IPD Project Details

Project ID: IPD6020

Project Title: Proteomic analysis of gut tissue of Labeo rohita infected with Aeromonas hydrophila

Description: This study aimed at performing the quantitative proteomics analysis of gut tissue, one of Labeo rohita, one of the important aquaculture fish species. Data was acquired using high resolution mass spectrometry followed by Label free quantification. After performing statistical analysis, a panel of differentially expressed proteins were identified which include extracellular matrix proteins, cytoskeletal proteins, immune related proteins and metabolic enzymes. This analysis revealed important signaling pathways and protein-protein interaction networks which could help in understanding disease pathogenesis. The data acquired in the study would provide a basis for further omics research and can help in elucidating biological processes in healthy or diseased fish.

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Sample Preparation: Tissue lysis was done using SDS buffer consisted of 5% SDS, 100mM Tris/HCl pH 8.5. Protease inhibitor cocktail (50x stock) were added to the tissue and incubated on ice for 30 minutes. Sonication was performed for 2 minutes with an amplitude of 40% with 5 sec pulse on and 5 sec off. Centrifugation was done to remove the debris and the clear supernatant was collected.

Peptide Separation: Protein in all the samples was quantified using BCA method and equal amount of protein was digested using filter assisted sample preparation (FASP) method. Peptides were desalted using c-18 cleaning 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 L. rohita uniprot database (ProteomeID- UP000290572, Taxonomy ID- 84645, downloaded on 18.06.2021)) using MaxQuant software (version 1.6.6.0). Label type was set as standard and under instrument option, Orbitrap Fusion was selected. Trypsin was set as protease and a maximum of two missed cleavages were allowed. Carbamidomethylation at

Cysteine (+57.021464 Da) and oxidation at Methionine (+15.994915 Da) were selected as fixed and variable modifications respectively. Protein and peptide identifications were performed at 1% false discovery rate (FDR). For quantitative analysis, label free quantification (LFQ) intensities were taken from the result file.

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: No PTMs

PubMed ID: 39292008