IPD Project Details

Project ID: IPD2081

Project Title: Bacillus subtilis under Curcumin treatment LC-MS/MS

Description: Comprehensive Analysis of Temporal Alterations in Cellular Proteome of Bacillus subtilis under Curcumin Treatment

Principal Investigator: Dr Sanjeeva Srivastava

PI Affiliation: Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai, India

Sample Preparation: Protein extraction was performed from control and curcumin (20, 60 and 120 min treatment) treated B. subtilis AH75 strains using TRIzol extraction protocol. Briefly, the bacteria were harvested at different time points of curcumin exposure (20, 60 and 120 min) and untreated control samples and washed with PBS buffer (pH 7.4) for 4 times to remove the media components. Cell lysis was performed with lysozyme (1 mg/mL) and sonication in presence of protease inhibitor cocktail (GE Healthcare). To the cell lysates, TRIzol and chloroform were added to remove RNA, and ethanol was added to remove DNA and chilled acetone was added to precipitate protein. Protein pellet was washed with guanidine-HCl and acetone to remove the phenol and salts. Protein pellets were air dried and finally dissolved in rehydration buffer containing 7 M urea, 2 M thiourea, 2% CHAPS (w/v) and traces of bromophenol blue. The protein concentration in each sample was measured using 2-D quant kit (GE healthcare) following the manufacturer’s instructions.

Peptide Separation: In-gel digestion of the differentially expressed protein spots (p ? 0.05) was performed following the same protocol as mentioned by Shevchenko et al. and Reddy et al. with minor modifications [52, 50]. The extracted trypsin digested peptides were further processed using Zip-Tip C18 pipette tips (Millipore, USA) following the manufacturer’s protocol for enrichment of the peptides and removal of salts. The protein identification was performed with MALDI-TOF/TOF mass spectrometer (AB Sciex, Framingham, MA) linked to a 4000 series explorer software (v.3.5.3) as described previously [50]. data analysis was performed by using MASCOT version 2.1 search engine with following parameters were specified; database- SwissProt, B. subtilis taxonomy, trypsin digestion with single missed cleavage, oxidation of methionine as a variable modification and carbamidomethylation of cysteine residue as a fixed

modification, mass tolerance 75 ppm for MS and 0.4 Da for MS/MS

Protein Characterization: Application of two complementary quantitative proteomic techniques; DIGE and iTRAQ in combination with high sensitive mass spectrometry effectively improved the proteome coverage. In silico pathway analysis using DAVID and KOBAS revealed modulation of fatty acid biosynthesis, peptidoglycan synthesis/ cell division, respiration and stress response proteins in response to curcumin.

Experiment Type: Bottom-up

Species: Bacillus subtilis subsp. subtilis str. 168 -224308

Tissue: Fat body (bto:0000442)

Cell Type:

Disease: Unknown

Instrument Details: LTQ Orbitrap Velos (MS:1001742)

Protein Modifications: methylthiolated residue, iodoacetamide derivatized residue, iTRAQ4plex-116 reporter+balance reagent acylated residue

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