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Comparative genomic analysis for phylogeny, physio-chemical and anti-microbial properties in unconventional dairy animals

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Abstract

Casein is a major milk protein that affects the chemical and anti-microbial properties of milk. Four casein proteins i.e. alphaS1, alphaS2, beta, and kappa— were computed for the construction of a phylogenetic tree. Moreover, computational analysis was also performed for the physio-chemical and anti-microbial properties of milk. Whole protein sequences were retrieved from the NCBI database. Physicochemical properties and anti-microbial peptides were predicted using the ProtParam tool and AMPA Tool, respectively. Phylogenetic analysis revealed strong relatedness between Camel and Yak as compared to Donkey. Moreover, most of the casein proteins were found to be unstable in the instability index. Few bactericidal stretches were found especially alphaS2 casein protein in Camel, Yak and Donkey. The average anti-microbial index was around 0.25 -0.26. This study revealed about phylogenetic relatedness of casein protein in different species. It also highlighted the importance of the bioinformatics approach for better prediction of physio-chemical and anti-microbial features of casein micelles.

Keywords: Unconventional, anti-microbial, phylogeny

Introduction

Milk forms an important dietary consumption of the human population around the world. It is rich of all essential nutrients such as protein and calcium. Milk consists of two major proteins such as casein, and whey protein. Out of which, Casein is an important milk protein and constitutes about 80% of total milk protein content. There are four types of casein protein components i.e. alpha-S1, alpha-S2, beta, and kappa-casein contribute colloidal stability of milk (Hassanin *et al.*, 2022) [3]. Bovine milk is the most commonly consumed milk around the world. In addition, conventionally buffalo, sheep and goat milk are also consumed in various parts of the world. However, milk consumption from unconventional species such as Camel, Donkey and Yak is also increasing. The reason being special medicinal benefits provided by milk of these species. Studies on Yak and donkey milk highlighted the presence of antioxidant and antidiabetic properties in casein protein (Salvo *et al.*, 2023) [8]. Additionally, these species reside in difficult terrains of the world and provide nutritious milk to the residents.

Phylogenetic analysis provides an insight towards evolutionary relatedness of casein protein from different species. Physicochemical and antimicrobial properties also provide better insights on bioactive agents present in the milk. Majority of studies on phylogenetic analysis, physicochemical and antimicrobial peptides have been conducted on conventional dairy species such as cattle, buffalo, sheep, goat. Few isolated studies have been there for unconventional species. A phylogenetic study of different casein proteins of *Camelus ferus* depicted distinct clusters of different proteins (Parveen *et al.*, 2023) [6].

Comparative genomic analysis provides a better insight on overall functionality and anti-microbial nature of abundant milk protein casein. Comparative analysis is an emerging field in computational bioinformatics field. However, no such study has been performed unconventional dairy animals i.e. Bactrian Camel, Donkey and Yak. Thus, this in-silico study was formulated to assess the phylogenetic relationship, physicochemical and anti-microbial properties

Materials and Methods

Retrieval and accession of protein sequences

Different types of casein protein sequences of Bactrian Camel, Yak and Donkey were retrieved from NCBI database (<https://www.ncbi.nlm.nih.gov/protein/>) to further process them for chemical analysis.

Multiple Sequence Alignment

Sequences were exported to MEGA v. 11 (Molecular Evolutionary Genetic analysis) software for alignment of protein sequences. Alignment was performed through Multiple sequence Alignment (MUSCLE) algorithm (Edgar, 2004) [3].

Phylogenetic analysis across species

Multiple sequence alignment file was exported in MEGA format and further utilized for phylogenetic analysis. Phylogenetic analysis was performed by MEGA software using Neighbour joining method. This method is commonly used for construction of phylogenetic tree based on distance between different species. It works on the principle of minimizing the sum of all the branch lengths of the resultant tree.

Physicochemical properties of casein

Physicochemical properties of different type of casein proteins such as pI, number of amino acid residues, aliphatic index and GRAVY (Grand average of hydropathicity) were calculated using ProtParam tool of EXPASY interface (<https://web.expasy.org/protparam/>).

Anti-microbial properties of milk

Anti-microbial properties were predicted using publicly accessible AMPA tool (<http://tcoffee.crg.cat/apps/ampa/do>). This tool is an automated server which predicts antimicrobial

peptides within particular protein sequence (Torrent *et al.*, 2012) [11]. Amino acid sequence of specific protein in FASTA format was run in the AMPA using the default parameter values, i.e., a propensity threshold of 0.225 and a window size of 7 amino acids.

Results and Discussion

Phylogenetic analysis

Phylogenetic analysis is an important means of studying average relatedness of a particular milk or viral protein among different species of animals or viruses (Singh *et al.*, 2024) [9]. Evolutionary relatedness of different casein proteins is related to evolutionary divergence of different dairy animals over time. Unconventional dairy animals such as Bactrian camel, Donkey and Yak belongs to different families. However, Camel is a pseudo-ruminant and forms a connection between ruminant Yak and Equidae family member, Donkey. Phylogenetic tree with genetic distance is illustrated in Figure. 1.

Table 1: Different protein sequences with accession number

Casein	Animal	Sequence Id
Alpha-S1 (CSN1S1)	Camelus Bactrianus	XP_010967691.1
	Yak	AXE74293.1
	Donkey	XP_014708642.1
Alpha-S2 (CSN1S2)	Camelus Bactrianus	WMS61012.1
	Yak	AIJ02700.1
	Donkey	CAV00691.1
Beta casein	Camelus Bactrianus	CDQ50355.1
	Yak	AXE74295.1
	Donkey	XP_044622644.1
Kappa casein	Camelus Bactrianus	CCI79380.1
	Yak	AFM93768.1
	Donkey	XP_014702750.1

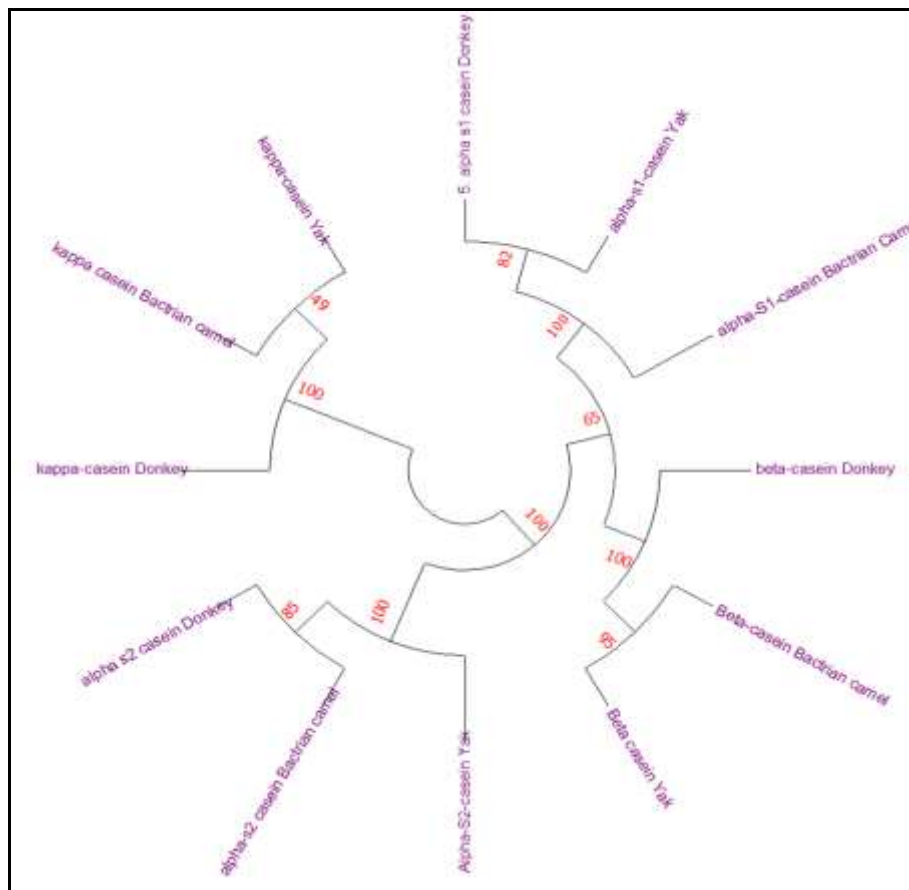


Fig 1: Phylogenetic tree of different casein micelles across different species using Neighbour-joining method

Physio-chemical properties of casein micelles

Physiochemical properties of different casein proteins include pI, aliphatic index, GRAVY (Grand average of hydropathicity) and instability index. The aliphatic index (AI) of a protein is the proportional volume that its aliphatic side chains occupy and is involved in protein thermal stability. Higher score of Aliphatic index proteins represents more thermally stability (Ikai, 1980)^[10]. GRAVY was calculated using the hydropathy values from

Kyte and Doolittle.

GRAVY values range from positive to negative values. Positive values indicate hydrophobic nature and negative value indicate hydrophilic nature of protein. Most of casein proteins were found to be unstable across different species. Physiochemical properties are tabulated in Table. 2.

Table 2: Physio-chemical features casein proteins across species.

Protein	Animal	Theoretical pI	Aliphatic index	Grand average of hydropathicity (GRAVY)	Instability index
Alpha-S1 (CSN1S1)	Bactrian Camel	5.36	99.35	-0.679	Unstable
	Yak	5.23	85.19	-0.464	Unstable
	Donkey	6.02	62.74	-0.827	Unstable
Alpha-S2 (CSN1S1)	Bactrian Camel	5.83	66.04	-0.669	Unstable
	Yak	5.23	75.05	-0.668	Unstable
	Donkey	5.87	70.59	-0.707	Unstable
Beta casein	Bactrian Camel	5.43	95.39	-0.135	Stable
	Yak	5.31	97.37	-0.154	Unstable
	Donkey	5.70	98.96	-0.221	Unstable
Kappa casein	Bactrian Camel	8.44	78.72	-0.152	Unstable
	Yak	5.28	80.98	-0.275	Unstable
	Donkey	9.21	93.63	-0.176	Unstable

Anti-microbial properties of casein components

Anti-microbial peptides were predicted through AMPA tool. Alpha-s2- casein protein of camel and Yak has 3 and 2 bactericidal peptides respectively. A study by Mohamed *et al.*, 2022^[5] has found the presence of several bioactive agents in camel milk such as lactoferrin, immunoglobulin G, peptidoglycan recognition protein-1, lysozyme and

lactoperoxidase. In addition, Yak has also one bactericidal peptide each for beta casein and kappa casein protein. Research by Cheng *et al.*, 2013^[11] have also found out anti-microbial effect of kappa casein protein in Yak. Average antimicrobial index of all proteins in different species ranges from 0.25 to 0.26. Table 3 depicts the anti-microbial properties of different casein proteins.

Table 3: Anti-microbial properties of casein micelles in different species

Protein	Species	Bactericidal stretches	Average antimicrobial index
Alpha-S1 (CSN1S1)	Camelus Bactrianus	0	0.267
	Yak	0	0.267
	Donkey	0	0.261
Alpha-S2 (CSN1S1)	Camelus Bactrianus	3	0.254
	Yak	2	0.252
	Donkey	0	0.258
Beta casein	Camelus Bactrianus	0	0.265
	Yak	1	0.263
	Donkey	0	0.264
Kappa casein	Camelus Bactrianus	0	0.253
	Yak	1	0.256
	Donkey	0	0.25

Conclusion

This bioinformatics study highlights importance of comparative genomics for better understanding of phylogenetic relatedness, chemical and anti-microbial properties of casein proteins. The unconventional dairy animals i.e. Bactrian Camel, Yak and Donkey were analysed in the above study. The milk from these species is well known for its anti-microbial properties and medicinal benefits. Overall, results enriched and confirmed knowledge database of chemical and anti-microbial properties of milk from these animals.

Conflict of interest

Authors declare that there is no conflict of interest.

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