylogeny of Osmanabadi GBD-1 Like at amino acid level

At the amino acid level change was noted at the position 13 where valine was found to be replaced in place of leucine amino acid (Fig 4). The structure of leucine is bigger so is its volume compared to valine. The structure of the goat β-Defensin-1 was predicted with Phyre2 (Fig 5). The changed amino acid is indicated with ball and stick representation. Whether this change affects protein targeting and translocation, signal sequence cleavage, and post cleavage events needs to be studied in future research.

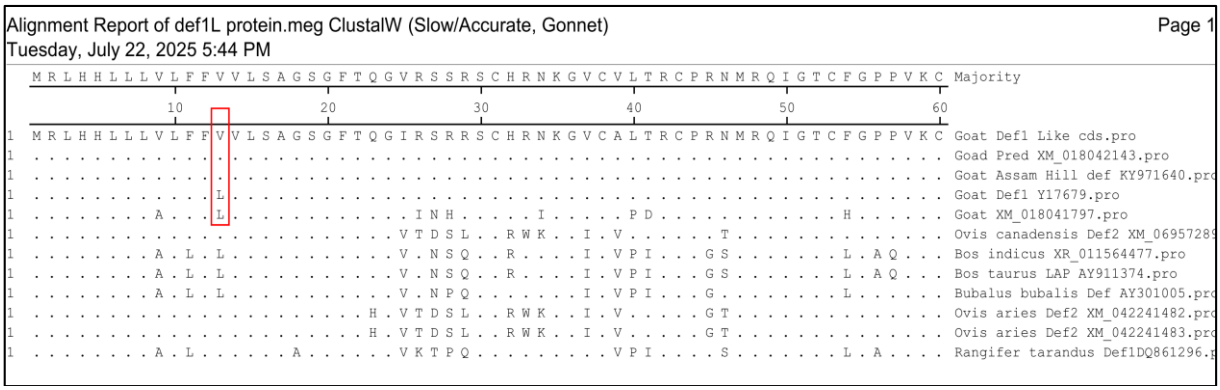

Fig 4: The alignment report of Osmanabadi GBD-1 Like generated using Clustal W.

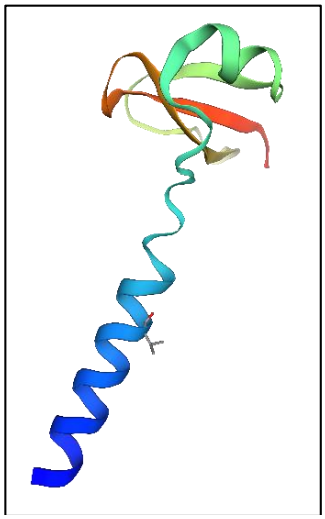

Fig 5: Amino acid changes in structure of Osmanabadi GBD-1 Like. The amino acid at position 13 valine in represented in ball and stick.

Acknowledgements

Authors are thankful to Associate Dean, KNP College of Veterinary Science Shirwal for supporting the research and necessary facilities.

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