IPD Project Details

Project ID: IPD2357

Project Title: Global proteomic analysis of changes in the Caenorhabditis elegans proteome during Klebsiella pneumoniae infection

Description: In this study, the changes in the global proteomics of C. elegans were analyzed by proteomics approach. The nematodes were exposed to K. pneumoniae for different time-points (12, 24 and 36 h) and the changes in the regulation were analyzed by employing mass spectrometry.

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Sample Preparation: The samples were then flash frozen in lysis buffer [7 M urea, 2 M thiourea, 4% CHAPS, and 30 mM Tris-HCl, pH 8.5 and protease inhibitor cocktail (Sigma)] and stored at -86 °C. When required, the samples were homogenized on ice for 1 min by employing sonicator. The total protein were immobilized in IPG strip and subjected to IEF using Ettan™ IPGphor 3 isoelectric focusing system. The differentially regulated protein spots were subjected to reduction and alkylation by DTT and iodoacetamide, respectively.

Peptide Separation: After in-gel digestion, the peptide extracts were pooled together and concentrated by using speed Vac. The peptides were dissolved in MS grade water and were desalted using ZipTipC 18 pipet tips (Millipore). About 0.5 ?l of ?-Cyano-4-hydroxycinnamic acid was mixed with equal volume of desalted samples and allowed to dry for 4-6 h and characterized by mass spectrometry analysis.

Protein Characterization: Data processing protocol The MALDI TOF/TOF analysis was performed using MALDI-TOF/TOF analyzer (AXIMA Performance, SHIMADZU BIOTECH) in a positive reflectron ion mode. With peptide mass fingerprint (PMF) and MALDI TOF/TOF, the proteins were identified against the all entries of C. elegans database using the MASCOT (Matrix Science Version 2.2) as a search engine. During search, the carbamidomethylation and oxidation was set as fixed and variable modifications, respectively. In the MASCOT search, the mass tolerance of 1.2 Da with one missed cleavage per peptide was allowed in all searches. Proteins with constant hit

against different mass tolerance and significant scores were considered.

Experiment Type: 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: monohydroxylated residue, iodoacetamide derivatized residue

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