Short Communication

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Proteomics-based milk whey proteome profiling of Indian Jersey crossbreed cows followed by chromosomal mapping

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Abstract

BACKGROUND: Milk contains a massive class of minor proteins that are known for their various biological and molecular functions. Many whey proteins transfer the host defense mechanism to the human body. In this assay, electrophoresis followed by a high-resolution mass spectrometry-based proteomic approach has been applied to identify the whey proteome of Indian Jersey crossbreed bovines.

RESULTS: Two search engines, MS Amanda and Sequest HT, have shown more than 29 minor proteins. Chromosomal mapping revealed that chromosomes 5 and 9 are expressing maximum proteins in the whey proteome. The principal component analysis, outlier plots, scree plots, score plots, and loading plots were generated to further assess the results.

CONCLUSION: The majorly expressed ones are glycosylation-dependent cell adhesion molecule-1, ubiquitin, desmoglein, annexin, glycoprotein, arginase, histones, peroxiredoxin, vimentin, desmin, catenin, peripherin, and 70 kDa heat shock protein.

Keywords: whey proteome; Jersey crossbreed; mass spectrometry; proteomics; chromosomal mapping

INTRODUCTION

Jersey (*Bos taurus*) is an exotic cattle breed widely utilized in breeding programs worldwide.[1] In India, these bovines have undergone crossbreeding with several zebu breeds (e.g. Sahiwal, Gir, Ongole) for better adaptability in harsh tropical conditions. Indian Jersey crossbreed cattle are the dominant milch animal of taurine origin, having broader breeding tracts in all parts of the country. These crossbreed animals are high milk-yielding bovines for milk production with a good percentage of milk fat. Bovine milk provides a variety of nutrients and immunologically beneficial components.[2] The milk proteins have been regularly examined, and knowledge about this crucial and complicated system has gradually grown in recent years. The number of studies on the analysis of proteins in bovine milk has significantly expanded as a result of growing knowledge of the beneficial proteins found in milk. Through the transmission of host defense proteins, milk provides a means of protection for human health. Milk proteome profiles from different animal species were previously analyzed for their functional and nutritional role.

Whey proteins comprise around one-fifth of milk proteins with a high abundance of β-lactoglobulin, α-lactoalbumin, lactoferrin, serum albumin, and so on.[3] Many of these proteins show their bioactivity in anticancer, antioxidative, antimicrobial activity, and immune modulation functions. The profiling of the whey proteome of different dairy animals could give a picture of their biological and functional roles. In recent years, proteomic-based studies coupled with mass spectrometry have been widely used to probe different dairy animals' proteomes.[4] Studies on the milk whey proteome have been carried out to identify the proteins involved in host defense and the immune system. Although a study on a proteome search was conducted in Jersey cattle, this is the first-time study of milk whey proteome profiling on Indian Jersey crossed animals and the chromosomal mapping of their whey proteome using bioinformatics tools.

MATERIALS AND METHODS

All chemicals, reagents, organic solvents, and markers were purchased from Himedia and Sigma-Aldrich (Bangaluru, India) and were of molecular grade.

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Milk samples collection and whey precipitation

Jersey-crossed cows ($N = 12$) with an unrelated lactation phase were selected from the dairy farm of the Banaras Hindu University, Varanasi, India, reared on similar management practices. Raw milk samples (100 mL) were drawn individually and were kept at −20 °C until analysis. For the preparation of the whey sample, trichloroacetic acid (TCA)–acetone precipitation was done using a 10% TCA–acetone solution,[5] followed by centrifugation at 18 000 × *g* for 10 min at 5 °C. The pH was maintained at 7.5 while sample pellets were resuspended in 2% diethanolamine and 1% phosphate-buffered saline. Protein concentration was measured using the Lowry assay with bovine serum albumin serving as the control protein.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis protein separation

With slight adjustments, the method of Andrews[6] was applied to conduct gel electrophoresis of the whey part of pooled milk samples to achieve the appropriate separation inside the gel. A 10 µg mass of whey protein that had been isolated from various Jersey-crossed milk samples was loaded in the polyacrylamide gel electrophoresis (PAGE) wells, consecutively after the pre-stained protein marker in the Mini PROTEAN system. For optimum in-gel digestion, colloidal Coomassie brilliant blue R-250 dye was used to stain the gel.

High-resolution mass spectrometry analysis

After the sodium dodecyl sulfate PAGE in-solution digestion process was done, 240 µg of pooled samples (20 µg each from all 12 animals) of TCA–acetone precipitation were processed separately. The reduction treatment was carried out using 0.005 mol L^{-1} tris(2-carboxyethyl)phosphine, followed by additional alkylation using 0.05 mol L^{-1} indole-3-acetic acid, and then trypsin degradation for 18 h at 37 °C (1:50, trypsin/lysate ratio). Peptides were then vacuum dried when the reaction was ended with 10% trifluoroacetic acid. A Thermo-Scientific Ultimate-TM 3000 RSLC-nano system coupled with QE Plus was used for the mass spectra analysis of the peptide mixture. In the C-18 column, gradients of chromatography were performed for 100 min. The Orbitrap was used to acquire the mass spectra at 70 000 mass resolution. All charged states of the precursor had been eliminated after a 10 s dynamic exclusion. All samples were evaluated, a file was produced, and a proteome discoverer was utilized to compare it with the UniProt KB custom proteome reference database (Fig. 1). The protein's false discovery rate was adjusted to 0.02 after matching the peptide spectra.[7]

Bioinformatics analysis

To get insights into the distribution of expressed proteins over different chromosomes, a Circos plot was drawn using three entities consisting of chromosome number, total proteins, and unique peptides, and the number of unique peptides detected for total proteins expressed by each chromosome was done. Multivariate principal component analysis was performed by using Minitab-19 for whole proteome analysis using coverage percentage, peptides, peptide spectrum matches, unique peptides, and sum posterior error probability scores. Outlier plots, scree plots, score plots, and loading plots were generated to further analyze the output.

RESULTS AND DISCUSSION

This study aims to analyze the comprehensive milk proteome profile of the Indicine crossbred bovine (Jersey crossed). This is the first study of a whey proteome search in a crossbreed bovine to generate a dataset. The method of TCA–acetone precipitation has been followed to isolate the whey from the milk matrix and was found to be the best among the alternatives.[8] After the sodium dodecyl sulfate PAGE electrophoretic run, digested samples were run on a high-resolution liquid chromatagraph coupled to a mass spectrometer and proteome mass spectrum obtained; the data were processed using two search engines: MS Amanda and Sequest HT. More than 90 peptides were identified; among these, 29 low-abundant proteins are listed in Table 1.

To date, most investigations on the whey milk proteome have emphasized pure bovine breeds.[8] In the past few years, interest in the complete characterization of bovine whey protein has risen significantly. Furthermore, the amount of knowledge on bovine peptide sequences accessible in datasets has grown progressively. For example, Tacoma *et al.*[9] examined the bovine milk of Holstein and Jersey breeds and they found a total of 43 unique low-abundance proteins were differentially expressed between the two dairy breeds. Later, the host defense peptides in cow milk were investigated.

Of the proteins identified that have shown different biological and molecular functions, a maximum of 16 proteins have a role in metabolic processes, 12 proteins work in cell organization and biogenesis, 11 proteins are regulatory in nature, and 10 proteins have a role in responses to stimulus. Other biological roles shown by these identified peptides are cell proliferation, defense responses, cellular movement, and homeostasis. In terms of molecular functions, a greater number of proteins are protein binding (19), then catalytically active (10), structural molecule active (8), and metal binding (7), and some other functions also

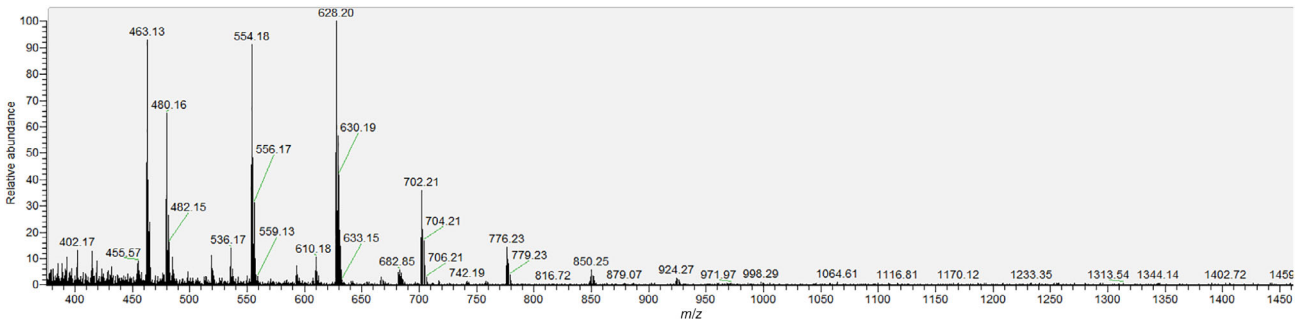

Figure 1. Illustration of the mass spectrum obtained from the high resolution accurate mass spectrometry analysis of whey proteome profile of Indian Jersey (*Bos taurus*) crossbred animals.

Table 1. Major low-abundant proteins identified in Jersey crossed bovine whey with respect to their functions, peptide spectrum match (PSM), calculated iso-electric point (Calc. pI), Pfam ID, and scores of the search engines

Accession	Description	Sum PEP score	Coverage (%)	Gene	PSM	Calc. pI	Biological function	Molecular function	Pfam ID	Score	
										MS Amanda 2.0	Sequest HT
Q8SPJ1	Junction plakoglobin	32.9	14	JUP	29	6.14	Cell organization and biogenesis; regulation of biological process; response to stimulus	Protein binding; signal transducer activity; structural molecule activity	Pf00514	3678.2	40.81
P0CG53	Polyubiquitin-B	9.569	56	UBB	16	7.47	Cell organization and biogenesis; cellular component movement; regulation of biological process; transport	Protein binding	Pf00240	1716.02	13.86
P63048	Ubiquitin-60S ribosomal protein L40	9.569	34	UBA52	16	9.83	Metabolic process	Protein binding; structural molecule activity	Pf00240	1716.02	13.86
P62992	Ubiquitin-40S ribosomal protein S27a	9.569	28	RPS27A	16	9.64	Metabolic process	Metal ion binding; protein binding; RNA binding; structural molecule activity	Pf00240	1716.02	13.86
P80195	Glycosylation-dependent cell adhesion molecule 1	7.464	12	GLYCAM1	12	6.68	—	Protein binding	Pf05242	1170.14	19.21
Q03763	Desmoglein-1	7.107	3	DSG1	5	5.08	—	Metal ion binding; protein binding	Pf00028	664.7	18.47
Q28161	Plakophilin-1	6.473	4	PKP1	4	8.95	—	Protein binding	Pf00514	600.31	16.75
P02470	Alpha-crystallin A chain	4.954	18	CRYAA	7	6.2	Cell death; cell organization and biogenesis; metabolic process; regulation of biological process; response to stimulus	Metal ion binding; protein binding; structural molecule activity	Pf00011	813.18	14.65
P04272	Annexin A2	4.077	7	ANXA2	4	7.31	Cell organization and biogenesis; metabolic process; regulation of biological process; transport	Enzyme regulator activity; metal ion binding; protein binding; RNA binding; transporter activity	Pf00191	486.64	15.52
Q28115	Glial fibrillary acidic protein	3.909	3	GFAP	2	5.45	Cell differentiation; cell organization and biogenesis; regulation of biological process; response to stimulus	Catalytic activity; motor activity; protein binding; structural molecule activity	Pf00038	331.75	13.31

Table 1. Continued

Accession	Description	Sum PEP score	Coverage (%)	Gene	PSM	Calc. pI	Biological function	Molecular function	Pfam ID	Score	
										MS Amanda 2.0	Sequest HT
P12763	Alpha-2-HS-glycoprotein	2.603	7	AHSG	3	5.5	Defense response; regulation of biological process; response to stimulus	Enzyme regulator activity; protein binding	Pf00031	218.86	15.75
Q2KJ64	Arginase-1	2.312	3	ARG1	4	6.54	Defense response; metabolic process; regulation of biological process; response to stimulus	Catalytic activity; metal ion binding	Pf00491	396.7	15.72
G3MYX0	Histone H4	2.073	10	LOC100847716	2	11.22	Cell organization and biogenesis	DNA binding; protein binding	Pf00125	178.72	22.76
Q5E947	Peroxiredoxin-1	1.884	5	PRDX1	2	8.4	Cell proliferation; cellular homeostasis; defense response; metabolic process; regulation of biological process; response to stimulus	Antioxidant activity; catalytic activity; protein binding; RNA binding	Pf00578	111.69	12.4
Q2YDE4	Proteasome subunit alpha type-6	1.721	5	PSMA6	2	6.76	Metabolic process	Catalytic activity	Pf00227	190.63	2.1
A5D984	Pyruvate kinase	1.617	2	PKM	2	7.85	Cell death; metabolic process; response to stimulus	Catalytic activity; metal ion binding; protein binding; RNA binding	Pf00224	152.16	1.73
A6QQJ3	Peripherin	1.584	2	PRPH	2	5.35		Structural molecule activity	Pf00038	182.45	2.1
P48616	Vimentin	1.584	2	VIM	2	5.12	Cell differentiation; cell organization and biogenesis; metabolic process; regulation of biological process; response to stimulus	Protein binding; RNA binding; structural molecule activity	Pf00038	182.45	2.1
O62654	Desmin	1.584	2	DES	2	5.27	Cell organization and biogenesis	Protein binding; structural molecule activity	Pf00038	182.45	2.1
Q9XSJ4	Alpha-enolase	1.487	2	ENO1	1	6.8	Metabolic process	Catalytic activity; metal ion binding	Pf00113		2.8
A7YWP4	Histidine ammonia-lyase	1.269	3	HAL	2	6.7	Metabolic process	Catalytic activity	Pf00221	192.28	3.1
P0CB32	Heat shock 70 kDa protein 1-like	1.211	2	HSPA1L	1	6.2	Cell organization and biogenesis; metabolic process	Nucleotide binding; protein binding	Pf00012	178.2	4.1

Table 1. Continued

Accession	Description	Sum PEP score	Coverage (%)	Gene	PSM	Calc. pI	Biological function	Molecular function	Pfam ID	Score	
										MS Amanda 2.0	Sequest HT
P10096	Glyceraldehyde-3-phosphate dehydrogenase	1.184	2	GAPDH	4	8.35	Cell death; cell organization and biogenesis; metabolic process; regulation of the biological process	Catalytic activity; nucleotide binding; protein binding	Pf00044	250.55	3.2
P68103	Elongation factor 1-alpha 1	1.038	2	EEF1A1	1	9.01	Metabolic process; response to stimulus	Catalytic activity; nucleotide binding; protein binding; RNA binding	Pf00009	215.9	2.43
F1MS40	Craniofacial development protein	0.904	3	CFDP2	2	5.33	—	—	Pf03372	94.93	2.3
A0JBZ9	Bucentaur-2	0.904	3	BCNT2	2	5.85	—	—	Pf03372	94.93	3.5
Q0VCX4	Catenin beta-1	0.877	1	CTNNB1	1	5.86	Cell communication; cell differentiation; cell growth; cell organization and biogenesis; cell proliferation; cellular component movement; metabolic process; regulation of biological process; response to stimulus; transport	DNA binding; protein binding; signal transducer activity	Pf00514	92.41	2.3
P68432	Histone H3.1	0.869	5	HIST1H3D; H3.1	2	11.12	Cell organization and biogenesis; regulation of the biological process	DNA binding; protein binding	Pf00125	170.38	2.17
G3MZU2	Phosphatidate cytidylyltransferase	0.864	2	CDS1	1	7.96	Metabolic process	Catalytic activity	Pf01148	111.3	2.3

Abbreviation: PEP, posterior error probability.

reported are DNA and RNA binding, enzyme regulation, and antioxidant activity. In similar probes done by Le *et al.*[10] using an ion-exchange methodology in colostral and mature whey, 293 distinct gene products were found, and Yang *et al.*[11] identified 183 proteins in the whey of the yak.

Plakoglobin is involved in regulation, response, and protein binding; molecular functions of ubiquitin, desmoglein, annexin, some glycoprotein, arginase, histones, peroxiredoxin, vimentin, desmin, catenin, peripherin, 70 kDa heat shock protein (HSP-70), and so on are mentioned in Table 1. Similar work has been done by Chopra *et al.*,[8] where they generated a comprehensive whey proteome for an Indian zebu breed (Sahiwal) to identify the low-abundant proteome. A comparative proteomic investigation has also been conducted on the Malnad Gidda breed in between different stages of lactation[12] and the expression of key proteins in the Jersey and Kashmiri cows.[13]

A Circos plot was made to study the expression pattern of proteins from the various chromosomes (Fig. 2). Chromosome 5 was highly expressed in the whey proteome, accounting for higher numbers of proteins for which 54 unique peptides were detected in proteome analysis. Chromosome 19 encoded 13 different proteins but a maximum number of unique peptides were detected, defining a higher abundance of the respective proteins in the proteome. Further, chromosome 23 and chromosome 6 contributed immensely to the whey proteome.

Principal component analysis showed high eigenvalues for the first component, accounting for a total variance of 78.9%, and with the second component of 9.5%. All the variables, like coverage percentage, unique peptides, sum posterior error probability scores, and peptide spectrum matches, are sufficiently correlated with the first component. The outlier plots based on Mahalanobis distance show few outliers showing a high correlation in the proteome dataset. The scree plot shows that the first, second, and third components reveal most of the variance in the proteome, whereas the first and second cumulatively define 90.9% variance. The score plot shows the distribution of milk whey proteins among the two components, where most of the observations are clustered together among the two components.[14]

The proteins are classified into different classes based on their functions. Many proteins that account for immune regulation are significantly correlated with the first coefficient and are sufficiently expressed. Ubiquitin is a bovine protein that reduces the risk of cancer cell proliferation and has been reported in the samples.[15] Several other proteins, in turn, promote innate immunity and have been found to be expressed in the whey proteome with adequate abundance. Glycosylation-dependent cell adhesion molecule (GlyCAM-1, lactophorin) acts as an L-selectin receptor ligand, regulates naive T-cell ability, and mediates their lymph node migration.[16] Heat shock proteins are seen in the results, which are highly conserved molecular chaperone proteins that are generated in response to stress.[17] The score plots classifying characteristic proteins of *B. taurus* show that most of the proteins are characterized, though some are not well characterized and have been annotated by using protein blast. These proteins have a high identity with similarly characterized proteins of different species. All these identified low-abundant whey proteins are excellent nutritional sources for human health.

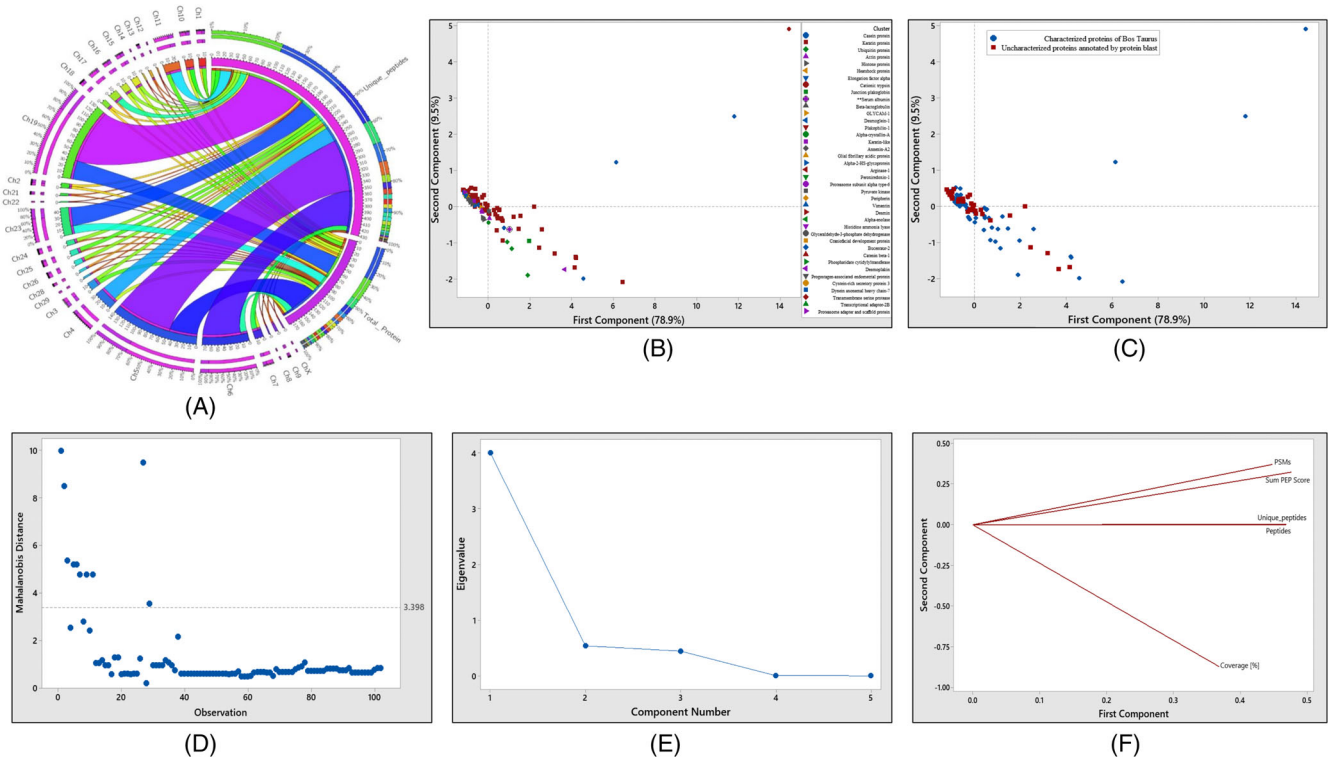

Figure 2. Different bioinformatics plots based on the result obtained from proteomic search databases. (A) Circos plot showing the total number of proteins expressed and their unique peptides detected in each chromosome. (B) Principal component analysis score plot for expressed proteins clustered based on their functional categories. (C) Principal component analysis score plots showing expressed protein clustered as characterized proteins and uncharacterized proteins annotated by blast analysis. (D) Outlier plot. (E) Scree plot. (F) Loading plot of coverage percentage, peptides, peptide spectrum matches, unique peptides, and sum posterior error probability score.

CONCLUSION

This study is a first-time comprehensive whey protein profiling of a crossbreed dairy animal. Jersey crossbreed cows are a large part of cattle resources throughout the world. The high resolution accurate mass spectrometry study on the isolated whey of this crossbreed animal has identified more than 29 low-abundant proteins in the proteome. The majority have a role in physiological, immune regulation, and defense systems in human health. It has been seen chromosomes 5 and 9 express the maximum number of proteins. A significant number of heat shock proteins shows the thermotolerance of these crossbreed animals. This study has also emphasized the future potential of more focused research on the proteome analysis of the different crossbred and indigenous dairy animals to understand their gene expression patterns and the functional value of the minor whey proteins.

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CONFLICT OF INTEREST

All the authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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