

IPD Project Details

Project ID: IPD6867

Project Title: Interactome identification of a glycosylphosphatidylinositol-linked aspartyl protease, CgYps1, in the pathogenic yeast *Candida glabrata*

Description: The major goal of this project is to identify proteins that interact with CgYps1 aspartyl protease in logarithmic-phase *Candida glabrata* cells.

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Sample Preparation: Two independent biological replicates of wild-type (wt) and mutant lacking eleven GPI-linked aspartyl proteases (Cgyps1-11?) strains were used for CgYps1 interactome analysis. Immunoprecipitation (IP) assay was performed using the polyclonal anti-Yps1 antibody (raised in mice). For IPs, cell extracts were prepared of YPD grown wt and Cgyps1-11? strains by glass bead lysis method. First, a preclearing step was performed with 2 mg of cell extracts, which were added to protein A-sepharose beads and the volume was made up to 500 μ l with lysis buffer (15 mM Na₂HPO₄, 150 mM NaCl, 2% Triton X-100, 0.1% SDS, 0.5% DOC, 10 mM EDTA, 0.02% NaN₃) containing protease inhibitors. Simultaneously, pre-washed beads were incubated with 20 μ l of CgYps1 antibody for bead-antibody conjugate. The volume was made up to 500 μ l by adding lysis buffer and samples were incubated for 2-3 h at 4°C. The supernatant from precleared cell lysate was mixed with antibody-conjugated beads and incubated overnight at 4°C. Next, beads were pelleted down at 1500 rpm for 2 min, followed by 3-4 washes with wash buffer (50 mM NaCl, 10 mM TRIS, 0.02% NaN₃). 30-50 μ l of 2X SDS dye was added and boiled for 5-10 minutes. The samples were loaded on 10% SDS gel. The gel was run till the dye entered into the resolving gel. Staining was performed with Coomassie Brilliant Blue, protein bands were excised and sent to the Taplin Biological Mass Spectrometry Facility, Harvard Medical School, Boston, USA (<https://taplin.med.harvard.edu>) for protein identification.

Peptide Separation: At the Taplin facility, gel pieces were subjected to in-gel trypsin digestion followed by microcapillary LC-MS/MS (Liquid chromatography-tandem mass spectrometry) using the LTQ OrbitrapVelos Pro ion-trap mass spectrometer. Sample with search name 68655 refers to wt replicate-1, 68656 refers to wt replicate-2, 68657

refers to Cgyps1-11? replicate-1 and 68657 refers to Cgyps1-11? replicate-2.

Protein Characterization: All generated fragmentation patterns were acquired and searched against the UniProt C. glabrata reference proteome database using the SEQUEST software to determine the peptide sequences. 1% false discovery rate was set as a cut-off to filter the identified peptides.

Experiment Type: Shotgun proteomics

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: dehydrated residue

PubMed ID: