IPD Project Details

Project ID: IPD5805

Project Title: Salivary proteins in buffalo with special emphasis to estrous cycle

Description: Saliva is considered as the best source for biomarker-discovery studies, since it is a non-invasive method when compared to other body sources. Usually, buffalo cannot express their estrus signs precisely. Hence, there is a need for concise methods to detect the time of estrus to ensure the success in artificial insemination. Therefore, we have established a reference proteome map on buffalo whole saliva during estrous cycle in order to document the estrus-specific proteins using SDS-PAGE and mass spectrometry. The present findings conclude that the proteomic approach adopted to identify the proteins from buffalo saliva around estrous cycle may provide a new tool for screening the estrus phase.

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Sample Preparation: SDS-PAGE The electrophoresis of whole saliva was done using 12% SDS-PAGE under denaturing condition. The saliva containing 40 µg protein from three phases viz., proestrus, estrus and postestrus was loaded on to the gel.

Peptide Separation: Trypsin in-gel digestion In order to make comprehensive identification of proteins, the SDS–PAGE gel plugs from each phase was divided into seven parts. The pieces were then destained using 100 µL of 50 mM Ammonium bicarbonate/50% (V) acetonitrile under 37°C. The destained gel pieces were dried under the speed-vac (Savant, Germany). The dried pieces were incubated in 100 mL of 2% ?-mercaptoethanol with 25 mM Ammonium bicarbonate for 30 min at room temperature under dark condition, for the reduction of salivary proteins. 10 % Vinyl pyridine and 25 mM Ammonium bicarbonate in 50% acetonitrile at equal volume were added and incubated for 20 min for the cysteine alkylation. Then the gel pieces were soaked with 1 mL of 25 mM Ammonium bicarbonate for 10 min then Speed-Vac until dryness. The completely dried pieces were incubated in 25 mM Ammonium bicarbonate containing 100 ng of modified trypsin (Promega, Germany) for overnight. The digested peptides were transferred into fresh tube and subjected to Speed-Vac and resuspended in 0.1% formic acid just before loading in the mass spectrometry. Mass spectrometry The in-gel

digested peptides were analyzed using LTQ-Orbitrap (Discovery) mass spectrometer with nanoelectrospray ionization source (Thermo Electron, San Jose, CA, USA) and fractionated with nano-flow high performance liquid chromatography (HPLC) system (Agilent Technologies 1200 series, Germany) with an Agilent C18 column having 100X0.075 mm and 3.5µm particle diameter along with mobile phase A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The peptide elution was noted at a linear gradient of 5%-35% B for the first 30 min, followed by rapid increase to 95% B for the next 10 min. The MS spectra (Survey Scan) were acquired with high resolution over the acquisition range of m/s 200-2000 and then the precursor ions were selected for further MS/MS scan.

Protein Characterization: The gathered mass spectrum was analyzed by Xcalibur software (version 2.0 SR1). The MS/MS spectrum ion scans were undergone with database search software, SEQUEST (TURBO) to identify the protein peptides. The mass tolerance for the precursor peptide ions was set to 3.5 and the fragment ion tolerance was set to 1. More than two matched peptides were used for the protein identification.

Experiment Type: Shotgun proteomics

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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