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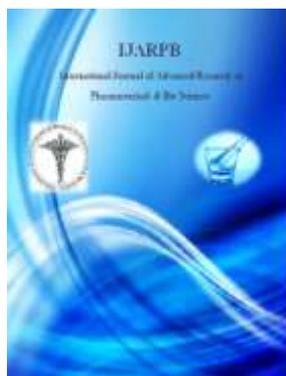
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Casein Protein: Phylogeny study of important domestic animals using computational method

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ABSTRACT

Casein is mammalian milk protein belong to phosphoproteins family and involved in a number of important physiological processes. We are analyzed four Subgroups of casein protein (Alpha S-1, Alpha S-2, Beta and Kappa) in four species (Cow, Camel, Domestic Sheep and Goat) reported in that family. In this study, a full length Amino acid Sequence of above four species in casein subgroup are find from public database. The candidate sequences are further analyzed by multiple sequence alignment, for phylogeny study using various tools such as Clustal X, NJ-Plot. The trees were calculated using the rooted Neighbor-Joining (NJ) method on distance matrix employing NEIGHBOR. The Default p-distance method was used for distance analysis. The results revealed that very similar relationship between AA sequences responsible for their common ancestor. Find large conserved region which is play an important role in phylogeny relationship.

KEYWORD: Casein protein, multiple sequence alignment, Neighbor Joining method, conserved region, phylogeny relationship

(Research Article)**INTRODUCTION**

The COW (*Bos Taurus*), Camel (*Camelus dromedaries*), Domestic Sheep (*Ovis aries*) and domestic Goat (*Capra hircus*);^[24]. Theirs milk plays very important role for medicinal, economical, religious and food. Milk proteins are currently of great scientific interest due to their beneficial health properties. Camel's milk consist insulin that is very useful in treatment diabetes (A-type)^[14]. Current research finds that milk protein support of muscle protein synthesis and muscle protein accretion in human being ^[17]. Phylogeny study of casein milk protein on the basis of protein sequences, it's more reliable than any other criterion. Adaptation of such a selection depends on the identification of candidate protein by determining the close relation between their animals milk.

Casein is milk protein secreted by mammary gland cells found in chromosome 6, arranged in tandem in the following order: α -s1, β , α -s2 and κ ^[15]. They constitute about 78-82% of milk protein. The common compositional factor is that caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues. Family of phosphoproteins is subdivided into four main groups: α S1-casein (molecular weight 23,000; 199 residues, 17 proline residues), α S2-casein (molecular weight 25,000; 207 residues, 10 prolines), β -casein (molecular weight 24,000; 209 residues, 35 prolines), and κ -casein (molecular weight 19,000; 169 residues, 20 prolines). The casein protein is divided into two groups, the calcium-sensitive and the non-calcium-sensitive. The κ -

casein is insensitive to calcium and α (S1)- and α (S2)-caseins and β -casein are calcium-sensitive ^{[18],[23]}, & (Dairy chemistry and physics; University of Guelph web page). Casein colloidal particle known as the casein micelle, biological function is to carry large amounts of highly insoluble calcium and phosphate to mammalian young in liquid form and to form a clot in the stomach for more efficient nutrition. Besides casein protein, calcium and phosphate, the micelle also contains citrate, minor ions, lipase and plasmin enzymes, and entrapped milk serum (Dairy chemistry and physics; University of Guelph web page).

MATERIAL AND METHOD**Sequences**

An exhaustive search was made in GenBank and EMBL databases ^[25] for κ casein milk protein Family. Protein sequences search was obtained and then filtered by removing partial and redundant sequences from the population and cross check by protein knowledge base UniportKb Data base. Complete protein representations by strain were included; our final working population consisted that each four sequences form sub family of casein protein for Alpha-S1 (P02662; ^[1], O97943; ^[4], P18626; ^[2], P04653; ^[3]) Alpha-S2 (P02663; ^[5], O97944; ^[4], P33049; ^[6], P04654; ^[7]) Beta Casein (P02666; ^[8], Q9TVDO; ^[4], P33048; ^[10], P11839; ^[9]) and Kappa casein (P02668; ^[11], P79139; ^[8], P02670; ^[12], P02669; ^[13]) as complete protein sequences.

(Research Article)**Table 1:** Employing Candidate Amino Acid Sequence for analyzing casein protein of their sub groups.

Organism, Accession no./ Sub groups	Bos taurus	Camelus dromedarius	Capra hircus	Ovis aries
Alpha-S1	P02662	O97943	P18626	P04653
Alpha-S2	P02663	O97944	P33049	P04654
Beta Casein	P02666	Q9TVDO	P33048	P11839
Kappa Casein	P02668	P79139	P02670	P02669

Multiple Sequence Alignment

General help for CLUSTAL X (2.0) software [22],[23] in (local System) was used for protein multiple Sequence Alignments (MSA) using the previously collected sequences with standard parameters. Input file for MSA are used in "fasta" format for casein family members obtain from Uniportkb database and MSA were

starting points for pair wise sequence identity was calculated in parameter (Gap opening; 10, Gap Extension; 0.2, Delay divergent sequence; 30%, Protein weight matrix; Pam 62) for all the combinations in respective family. Aligned output file save in local hard drive in Clustal aligned (.aln) format. Table (1) shows the score of MSA in candidate family members.

Table 2: After MSA Score of Alpha S-1, Alpha S-2, Beta and Kappa Casein Protein

Family Member	No of Member	Length	Pair wise Identity (%)	Identical Sits (%)
Alpha-S1	4	344	67.0 %	203;59.4%
Alpha-S2	4	344	74.0%	234;68.6%
Beta Casein	4	302	81.2%	206;70.8%
Kappa Casein	4	259	74.3%	167;65.2%

Phylogeny tree construction

The bootstrapping of the 'alignment dataset' was restricted to 1000 times owing to the large number of sequences in the alignment. Protein distances were calculated employing Clustal

x2.0 package [18], [19]. The trees were calculated using the rooted Neighbor-Joining (NJ) method [21] on distance matrices employing NEIGHBOR from the Clustalx package. The Default p-distance method was used for distance

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analysis. These trees were analyzed employing of Clustalx package to derive a consensus tree. A rooted tree was plotted using NJ Plot software package [21]. Sequences with more than 50% bootstrap support values were confirmed and grouped.

Result and Discussion

Multiple Sequence alignment

The MSA of protein sequences obtained from Clustalx [19],[20] , including the Family of candidate protein sequences, the table (1) show the maximum pair wise similarity in Beta Casein groups (81.2 %) and minimum alpha S-1 (67.0 %). In other hand identity sits maximum in Beta Casein (206, 70.8 %) or minimum Alpha S-1 casein (203, 59.4%).

Alpha S-1

	1	10	20	30	40	50	60
sp P02662 CASA1_BOVIN	MKLLILTCLVAVALARPKHP			IKHQGLPQ	-----	EVLNE-NLLRFFV	APFPEVFGKEK
sp P18626 CASA1_CAPHI	MKLLILTCLVAVALARPKHP			INHRGLSP	-----	EVPNE-NLLRFV	VAPFPEVFRKEN
sp P04653 CASA1_SHEEP	MKLLILTCLVAVALARPKHP			IKHQGLSS	-----	EVLNE-NLLRFV	VAPFPEVFRKEN
sp O97943 CASA1_CAMDR	MKLLILTCLVAVALARPKYPLRY			PEVFQNE	PDSIE	EVLNKRKILEL	AVVSPI-QFRQEN
sp P02662 CASA1_BOVIN	VNELSKD		-----	IGSESTEDQAMEDIK	-----	QMEAESISSSEE	
sp P18626 CASA1_CAPHI	INELSKD		-----	IGSESTEDQAMEDAK	-----	QMKAGSSSSSEE	
sp P04653 CASA1_SHEEP	INELSKD		-----	IGSESIEDQAMEDAK	-----	QMKAGSSSSSEE	
sp O97943 CASA1_CAMDR	IDELKD		-----	TRNEPTEDHIMEDTE	-----	RKESGS-SSSEE	
sp P02662 CASA1_BOVIN	IVPNSVEQKHIQK-EDVPSERYLGYLEQLLRLKKYKVPQLEIVPMS						
sp P18626 CASA1_CAPHI	IVPNSAEQKYIQK-EDVPSERYLGYLEQLLRLKKYKVPQLEIVPKS						
sp P04653 CASA1_SHEEP	IVPNSAEQKYIQK-EDVPSERYLGYLEQLLRLKKYKVPQLEIVPKS						
sp O97943 CASA1_CAMDR	VVSSTTEQKDILK-EDMPSQR---YLEELHRLNKYKLLQLEAIRDQKL						
sp P02662 CASA1_BOVIN	-----						
sp P18626 CASA1_CAPHI	-----						
sp P04653 CASA1_SHEEP	-----						
sp O97943 CASA1_CAMDR	-----						
sp P02662 CASA1_BOVIN	-----AEEERLHSMKEGIHAQQKEPMIGVMQELAYFYPELFRQFYQLDAY						
sp P18626 CASA1_CAPHI	-----AEEQLHSMKEGNPAHQKQPMIAVNMQELAYFYPLFRQFYQLDAY						
sp P04653 CASA1_SHEEP	-----AEEQLHSMKEGNPAHQKQPMIAVNMQELAYFYPLFRQFYQLDAY						
sp O97943 CASA1_CAMDR	-----IPRVKLSSEHYLEQLYRINEDMHPQLGEPVKVVTQEQAYFHLEPFPQFFQLGAS						
sp P02662 CASA1_BOVIN	PSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEKT-TMPLW---						
sp P18626 CASA1_CAPHI	PSGAWYYLPLGTQYTDAPSFSDIPNPIGSENSGKT-TMPLW---						
sp P04653 CASA1_SHEEP	PSGAWYYLPLGTQYTDAPSFSDIPNPIGSENSGKI-TMPLW---						
sp O97943 CASA1_CAMDR	PYVAWYYPPQVMQYIAHPSSYDTPEGIASEDGGKTDVMPQW---						

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Alpha S 2

	1	10	20	30	40	50	60
sp P33049 CASA2_CAPHI	MKFFIFTCLL	LAVALAKH	MEHVSSSEEP	-INIFQEIYKQ	EKNMAIHP	PRKEKLC	TTSCEEV
sp P04654 CASA2_SHEEP	MKFFIFTCLL	LAVALAKH	MEHVSSSEEP	-INISQEIYKQ	EKNMAIHP	PRKEKLC	TTSCEEV
sp P02663 CASA2_BOVIN	MKFFIFTCLL	LAVALAKN	IMEHVSSSEES	-I-ISQET	YKQEK	NMAINP	SKENL
sp O97944 CASA2_CAMDR	MKFFIFTCLL	AVLAKHE	MDQSSSEES	-INVSQ	QKFKQ	VKVAIHP	SKEDIC
sp P33049 CASA2_CAPHI	VRNANEEYSIR	-----	SSSEESA	EA	VAPEE	EIKITV	DDKH
sp P04654 CASA2_SHEEP	VRNADEEYSIR	-----	SSSEESA	EA	VAPEE	EIKITV	DDKH
sp P02663 CASA2_BOVIN	VRNANEEYSIG	-----	SSSEESA	EA	VATEE	EIKITV	DDKH
sp O97944 CASA2_CAMDR	VRNIKEV	-----	-----	ESAEV	P	TEN	-----
sp P33049 CASA2_CAPHI	KFPQYLQY	YQGP	I	VLN	P	WD	Q
sp P04654 CASA2_SHEEP	KFPQYLQY	YQGP	I	VLN	P	WD	Q
sp P02663 CASA2_BOVIN	KFPQYLQY	YQGP	I	VLN	P	WD	Q
sp O97944 CASA2_CAMDR	KFLQYLQAL	HGGQ	I	V	M	N	P
sp P33049 CASA2_CAPHI	-----	-----	-----	NSKKT	ID	ME	ST
sp P04654 CASA2_SHEEP	-----	-----	-----	NSKKT	ID	ME	ST
sp P02663 CASA2_BOVIN	-----	-----	-----	NSKKT	VD	ME	ST
sp O97944 CASA2_CAMDR	-----	-----	-----	-----	ST	VE	TE
sp P33049 CASA2_CAPHI	YYQKFAW	PQYLK	T	V	D	O	H
sp P04654 CASA2_SHEEP	YYQKFAW	PQYLK	T	V	D	O	H
sp P02663 CASA2_BOVIN	YYQKFAW	PQYLK	T	V	D	O	H
sp O97944 CASA2_CAMDR	YYQTF	FLW	P	E	Y	L	K

Beta Casein

	1	10	20	30	40	50
sp P02666 CASE_BOVIN/1-224	MKVLILACL	VALALARE	LEELNV	PC	E	IVESLSS
sp P33048 CASE_CAPHI/1-222	MKVLILACL	VALALARE	QEELNV	V	G	ETVESLSS
sp P11839 CASE_SHEEP/1-222	MKVLILACL	VALALARE	QEELNV	V	G	ETVESLSS
sp Q9TVDO CASE_CAMDR/1-232	MKVLILACR	VALALARE	KKEFKT	A	C	EAL
sp P02666 CASE_BOVIN/1-224	KFQSEEQ	--Q	TEDE	LQDK	IHP	FAQ
sp P33048 CASE_CAPHI/1-222	KFQSEEQ	--Q	TEDE	LQDK	IHP	FAQ
sp P11839 CASE_SHEEP/1-222	KFQSEEQ	--Q	TEDE	LQDK	IHP	FAQ
sp Q9TVDO CASE_CAMDR/1-232	KFKIEREQ	--Q	TEDE	EQDK	IYTF	PQP
sp P02666 CASE_BOVIN/1-224	-----	FLQPE	V	M	G	V
sp P33048 CASE_CAPHI/1-222	-----	FLQPE	I	M	G	V
sp P11839 CASE_SHEEP/1-222	-----	FLQPE	I	M	G	V
sp Q9TVDO CASE_CAMDR/1-232	-----	FLQPK	V	M	D	V
sp P02666 CASE_BOVIN/1-224	+QSLT	LD	V	E	N	L
sp P33048 CASE_CAPHI/1-222	+QSLT	LD	V	E	K	L
sp P11839 CASE_SHEEP/1-222	+QSLT	LD	V	E	K	L
sp Q9TVDO CASE_CAMDR/1-232	+QSLT	LD	L	E	N	L
sp P02666 CASE_BOVIN/1-224	SKVLP	V	P	Q	K	--
sp P33048 CASE_CAPHI/1-222	PKVLP	V	P	Q	K	--
sp P11839 CASE_SHEEP/1-222	PKVLP	V	P	Q	K	--
sp Q9TVDO CASE_CAMDR/1-232	FKVLP	V	P	Q	K	--
sp P02666 CASE_BOVIN/1-224	--	--	--	--	--	--
sp P33048 CASE_CAPHI/1-222	--	--	--	--	--	--
sp P11839 CASE_SHEEP/1-222	--	--	--	--	--	--
sp Q9TVDO CASE_CAMDR/1-232	--	--	--	--	--	--

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Kappa Casein



Figure -: 1-4 show MSA of Alpha S-1, Alpha S-2, Beta and Kappa Casein Protein groups in Clustal x 2.00 software package and the highlighted colour rectangle shaped indicated conserved region of candidate sequence. Alignment was done employing Amino Acid sequence.

There are maximum 5 clusters mines from conserved region in AA sequences.

Table 3: Conserved region of aligned sequences show in different colour rectangle shaped

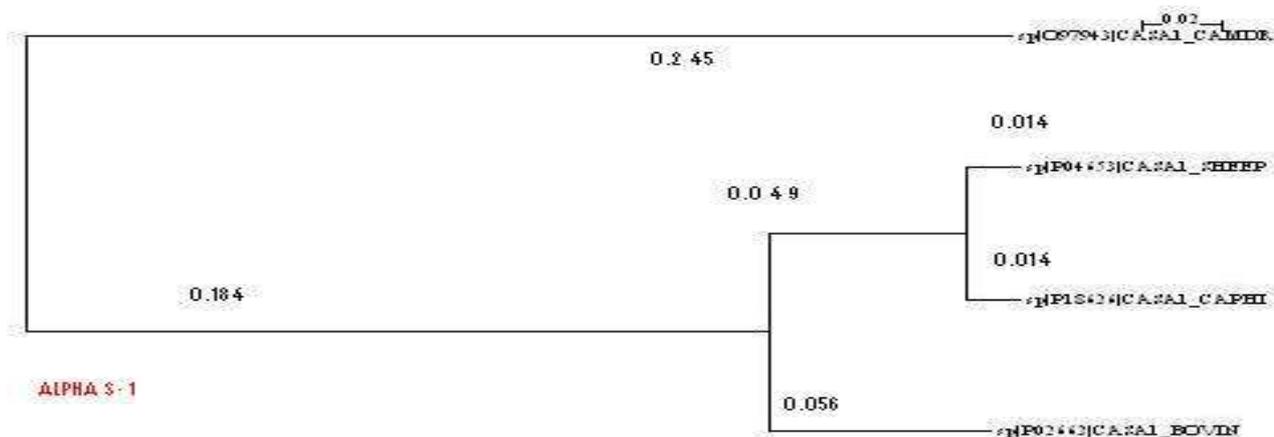
Conserved Region no/ color	Alpha S-1	Alpha S-2	Beta	Kappa
1 (Red)	MKLLILTCL VAVALARP KHPIKHQG LPQ	MKFFIFTCLLAVALAKHKMEH VSSEEP- INIFQEIYKQEKNNMAIHPRKE KLCTTSCEEVVRNANEEEEYS IR	MKVLILACLVALAL ARELEELNVPGEI VESLSS	MKSFFLVVTILAL TLPFLGAEVQMQ EQPTCFEKVERL LNEKTVKYFPIQF VQSRYPSTY INYYQHLAVP
2 (Green)	EVLNE- NLLRFFVA	SSSEESA EVAPEEIKITVDDK HYQKALNEINQFYQ	SEESITRINK-KIE KFQSEEQ--	INNQFLPYPYYAK PVAVRSPAQTLQ

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	PFPEVFGK EKVNELSK D		QQTEDELQDKIHP FAQTQSLVYFPF-- GPIP- SLPQNIPPLTQTP VVVPP	WQVLPNAVPAKS CQDQPTAMARH PRRPSFIAIPPK KTQDKTVNPAIN TVATVEPP
3 (Blue)	IGSESTED QAMEDIK	KFPQYLQYPYQGPIVLNPWD QVKRNAGPFTPTVN-- REQLSTSEE	FLQPEVMGVSKV KEAMAPKHKEMP FPKYP-VEPFTES	PTAEPAVN
4 (Purple)	QMEAESIS SSEEIVPNS VEQKHIQK- EDVPSERY LGYLEQLL RLKKYKVP QLEIVPNS	NSKKTIDMESTEVFTKTKLT EEEKNRNLNFKKISQ YYQKFAWPQYLKTVDQHQQ AMKPWTQPKTN-- AIPYVRYL	QSLTLTDVENLHL PLPLLQSWMHQP HQPLPPT	TVVIAEASSEFI
5 (Black)	AEERLHSM KEGIHAQQ KEPMIGVN QELAYFYP ELFRQFYQ LDAYPSGA WYYVPLGT QYTDAPSF SDIPNPIGS ENSEKT- TMPLW	Nil	VMFPPQSVLSLSQ SKVLPVPQK-- AVPYPQRDMPIQA FLLYQEPVLGPVR GPFPIIV	TSTPETTTVQITS TEI

Table no.3 show major conserved region in aligned sequence that's defined molecular functioning, genetically, structural and evolutionary relationship similarity in candidate protein sub family and crosscheck in online tool of expasy server (<http://www.expasy.org/cgi-bin/peptidecutter>) were enzyme and chemical compound viz. (Chymotrypsin-high specificity(c term to), Asp -N endopeptidase + N-terminal Glu, pepsin (pH 1.3; pH>2) protenase K and thermolysin) are maximum cleavage in respective sequences.

Phylogeny analysis



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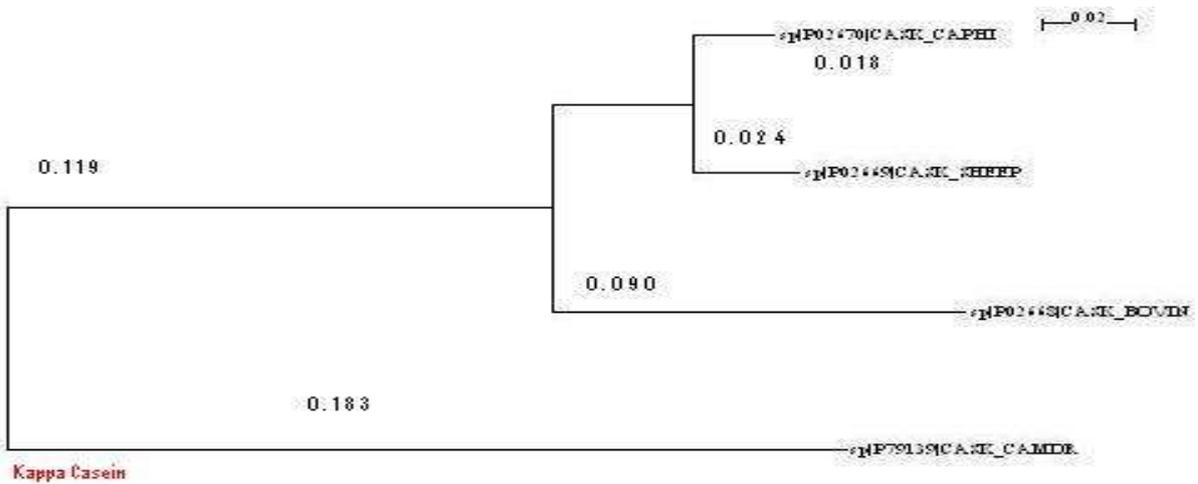
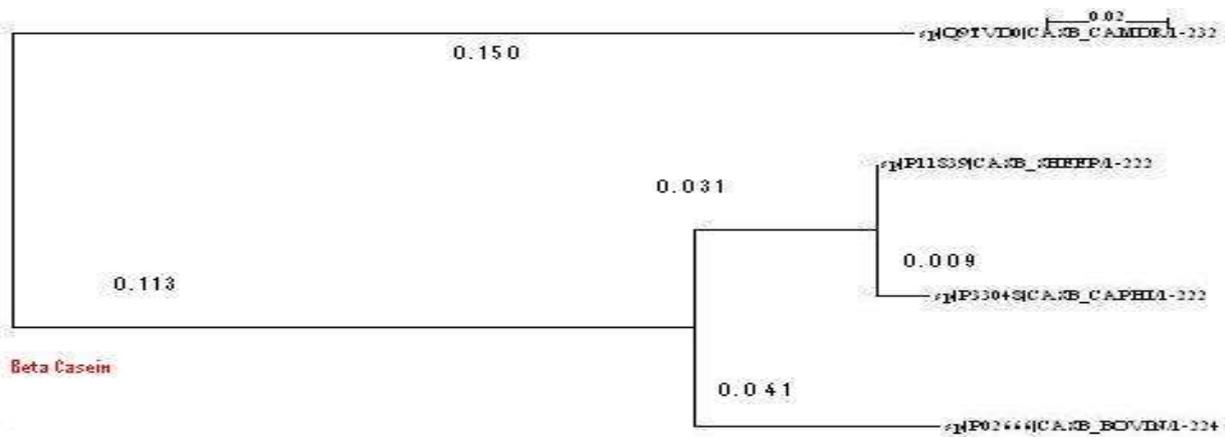
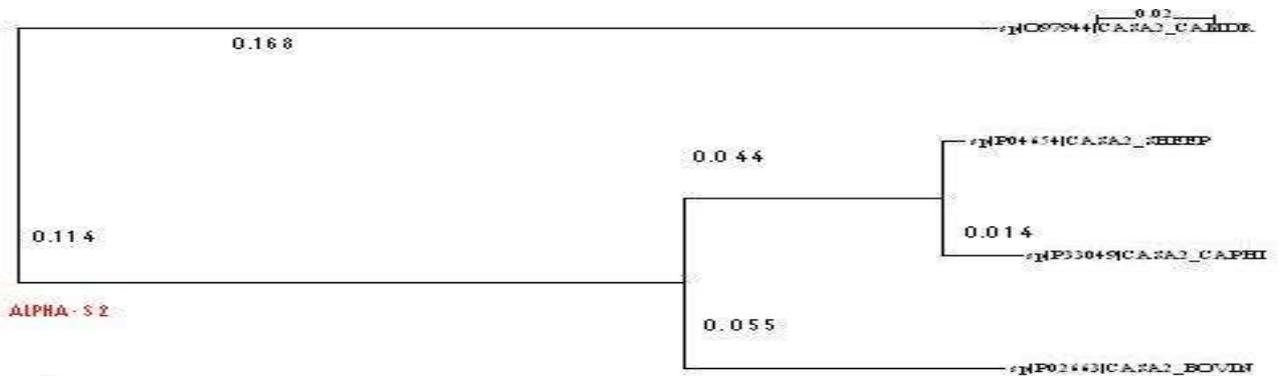


Figure: - (4 to 8) Phylogenetic tree based on casein subfamily (with name caption) calculated by NJ algorithm in Clustalx and Tree generated in NJ plot software package marked define the evaluator bootstrap value

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We generated a cladogram protein directed tree [22] of casein subgroup family sequences, and considered tree branches to be confirmed if they were found in 1,000 bootstrap steps. This approach finally results in four casein subgroups, each and every group find four leaf nodes. The observed data assuming that it has evolved in accordance with a chosen very close evolutionary model. Phylogenies are then inferred by finding tree and the parameters that yield the highest like hood. The mismatch of protein sequence seems to be diverged two sub nodes in all trees.

Most interesting result of our analysis is that Casein each subgroup divided in two sub group which one consist only one species (Camelus dromedaries)

another are again divide in two group where contain one (Bos Taurus) and another (Ovis aries and Capra hircus) show common ancestor according to the AA sequence. The position of Camel (Camelus dromedaries) in all sub family (Accession no. O97943, O97944, Q9TVDD and P79139) are very similar tree topology but rest of all species topologies mostly same in all trees. Ovis aries and Capra hircus AA sequences are unique Common ancestor in all trees topologies (P18626-P04653; P33049-P04654; P33048-P11839; P02670-P02669) and their distances are quite similar.

Table 4-7: calculate distance matrix of four species in Alpha S-1, Alpha S-2, Beta and Kappa Casein in respective tree topology.

Alpha S-1	Camelus dromedaries	Bos Taurus	Ovis aries	Capra hircus
Camelus dromedaries	0	0.485	0.492	0.492
Bos Taurus	0.485	0	0.119	0.119
Ovis aries	0.492	0.119	0	0.014
Capra hircus	0.492	0.119	0.014	0

Alpha S-2	Camelus dromedaries	Bos Taurus	Ovis aries	Capra hircus
Camelus dromedaries	0	0.337	0.326	0.34
Bos Taurus	0.337	0	0.099	0.113
Ovis aries	0.326	0.099	0	0.014
Capra hircus	0.326	0.113	0.014	0

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Beta	Camelus dromedaries	Bos Taurus	Ovis aries	Capra hircus
Camelus dromedaries	0	0.304	0.294	0.303
Bos Taurus	0.304	0	0.072	0.081
Ovis aries	0.294	0.072	0	0.009
Capra hircus	0.303	0.081	0.009	0

Kappa	Camelus dromedaries	Bos Taurus	Ovis aries	Capra hircus
Camelus dromedaries	0	0.392	0.326	0.320
Bos Taurus	0.392	0	0.114	0.108
Ovis aries	0.326	0.114	0	0.042
Capra hircus	0.320	0.108	0.042	0

In (Table: - 4-7) calculate distance matrix [16] for each casein protein groups in between four AA sequences of respective animals. In alpha S-1 casein groups maximum distance both Ovis aries and Capra hircus contain score 0.492 (P04653-P18626) between Camelus dromedaries AA sequences (O97943) and minimum 0.014 between Ovis aries and Capra hircus. In Alpha -S2 maximum distance find between (O97944) Camelus dromedaries and (P02663) Bos Taurus (0.337) and minimum between Capra hircus (P33049) and Bos Taurus (0.113). In Beta Casein, maximum distance find between (Q9TVD0) Camelus dromedaries - (P02666) Bos Taurus (0.304) and minimum between (P11839) Ovis aries - (P33048) Capra hircus (0.009). In Kappa Casein maximum distance find between (P79139) Camelus dromedaries and (P02668) Bos Taurus (0.392) and minimum between minimum 0.042 between Ovis aries (P02669) and Capra hircus (P02670) according to tree topologies. Most focusing point that is Camelus dromedaries and Bos Taurus Casein (Alpha- S2, Beta and Kappa Casein) are diverting in their AA sequence and position of that sequence might be the early origin to their

common ancestor. It is remarkable that early research find that three related calcium sensitive casein gene (Alpha S-1, Alpha S-2, and Beta) arose from the common ancestors through intra- and intergenic duplication and exon shuffling.

CONCLUSION

The present study is a step forward towards a better understanding of the distribution of casein Family in the Milk protein genera. The study has identified the close relationship in their subgroups in four species on bases of phylogeny analysis. This Protein Sequence studies is used for the sustainable research for carrying out genetic and phylogenetic studies.

Hopefully, this Study has revealed the importance and utility of bioinformatics resources that are valuable for current Proteomics research. The resources and tools reviewed in this paper are used mostly for Sequence analyses and Phylogeny analyses, which are essential for finding evolutionary relationship and genetics similarities.

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