IPD Project Details

Project ID: IPD4029

Project Title: p38-MAPK recruits the proteolysis pathways in Caenorhabditis elegans during bacterial infection

Description: TiO2 column chromatography, MALDI-ToF-MS, SUMOylation, Coimmunoprecipitation and LC-MS/MS

Principal Investigator: Dr Krishnaswamy Balamurugan

PI Affiliation: Professor Department of Biotechnology Science Campus Alagappa University Karaikudi - 630 003 Tamil Nadu India

Sample Preparation: 100 µg proteins from control and S. Typhi exposed lysates/column elutes were taken for in-solution digestion. Disulfide bonds were reduced using 20 µl of dithiothreitol (20 mM final concentration) for 1 h at 56 ?C and free thiol groups were alkylated using 20 mM IAA at room temperature for 1 h in dark.

Peptide Separation: The urea concentration (if urea buffer was used during sample preparation) was diluted below 0.5 M using 100 mM ammonium bicarbonate and the proteins were digested overnight (14 - 16 h) using MS grade trypsin enzyme at a protein-to-enzyme ratio of 25:1 at 37 ?C. Finally, the digested peptides were subjected to LC-MS/MS analysis, respectively.

Protein Characterization: Positive-ion mode of MALDI-MS analysis has been performed using AXIMA Performance MALDI-ToF-ToF mass spectrometer. Equal ratio of desalted samples were mixed with 2, 5-dihydroxybenzoic acid (2,5-DHB) (prepared with 0.1 % Triflouroacetic acid in 50 % acetonitrile) along with 1 % phosphoric acid as an additive to improve the phosphopeptide ion signals (Kjellström and Jensen, 2004). The m/z values (700 – 3500 m/z range) of the each sample from MALDI-MS spectra were subjected to MS-Fit-Protein Prospector analysis (http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msfitstandard) (Prasath et al. 2019). The parameters set as follows: Taxonomy - Caenorhabditis elegans; Database - UniProt; Constant Modifications - Carbamidomethylation (C); Possible/variable modifications – Oxidation of Met, Acrylamide modified Cys, Phospho (STY); Peptide tolerance – 20 ppm. The resulting protein hits, along with the phosphorylation sites, were manually retrieved and tabulated. Meanwhile, experimental

phosphorylation sites of the respective proteins were acquired from the UniProt database and tabulated. The MALDI-MS identified proteins were taken to “PHOSIDA” bioinformatics tool (http://141.61.102.18/phosida/index.aspx) to predict the possible phosphorylation sites (Gnad et al. 2007). The overlapping proteins between the datasets were identified using online venn diagram platform (Oliveros, 2007).

Experiment Type: Shotgun proteomics, Affinity purification coupled with mass spectrometry 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: iodoacetamide derivatized residue

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