IPD Project Details

Project ID: IPD6532

Project Title: Total membrane proteome analysis of Candida glabrata wild-type and eleven CgYapsins-deficient strain Cgyps1-11?.

Description: The project is aimed at characterizing the effect of loss of a family of eleven aspartyl proteases (Cg Yapsins) on the global membrane proteome of Candida glabrata.

Principal Investigator: Dr Rupinder Kaur

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

Sample Preparation: C. glabrata wild-type and Cgyps1-11? strains, used for the proteome analysis, were grown in YPD medium till logarithmic-phase. Next, cells were harvested and washed twice using ice-cold water. The cells were normalized to 20 OD600 and suspended in buffer 1 [0.1 M Tris (pH 10.7), 5 mM EDTA, 2 mM dithiothreitol (DTT) and 1X protease inhibitor cocktail solution]. The 0.5 mm acid-washed glass beads were added to cells, and the cells were lysed using mechanical force. The cell homogenates were diluted using the buffer 2 [0.1 M Tris-HCl (pH 8.0), 0.33 M sucrose, 5 mM EDTA and 2 mM DTT], and centrifuged at 1000g for 3 min at 4°C. The supernatant was transferred into a new centrifuge tube, followed by centrifugation at 3000g for 5 min at 4°C to remove unbroken cells and cell debris, if any. Next, the supernatant was centrifuged at 19000g for 45 min at 4°C to obtain total membrane fraction pellets. The membrane pellets were washed with the buffer 2 [0.1 M Tris-HCl (pH 8.0), 0.33 M sucrose, 5 mM EDTA and 2 mM DTT] and suspended in buffer 3 [membrane suspension buffer; 20% glycerol (v/v), 0.1 mM EDTA, 0.1 mM DTT and 10 mM Tris-HCl (pH 7.5)]. Protein quantification was performed using the BCA protein assay kit. 200 µg TMFs were resolved on a 10% SDS-PAGE gel till bromophenol blue dye in the sample buffer entered about 1 cm into the gel. After staining with Coomassie Brilliant Blue, protein bands were excised using a scalpel and sent to the Taplin Biological Mass Spectrometry Facility, Harvard Medical School Spectrometry Facility, Harvard Medical School, Boston, USA (https://taplin.med.harvard.edu) for global protein identification. All samples were prepared and sent from two independent biological replicates.

Peptide Separation: At Taplin facility, gel pieces were subjected to in-gel trypsin

digestion at 37ºC followed by microcapillary LC-MS/MS (Liquid chromatography-tandem mass spectrometry) using the LTQ Orbitrap Velos Pro ion-trap mass spectrometer. Sample with search name 50050 refers to C. glabrata wild-type replicate 1, 50051 refers to C. glabrata wild-type replicate 2, 50052 refers to Cgyps1-11? replicate-1, and 50053 refers to Cgyps1-11? replicate-2.

Protein Characterization: The acquired peptide fragmentation data were analysed using the Sequest software, run against the UniProt C.glabrata reference database, and identified peptides were filtered to 1% false discovery rate.

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: dehydrated residue

PubMed ID: 35051415