IPD Project Details

Project ID: IPD8171

Project Title: Organ wise proteomic profiling of Indian major carp, Labeo rohita

Description: The aim of this study was to develop an organ wise proteome map for Labeo rohita. Using LC-MS/MS, we have performed in-depth proteomics analysis of 19 different sample types, including 17 tissue samples, plasma from female fish and embryo 4-day post fertilization. Whole analysis resulted in the identification of more than more than 8000 proteins with 1% FDR of which more than 76% were identified with two or more than two unique peptides. The dataset show organ wise pattern of protein expression along with extensive catalogue of orgsn wise Post translational modification. This proteomic information would complement the recently published genome to accelerate further research.

Principal Investigator: Dr Sanjeeva Srivastava

PI Affiliation: Department of Biosciences and bioengineering, IIT Bombay, India, 400076

Sample Preparation: Protein extraction from 17 tissue samples was done using urea lysis buffer composed of 8M urea, 50 mM Tris-HCl, 75 mM NaCl and 1 mM MgCl2. Tissues were first homogenised, sonicated, bead-beated for 90 sec followed by centrifugation at 8,000 x g for 15 minutes at 4-degree C. For embryo sample, conventional trizol method was followed for extracting proteins. Plasma was directly taken as protein extract.

Peptide Separation: Protein in all the samples was quantified using Bradford assay and fractionated on 1D-SDS-PAGE. Fractionated protein bands were performed in-gel digestion using trypsin as protease. Protein digest was extracted from gel pieces using gradient solution of Acetonitrile. Peptides were desalted using c-18 zip tips, vacuum dried and stored till further use for mass spectrometry. For LC-MS/MS, peptides were quantified and around 1 ug of peptides were subjected to mass spectrometry.

Protein Characterization: The raw MS data was searched against the NCBI L. rohita database using Proteome Discoverer 2.2 (Thermo). Briefly, the parameters included maximum two missed cleavages, oxidation of methionine and N-terminus acetylation as variable modifications, Carbamido-methylation of Cys as static modification, FDR< 0.01,

mass error tolerance of 0.05 Da and 10 ppm for fragment and precursor ions respectively. For post translation modification analysis, the raw files were converted to Mzml and analysed using Trans Proteomic pipeline.

Experiment Type: Shotgun proteomics, Gel-based experiment

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: No PTMs

PubMed ID: 34962809