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Sequencing and bioinformatics analysis of the partial promoter region of κ-casein (CSN3) gene in Iranian Bacterianus and Dromedaries camels

Ali Akbar Khabiri*, Mojtaba Tahmoorespur, Mohammad Reza Nassiri, Mohammad Hadi Sekhavati

Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran.

ABSTRACT

κ-casein is a glycosilated protein belonging to a family of phosphoproteins (αs1,β, αs2,κ) that represents the major protein component in mammalian milk. κ-casein plays an essential role in the case of micelle stabilization, determining the size and the specific biological function. In the present study, we report the characterization of the partial sequence of κ-casein promoter region in the Iranian *Bacterianus* and *Dromedaries* camels. κ-casein partial promoter fragment (1212 bp) was successfully amplified, and sequenced. The sequence analysis results showed that there was a high homology (>95%) in the region of sequenced camel partial promoter fragment and related sequences in the vast range of species. Furthermore, four and three haplotypes observed in *Bacterianus* and *Dromedaries* camels, respectively.

Key words: κ-casein, Bioinformatics analysis, Camels

INTRODUCTION

The camel stock is at present estimated to be about 23 million in the world. Iran holds almost 0.6% of the whole camel population (100 *bactrian*; 150000 *Dromedary*) (Ansari-Renani, Salehi et al., 2010). In the last forty years (1970 –2013), the number of camels has increased of almost 45% (www.faostat.fao.org). Camels were raised mainly for the milk and meat production in Iran. The daily camel milk production is in the range of 3-10 Lit during a lactation period (12–18 months) (Farah et al., 2007). Camel milk protein and fat content was reported 2.9% and 3.1%, respectively (Al-haj and AlKanhal., 2011). The performance of technological processes of cheese production depends on the structure of κ-casein protein which encoded by CSN3 gene (Zaton et al., 1999). Casein gene is organized as a tightly linked cluster on chromosome 6 in cattle, sheep and goat (Rijnkels et al., 2002). κ-casein plays an important role in the formation, stabilization and aggregation of the casein micelles; thus, altering the manufacturing properties and digestibility of milk (Jann et al., 2004). The κ-casein polymorphism is well described in the cattle (Prinzenberg et al., 2008), goat (Jann et al., 2004; Prinzenberg et al., 2005) and sheep (Ceriotti et al., 2004; Coral et al., 2010). Goats and cows represent the most allelic polymorphism in CSN3 gene among the farm animals (Caroli et al., 2006; Ramunno et al., 2004). Yahyaoui et al (2003) showed that allelic polymorphism in the C-terminal region of κ-casein gene affected on casein solubility and over a broad range of calcium ion concentrations and contains a hydrophilic. However, the κ-casein coding region sequence analysis is not always consistent in the association studies (J. M. L. Heck, A. Schennink 2009) and this might be attributed to the presence of intragenic haplotypic combination of variants in the regulatory and noncoding regions (Prinzenberg et al., 2002). Regulatory regions allelic polymorphism

Corresponding Author E-mail: aa.khabiri@gmail.com

may change gene expression or alter the amino acid profile which affects the functional properties of the protein. In this case, Prinzenberg et al (2002) indicated that casein gene expression regulated by hormones and most of the potential hormone receptor binding sites which occur within the 5′-flanking region of casein genes. Moreover, the results showed that mutations at the κ-casein 5′-flanking region might also have enduring effect on milk protein gene regulation at transcriptional level (Prinzenberg et al., 2002; Szymanowska et al., 2004). Based on our knowledge, there is no report on allelic polymorphism analysis on κ-casein 5′-flanking region in Iranian camels. The objective of this study was to sequencing and bioinformatics analysis of the partial promoter region of κ-casein (CSN3) gene in Iranian *Bacterianus* and *Dromedaries* camels.

MATERIALS AND MTHODS

Blood samples and DNA extraction

The blood samples were collected randomly from 10 *Dromedaries* and 5 *Bacterianus* camels were raised in north and northwest of Iran. DNA was extracted from 100 µl of blood, using a commercial kit (Diatom DNA Prep100, ISO Gene, Moscow, Russia) following the manufacturer's protocol. The quality of the extracted DNA was analyzed by electrophoresis on 0.8% agarose gel and the purity of the obtained DNA was verified by Nano Drop ND-2000 spectrophotometer (Thermo, USA).

PCR amplification

DNA regions (-1162 to +50of CSN3 gene cluster) were amplified using the Personal Cycler™ thermo cycler (Biometra, Germany). The specific primers were designed using Primer Premier 5, according to the available nucleotide sequences on the NCBI GenBank (EMBL ID HE863813) database. The specific primers were as follows:

CSN3- F: 5′- TTGAAACTCTGCCATCTTTCTC-3′

CSN3- R: 5′- TGTGCCTGTCAGGTCTTGC-3′

The PCR was performed in a total volume of 25 µl with the reaction mixture containing 2.5 µl of 10X PCR buffer, 2 µl $MgCl_2$ (50mM) and 2 µl dNTPs (Mix), 1.5 µl of the DNA solution (50 to 100 ng/µl), 1 µl of mix primer (5 pmol/µl), 0.125 U/µl of *EX Taq* DNA polymerase (Takara, Japan), and deionized water up to 25 µl final volume. PCR program was performed with an initial denaturation step at 94°C for 10 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 45 sec, and a final extension at 72°C for 10 min. The PCR products were assessed by 0.8 % agarose gel electrophoresis and were sequenced by specific designed primers (Macro Gen, South Korea).

Bioinformatics analysis

SNP discovery and multiple alignments were accomplished using Basic Local Alignment Search Tool (BLAST) and CLC Main workbench version 5.5. Evolutionary divergence for CSN3 promoter sequences among different species was conducted by the maximum composite likelihood method (MEGA version 5.0). Phylogenetic tree was constructed using the Neighbor-Joining method by using the same software.

RESULTS AND DISCUSSION

The partial region of Iranian *C. Bactrianus* and *C. Dromedaries* CSN3 promoter (1112 bp) plus the first 100 bp from exon 1 was successfully amplified. The accuracy of these fragments was visualized on 0.8% agarose gel electrophoresis (Figure1).

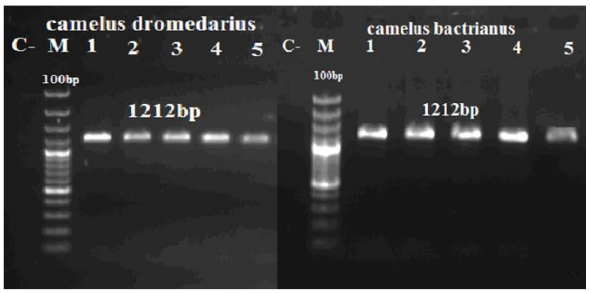

Figure1: The amplified PCR products on agarose 0.8%

Obtained sequences have been submitted on NCBI gene database with KJ755350 and KJ755349 accession numbers for *Dromedarius* and *Bactrianus*, respectively. Based on the results of sequencing quality, the 1050 nucleotides were considered for bioinformatics analyses. This region was compared with other promoter regions of CSN3 sequences in different species for drowning Phylogenetic tree. The amplified sequences were found to have 100% homology with the previously reported sequences in camel (HE863813 and HE863814) and had the lowest similarity with *H. Sapiens* (Table 1). These findings were in parallel to Pauciullo et al. (2013) results.

Table 1. Genetics distance between sequences of the Iranian *Bacterianus* and *Dromedaries* camels.

	شماره دسترسی	۱	۲	۳	۴	۵	۶	۷	۸	۹	۱۰	۱۱	۱۲	۱۳	۱۴
C. dromedarius*	KJ755350														
C. bactrianus*	KJ755349	0.000													
C. dromedarius	HE863813	0.000	0.000												
C. bactrianus	HE863814	0.000	0.000	0.000											
B. taurus	AY380228	0.168	0.168	0.168	0.168										
B. bubalis	AM900443	0.171	0.171	0.171	0.171	0.010									
C. hircus	Z33882	0.188	0.188	0.188	0.188	0.041	0.037								
O. aries	L31372	0.197	0.197	0.197	0.197	0.051	0.048	0.017							
E. zebra	EU429802	0.223	0.223	0.223	0.223	0.224	0.248	0.256	0.267						
E. asinus	EU429803	0.229	0.229	0.229	0.229	0.239	0.243	0.251	0.267	0.003					
E. caballus	AY579426	0.234	0.234	0.234	0.234	0.244	0.248	0.256	0.267	0.007	0.003				
O. cuniculus	AJ309572	0.381	0.381	0.381	0.381	0.284	0.385	0.422	0.427	0.388	0.381	0.388			
M. musculus	AJ309571	0.480	0.480	0.480	0.480	0.499	0.497	0.510	0.504	0.565	0.556	0.565	0.596		
H. sapiens	U429802	0.940	0.940	0.940	0.940	1.013	1.005	1.009	1.013	0.909	0.915	0.921	0.870	0.955	

* indicated the sequences which obtained in the present study.

Phylogenetic tree was drawn for 14 species. Phylogenetic data confirmed that camels belong to their own branch (Fig. 2). κ-casein promoter sequences showed more homology to other previously reported sequences in camels (HE863813 and He863814) and also to *B. taurus* (AY380228) (average homology > 97%). *H. Sapiens* showed the lowest similarity (average homology > 0.06%).

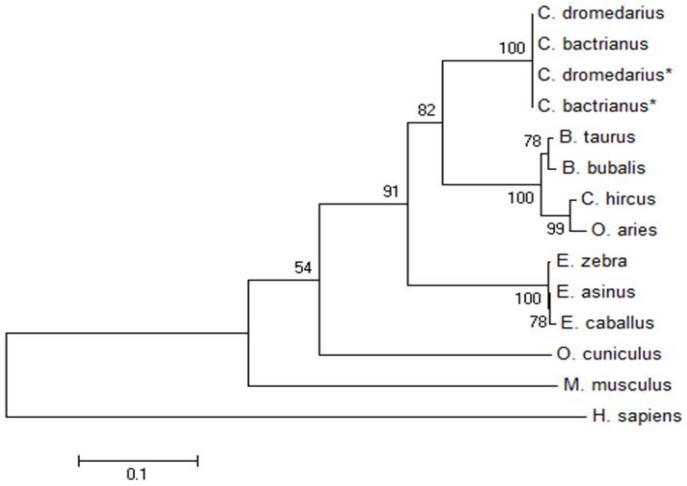

Figure 2: Phylogenic tree for the 1050 bp of promoter region of CSN3 in Iranian *Bactrianus* and *Dromedaries* camels.

Table 2 showed the ratio of T/A content to G/C in CSN3 gene. The promoter region of camel`s CSN3 gene is also characterized by high T/A content compared to G/C (69.35% vs 30.65%). However, this feature seems to be conserved among the species (Ward et al. 1997; Pauciullo et al. 2013).

Table 2: The ratio of T/A content to G/C in different species

گونه	شماره دسترسی	T/A	G/C
C. dromedarius	HE863813	68.66	31.34
B. taurus	AY380228	66.25	33.75
B. bubalis	AM900443	66.49	33.51
C. hircus	Z33882	65.17	34.83
O. aries	L31372	68.48	31.52
E. zebra	EU429802	65.75	34.18
E. asinus	EU429803	63.4	36.6
E. caballus	AY579426	63.6	36.4
O. cuniculus	AJ309572	66.53	33.47
M. musculus	AJ309571	62.13	37.87
H. sapiens	U429802	67.71	32.29

The bioinformatics analysis showed that there was four and three haplotypes in *Bacterianus* and *Dromedaries* camels, respectively (Table 3). As indicated in Table3, the high variety in nucleotide sequences were detected in the region of 278-933 bp among 5 *Bacterianus* camel`s CSN3 promoter, whereas this variation was observed through overall sequenced *Dromedaries* camel`s CSN3 promoter. Totally, 12 and 6 SNP were observed in *Bacterianus* and *Dromedaries* camels, respectively (Table 3).

Table 3: Bioinformatics analysis for detection of haplotypes and mutation position in Iranian *Bacterianus* and *Dromedaries* camels.

Species	Haplotype	Sample	Mutation Position											
			278	606	630	631	650	692	698	714	835	836	888	993
Bacterianus	1,2	1	A	A	A	A	A	T	T	A	T	A	C	C
	3	2	.	.	.	C	.	.	.	T	.	.	.	.
	4	3	.	.	G	.	.	.	.	T	.	G	.	.
	5	4	C	G	.	.	C	A	G	.	G	G	T	A
			61	716	838	855	994	1043						
Dromedarios	1-8	1	T	T	G	C	A	T						
	9	2	.	.	.	.	.	G						
	10	3	C	A	A	A	C	.						

* 1,2: There was 100% similarity between two Bacterianus camels obtained sequences.

* 1-8: There was 100% similarity between eight Dromedaries camels obtained sequences.

CONCLUSION

Allelic polymorphism in regularity region, non-coding and coding sequence could alter the rate of gene expression and amino acid profile which causes the functional properties of the protein. In the current study the partial region promoter for CSN3 gene in Iranian *Bacterianus* and *Dromedaries* camels were sequenced and bioinformatics analysis was performed. This research was the first study on the CSN3 promoter region in Iranian camels.

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