

IPD Project Details

Project ID: IPD4495

Project Title: B. subtilis, Tatarol, LC-MS/MS

Description: Comprehensive Proteomic Analysis of Tatarol Induced Alterations in Bacillus subtilis by Multipronged Quantitative Proteomics.

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Sample Preparation: The B. subtilis AH75 strain having a spectinomycin antibiotic marker was grown overnight at 37 °C in Luria Broth (LB) containing 100 µg/mL spectinomycin. This culture was diluted with fresh LB media to maintain the OD600 at 0.05 and subsequently incubated again at 37 °C till the OD600 reached 0.1. B. subtilis growth was measured by monitoring the OD at 600 nm for the untreated control and IC50 (1.5 µM) and MIC (2 µM) tatarol treated cultures at 37 °C.

Peptide Separation: Trypsin solution (Trypsin Gold; Promega, Madison, USA) was added to the dried gel pieces keeping the ratio of trypsin:protein around 1:10 (w/w). After addition of the trypsin solution, the gel pieces were kept at 37 °C overnight in a dry bath. The digested peptide fragments were extracted with an extraction solution containing a gradient of ACN (50–80%) and 0.1% TFA. Finally, trypsin digested samples were further processed using Zip-Tip C18 pipette tips (Millipore, USA) following the manufacturer's protocol and subjected to a 4800 MALDI-TOF/TOF mass spectrometer (ABSCIEX, Framingham, MA) linked to a 4000 series explorer software (v.3.5.3).

Protein Characterization: SEQUEST (SCM build 59) search engine, Proteome Discoverer 1.3 workflow, UniProtKB B. subtilis 168 database, The search parameters include: 20 ppm and 0.1 Da mass tolerances for MS and MS/MS respectively, trypsin as a proteolytic enzyme with one missed cleavage, iTRAQ modification at N-terminal and lysine and alkylation of cysteine as fixed modifications and oxidation of methionine as variable modification.

Experiment Type: Shotgun proteomics

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

Tissue: Unknown

Cell Type: Unknown

Disease: Unknown

Instrument Details: LTQ Orbitrap Velos (MS:1001742)

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

PubMed ID: [25464363](#)