IPD Project Details

Project ID: IPD8281

Project Title: Quantitative secretome profiling of Candida glabrata wild-type and aspartyl protease-deficient strains

Description: The project is aimed at deciphering the proteins, which are differentially abundant in the secretome of Candida glabrata wild-type and a mutant strain deleted for eleven cell surface-associated aspartyl-proteases (CgYapsins).

Principal Investigator: Dr Rupinder Kaur

PI Affiliation: Laboratory of Fungal Pathogenesis, Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India

Sample Preparation: The supernatants of YNB medium-grown logarithmic-phase cultures of wild-type (wt) and Cgyps1-11? strains of C. glabrata cells were collected in duplicates and passed through 0.4 µm membrane or syringe filters to remove the residual cells. The resultant filtrates were concentrated using Amicon Ultra-15 and Ultra-0.5 (10 kDa cutoff) centrifugal filter units, and analysed using the EASY-nLC 1000 system coupled to the QExactive mass spectrometer. For this analysis at the Valerian Chem Private Limited (Vproteomics), New Delhi, India, protein were first reduced with TCEP solution followed by alkylation with iodoacetamide (50 mM) and trypsin digestion.

Peptide Separation: The trypsin-digested samples were resolved on a 50-cm long PicoFrit column and peptides were eluted with a 5-15% gradient of the Buffer-B (95% acetonitrile, 0.1% formic acid) for 85 min, 15-40% gradient for 80 min, followed by 95% gradient for 6 min at a flow rate of 250 nl/min for the total run time of 180 min. The quantitative label-free relative protein quantification, following LC-MS, was done using the Minora Feature Detector Node of the Proteome Discoverer 2.2.

Protein Characterization: The MS data were acquired using a data-dependent top10 method dynamically choosing the most abundant precursor ions from the survey scan. The raw data files for all four samples were analysed using the Proteome Discoverer 2.2 software against the UniProt C. glabrata reference proteome database (containing 8078 entries). For Sequest HT and MS Amanda 2.0 search, the precursor and fragment mass tolerances were set at 10 ppm and 0.5 Da, respectively. The enzyme specificity for trypsin/P was set as cleavage at the C terminus of ‘K/R’, unless followed by “P”, along

with two allowed missed cleavage sites. Carbamidomethyl on cysteine as fixed modification, and methionine oxidation and N-terminal acetylation were considered as variable modifications for database search. Both the peptide spectrum match and the protein false discovery rate were set to 0.01 and determined using the percolator node. Relative protein quantification was performed using the Minora Feature Detector Node of the Proteome Discoverer 2.2 with default settings. The peptide spectrum matches with high confidence were only considered.

Experiment Type: Shotgun proteomics

Species: Data in species_details No Data

Tissue: Unknown No Data

Cell Type: Unknown No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: iodoacetamide derivatized residue

PubMed ID: