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Expression kinetics of different classes of proteases in milk derived somatic cells during lactation cycle of Sahiwal cows and Murrah buffaloes

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Proteases are important class of enzymes that play physiologically significant roles and affect flavour, texture and longevity of milk and its products. The expression of these endogenous proteases varies from species to species as well as across different stages of lactation. However, the expression kinetics associated with proteases are poorly understood in Indian native cattle as well riverine buffalo-the major dairy species of India. The present study was conducted to understand the transcriptional profiling of different classes of proteases in riverine buffalo and indicine cattle across various lactation stages. To study the expression profile of protease-pathways associated genes, a total of 40 animals including 20 Sahiwal cows and 20 Murrah buffaloes, were selected from cattle yard of National Dairy Research Institute (NDRI), Karnal based on the data of their previous parities. Milk samples were collected from these animals covering different stages of lactation viz. early (5-25 days), peak (30-60), mid (90-120 days) and late (>240) lactation to delineate the expression pattern of protease-pathways associated genes in milk-derived somatic cells across various lactation stages. RNA isolation was performed from milk derived somatic cells using Trizol method and cDNA was synthesized using Invitrogen cDNA Synthesis Kit. Before expression analysis of target gene, most suitable internal control genes (ICGs) from a panel of 10 known reference genes from different functional categories were evaluated to select most stable ICGs so as to normalize qPCR expression data. The data analyzed using three softwares: GeNorm, Normfinder and Bestkeeper, identified RPL4, RPS23 and GAPDH gene to be the most appropriate ICGs. Further, qPCR was performed on all the target genes i.e Cathepsins, Plasminogens and ubiquitous-proteosomes and data was normalised using identified ICGs. The analysis revealed that expression of proteases is low during early and peak lactation stages while it increases during mid and late lactation stages indicating that expression of proteases increases as lactation stage advances. The observed trend of proteolytic activity was similar in both cattle and buffalo however, the expression levels were comparatively higher in buffalo. The findings of this study have implications in the dairy sector involved in processing of milk especially companies making infant milk formulas.

Biography

Manjula Miglani has completed her BTech in Biotechnology in 2012 from Kurukshetra University and MTech in Biotechnology in 2014 from SRM University, Chennai. She has recently started her PhD in Biotechnology from Jaypee institute of Information Technology.

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