Genetic variability detected at the (c-type) milk lysozyme encoding gene in donkey

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Lysozyme is known to be a natural antimicrobial agent since it catalyses the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. It inhibits the development of many pathogens bacteria, thus making the milk somewhat selective in regards to the milk bacteria content. Three major distinct types of lysozymes have been identified: chicken-type (c-type), invertebrate-type (I-type), and goose-type (g-type). In particular, there are at least 4 non-stomach lysozyme genes in ruminants (i.e., mammary gland, kidney, trachea, intestinal). Lysozymes in ruminants and equine milk are considered to be the c-type because of their similarity to chicken egg white lysozyme. The c-type lysozyme content in donkey’s milk varies during the different stages of lactation with a mean value of 1.0 mg/mL and proved to be higher than that in bovine, ovine, caprine (I-type), whereas it was very close to mare’s milk. In the equine species, the c-type lysozyme encoding gene (4 exons) maps on chromosome 5 and transcribes a mRNA of 1323 bp, coding for a protein of 440 aa. To our knowledge, no information on genetic variability has been reported so far at this locus in donkey. Consequently, in order to detect variability, total RNA was extracted from milk somatic cells of 6 unrelated Ragusara donkeys reared in Central Italy. The mRNA fragment comprised between the last 54nt of exon 1 and the first 265nt of exon 4 was amplified by RT-PCR and sequenced. Primers (For CCAAAGTCCTTACAAACTAC and Rev ACCAGCATTAGTCTATCAGG) were designed using as template the generic donkey sequence (EMBL ID: NW_014638180). The obtained sequence (463nt) is relative to the cDNA tract spanning the last 64nt of exon 1 to the 238nt of exon 4. Stop codon is located at the 65th–67thnt of exon 4. Sequences comparison showed a transition G-C at the 169thnt of exon 2 (NW_014638180:g:1784G>C:T) responsible for the aa change Asp25→Glu. The presence of the codon CCA at exon 2 of the donkey milk lysozyme encoding gene might represent the ancestral condition of the gene in equids, as it has been also found in other donkey and mule sequences. The identification of this SNP could represent the first report of polymorphism at this locus in donkey. Next step of the research will be the analysis of a large number of samples in order to establish the frequency of this mutation in donkey species and to evaluate if and how the new genetic variant may influence functional and biological properties of donkey’s milk.