# IPD Project Details

**Project ID:** IPD9272

**Project Title:** From Omics to Function: NCU05627 Emerges as a Pivotal Sugar Transporter for Cellulase Secretion in Neurospora crassa

**Description:** This study investigates the role of sugar transporters in cellulase secretion and polysaccharide utilization in Neurospora crassa using an LC–MS/MS-based proteomics approach. Comparative proteomic profiling identified 2480 proteins shared between the experimental conditions, including several upregulated sugar transporters and carbohydrate-active enzymes (CAZymes) associated with biomass degradation. Functional annotation through Gene Ontology analysis revealed enrichment of proteins involved in catalytic activity and metabolic processes. Among the identified sugar transporters, the putative xylose transporter NCU05627 was selected for further characterization due to its previously uncharacterized role in cellulase induction. Phylogenetic analysis grouped NCU05627 within the hexose transporter-enriched clade, suggesting a conserved role in sugar uptake. Functional studies demonstrated that deletion of NCU05627 reduced the secretion of plant cell wall-degrading enzymes. This dataset provides comprehensive insight into the sugar transportome of N. crassa and identifies transporter proteins potentially involved in cellulose degradation and fungal biomass conversion.

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**Sample Preparation:** Mycelia were ground in liquid nitrogen, and 1 mL of disruption buffer (Tris-HCl, EDTA, PMSF) with silica beads was added. The mixture was vortexed for 1 hour at 4 °C, then centrifuged at 10,000 × g for 10 minutes at 4 °C to remove cell wall proteins. Membrane pellets were isolated by ultracentrifugation at 150,000 × g for 60 minutes at 4 °C and washed twice to remove contaminants. The protein pellet was dissolved in 100 mM ammonium bicarbonate (volume adjusted based on pellet miscibility) and incubated for 1 hour at 37 °C. Membrane proteins were solubilized using a 2× solution of 20 mM HEPES (pH 7.6) containing 1 M 6-aminocaproic acid and 10 g/L n-dodecyl-?-D-maltoside, with stirring at 1,000 rpm for 1 hour at 20 °C. Samples were then sonicated for 15 minutes in a water bath and centrifuged at 22,000 × g for 30 minutes. The supernatant was collected and sent for mass spectrometry analysis.

**Peptide Separation:** Peptides were separated using an Easy-nLC-1000 system (Thermo Fisher Scientific) coupled with a Picofrit C18 column (1.8 ?m resin, 15 cm length). Samples were loaded in buffer A containing 2% acetonitrile and 0.1% formic acid, followed by gradient elution using 0–38% buffer B (80% acetonitrile, 0.1% formic acid) at a flow rate of 500 nL/min for 96 min. This was followed by 90% buffer B for 11 min and column equilibration for 3 min prior to LC–MS/MS analysis.

**Protein Characterization:** RAW LC–MS/MS data were processed using Proteome Discoverer v2.5 against the UniProt reference database for Neurospora crassa. Protein identification was performed using Sequest and Amanda search algorithms with precursor and fragment mass tolerances of 10 ppm and 0.02 Da, respectively. Trypsin/P was used as the proteolytic enzyme specificity. Carbamidomethylation of cysteine was set as a fixed modification, whereas oxidation of methionine and N-terminal acetylation were considered variable modifications. Peptide spectrum match and protein false discovery rates were maintained at 1% FDR. Identified proteins were further subjected to functional annotation, including Gene Ontology (GO), carbohydrate-active enzyme (CAZyme), and sugar transporter classification analyses.

**Experiment Type:** Bottom-up

**Species:** Data in species_details  No Data

**Tissue:** Data in tissue_details  No Data

**Cell Type:** Data in cell_details  No Data

**Disease:** Data in disease_details  No Data

**Instrument Details:** Data in instrument_details  Data in instrument_details

**Protein Modifications:** Carbamidomethylation of cysteine was set as a fixed modification, while oxidation of methionine and protein N-terminal acetylation were considered variable modifications.

**PubMed ID:**