IPD Project Details

Project ID: IPD3461

Project Title: Identification of differentially regulated proteins of Caenorhabditis elegans during Salmonella enterica Serovar Typhi exposure using Mass Spectrometry

Description: Recently, proteomics based studies have become the primary contributors for the understanding of host-pathogen interactions and discovery of various drug targets. Herewith, we have used the popular eukaryotic model organism Caenorhabditis elegans to study the host pathogen interactions at protein levels. Especially, we have employed proteomics approach to monitor the protein players that underwent for differential regulation during host-pathogen interaction through label-free quantitation LC-MS/MS technique. As a result of LC-MS/MS analysis, a total of 3747 differentially regulated proteins were identified during pathogenic condition among which 12 and 04 proteins were upregulated and downregulated with at least 2 folds, respectively. Bioinformatics analyses result suggested that the upregulated proteins have crucial roles in TCA cycle, whereas downregulated proteins were majorly involving in the N-glycosylation synthesis. Additionally, the mRNA levels of up and downregulated proteins were validated using qPCR analysis. Overall, the study suggested that the bacterial infection could target multiple molecular mechanisms to modulate the host machinery which favour the bacterial pathogen for their pathogenesis.

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Sample Preparation: 100 µg of proteins from both control and S. Typhi exposures were taken for SDS-PAGE. In-gel trypsin digestion was performed after a short run of about only 1 cm across the stacking gel and the entire gel area was excised for analysis (Mendes et al. 2016). Prior to in-gel trypsin digestion, the proteins samples were destained using 55 mM ammonium bicarbonate (AmBiC) prepared in 50 % ACN (Acetonitrile) followed by dehydrated with 100 % ACN and reduced using 20 ?l of 10 mM DTT (Dithiothreitol) prepared in 50 mM AmBiC at 60 °C for 30 min. Subsequently, the protein samples were alkylated using 20 ?l of 20 mM IAA (Iodoacetamide) at 37 °C for 30 min in dark and vacuum dried. Afterwards, the proteins were added with 5 ?l of trypsin solution (80 ng/?l) prepared in 50 mM AmbiC with 10 % ACN and incubated at 37 °C for 16 h.

Peptide Separation: After incubation, the trypsinization was inhibited by the addition of 2 ?L of formic acid to the microfuge tubes for 20 min. Finally, the trypsin digested peptides were centrifuged for 5 min at 20,817 ×g at 4 °C and the supernatants were subjected to LC-MS/MS analysis (Mir and Balamurugan, 2019). The label-free quantitation and protein profiling analysis has been outsourced at Rajiv Gandhi Centre for Biotechnology, Kerala, India (website: https://rgcb.res.in/mass-spectrometry-and-proteomic-core-facility.php).

Protein Characterization: The trypsin digested peptides were subjected to LC-MS/MS analysis by employing liquid chromatography using 2D nanoacquity UPLC® system coupled with qTOF (quadrupole-Time of Flight) MS (SYNAPT-G2-HDMS, Waters, UK) and operated/controlled by MassLynx4.1 SCN781 software. Initially, fractionation of peptides was performed using 1st Reversed Phase (RP) column at high pH (pH 8.0) which is the first dimension separation, subsequently subjected to 2nd RP column at a low pH (pH 2.0) which is the second dimension. In connection to the 2D separation, peptides from the nano-LC column were subjected to MS analysis. The parameters used in this study were previously described by Dharmaprakash et al., 2014. The LC-MS/MS raw data from each sample and each replicate were subjected to ProteinLynx Global SERVER™ v2.5.3 (Waters, UK) for the protein identification as well as label free quantitation (relative). The C. elegans protein sequences were retrieved from NCBI which was used for protein identification and the FDR (False Discovery Rate) of proteins was set to 4 %. Meanwhile, the post processing of LC-MS/MS raw data was planned in such a way that the resulting protein must have at least one fragment ion match and one peptide match. Trypsin was selected as the primary enzyme with a specificity of at least one missed cleavage site. Mass tolerance of precursor ions was set to 10 ppm and 20 ppm for their respective fragment ions. Carbamidomethylation (cysteine residues) was selected as fixed modification and oxidation (methionine residues) was selected as variable modification.

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

PubMed ID: