Title: The proteopeptidomic profiling of colostrum from ladakhi Yak reveals key molecules with bioactive attributes

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Abstract:

We investigated Ladakhi yak colostrum to identify bioactive peptides. Ladakhi yaks are adapted to high altitude one of the world's highest inhabited regions situated roughly between 32 to 36 degree north latitude and 75 to 80 degree East longitude and altitude ranging from 2300 mtrs to 5000 mtrs above. Harsh climate is characterized by extreme temperature (-40^{0}C in winter and 35^{0}C in summer), low humidity (25-40%), low precipitation (80-300mm), low oxygen level, high UV level and wind speed. The Yak colostrum and milk are among the main food items of the people of this region. We compared the major proteins and peptides present in the colostrum and found that though majority of the endogenous peptides (EPs) are originating from caseins, the other major proteins of milk remain protected from proteolysis. The other low abundant proteins contribute to the pool of EPs significantly. **P**roline **R**ich **P**eptides (PRPs) are highly abundant in colostrum which falls down as the colostrum transitions to mature milk. The bioinformatics analysis revealed over 3200 EPs in colostrum using trichloroacetic acid (TCA) and a molecular weight cut off (MWCO) extraction method. A substantial proportion (exceeding 25%), EPs were above 0.5 in PeptideRanker, indicating a high-confidence of being bioactive. Our study selected a few of these EPs which were custom synthesized and evaluated for their immunomodulatory action. The study confirms that the presence of highly bioactive EPs in colostrum and milk are responsible for conferring strong immunomodulatory properties to the people subsisting on milk and milk products derived from ladakhi yak. When we compare the gel visualization of major proteins in colostrum and milk with in-sol digestion and identification of proteins in milk, we find huge difference in the number of total identified proteins. This raises concerns on the reality of exact number of milk proteins present in milk and colostrum naturally. The race for reporting the highest number of identification in any given sample, along with technical differences in extraction protocol and the differential efficiency of mass spectrometry results in highly variable reports on milk proteome. We suggest that care should be taken in identifying the real milk proteins (secretome from mammary epithelial cells) without considering the contaminant proteins (low abundant proteins) which may probably originate from lysed leukocytes present in milk.

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Biography

Sudarshan Kumar is a distinguished Senior Scientist with over 15 years of experience in Biotechnology, specializing in proteomics, molecular biology, and high-throughput analysis for advancing dairy science. Affiliated with the National Dairy Research Institute (NDRI), Karnal, India, his innovative research significantly impacts the dairy and animal health sectors. In 2022, he was awarded the prestigious **Fulbright-Nehru Academic and Professional Excellence Fellowship**, enabling him to undertake a nine-month teaching and research program at **Oregon State University, Corvallis, Oregon, USA** (March–December 2023). His work at OSU focused on **proteomics-based approaches** using Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) to explore bacterial responses to antimicrobial peptides (AMPs). His broader research encompasses proteomics methodologies to investigate lactation and associated disorders in bovines and the use of mass spectrometry to identify and quantify residual veterinary drugs and pesticides in milk. Pregnancy diagnosis in bovine, semen sexing, and lactation are the the forefront of his research area. His research strength includes- High-throughput nano-LC Mass Spectrometry, quantitative proteomics tools such as iTRAQ, TMT, SILAC, and LFQ. Cloning, protein expression, and purification using bacterial, yeast, and mammalian cell systems. Mammalian cell culture-based assays to study protein function and drug efficacy and Targeted quantification of veterinary drugs and pesticides in milk.

Key Research Contributions

1. **Novel Antimicrobial Peptides:** Research on AMPs to combat bacterial resistance (**Scientific Reports, 2021**).
2. **Bovine Pregnancy Diagnosis:** Development of a pregnancy diagnosis kit for bovines (**YouTube Presentation**).
3. **Subclinical Mastitis Biomarkers:** Identification of protein-based biomarkers for early detection (**Scientific Reports, 2020**).
4. **Buffalo Cell Line Development:** Created the first-ever buffalo mammary gland epithelial cell line, fostering "Open Access Science" for researchers worldwide (**PLOS ONE, 2011**).
5. **Urine Biomarker Discovery:** Discovered 1,550 proteins in cow urine and identified potential biomarkers for early pregnancy detection (**Clinical Proteomics**).
6. **Melanocyte Cell Line Development:** Developed a Tharparkar cow-derived cell line to study stress-related skin pathophysiology, with implications for human health (**Journal of Dermatological Science**).
7. **Recombinant Protein Production:** Produced recombinant molecules like Interferon tau (IFN-τ), Leukemia Inhibitory Factor (LIF), and others, which have diverse applications in dairy animal reproduction.