# IPD Project Details

**Project ID:** IPD3843

**Project Title:** Comparative proteomic analysis of secretomes from engineered Penicillium funiculosum modulated with sugarcane bagasse

**Description:** Sugarcane bagasse (SCB), one of the most abundant plant feedstocks is at the leading front of the biofuel industry based on the potential to promote economic, social and environmental development worldwide through sustainable set-ups related to energy production. This complex biomass requires a vast array of carbohydrate active enzymes (CAZymes) mostly from filamentous fungi for its deconstruction to monomeric sugars for the production of value-added fuels and chemicals. In this study, we attempted to evaluate the comprehensive repertoire of proteins in the secretome of an engineered Penicillium funiculosum, (PfMig188) in response to SCB. For this, a systematic approach was utilized for the cultivation of the fungus towards production of tailored enzymes specific for saccharification of SCB. This was done through the modulation of the production media with different concentrations of pretreated SCB (0 â€“ 45 g/L) and the array of secreted proteins were identified by enzyme activity assay and liquid chromatography-tandem mass spectrometry (LC-MS/MS). A total of 280 proteins were detected in the secretome of PfMig188 with 46% of them being CAZymes. Modulation of the cultivation media with SCB up till 15 g/L enhanced the secretion of some importanthemicellulasesand cell wall modifying enzymes such as Î²-xylosidase (GH5, GH43), Î²-1,3-galactosidase (GH43), endo-1,3(4)-beta-glucanase (GH16), cutinase (CE5) and hydrophobic surface binding protein (HsbA)that works in synergy with the cellulases in the secretome to improve SCB saccharification by 20%. Our findings provide more insight on the enzyme system of PfMig188 for degradation of complex biomass such as SCB and highlights the important role of adjusting the culture medium composition with SCB for secretion of enzymes specific for its saccharification.

**Principal Investigator:** Dr Syed Shams Yazdani

**PI Affiliation:** ICGEB

**Sample Preparation:** To prepare the samples for proteomic studies, secretome aliquots containing 50 µg of total protein were precipitated following the method described by Crowell et al. The precipitated protein was then vacuum-dried, reconstituted in 150 µL of resuspension buffer containing 8 M urea, 7.5 mM NaCl, 50 mM Tri-ethylammonium bicarbonate (TEAB)pH 8.2, 10 mM dithiothreitol (DTT), and incubated at 56 °C for 45

min as described by Ogunmolu et al. This was followed by the addition of 20 ?Lof 200 mM iodoacetamide prepared in 50 mM TEAB and incubated at 25 °C in the dark for 60 min. DTT was then added to a final concentration of 10 mM to consume any unreacted iodoacetamide and the reaction mixture was further incubated for additional 60 min in the dark. After incubation, the reaction mixture was diluted five-fold in 50 mM TEAB, pH 7.6, followed by the addition of CaCl2 to a final concentration of 1 mM.

**Peptide Separation:** Samples were then digested using sequencing grade trypsin (1 µg per 50 µg of total protein;Pierce Biotechnology, USA) overnight at 37 °C. The enzymatic digestion was terminated by the addition of formic acid till the pH was in the range of 3-4. The tryptic peptides were desalted using C18 spin columns (Thermo Fisher Scientific). Eluted samples were vacuum-dried and reconstituted in 0.1% (v/v) formic acid before being subjected to LC-MS/MS.

**Protein Characterization:** LC-MS/MS analysis was performed using Orbitrap Fusion Lumos Tribrid Mass Spectrometer equipped with nano-LC Easy nLC 1200 (Thermo Fischer Scientific, Singapore). Liquid chromatography separation was performed with a flow rate of 300 nl/ml on a C18 pre-column (Acclaim PepMapTM100, 75um X 2 cm, nanoViper, P/N 164946, Thermofisher Scientific Incorporation) followed by analytical column (Acclaim PepMapTMRSLC C18, 75um X 50 cm, 2um, 100 Å, P/N ES803). The peptides were separated using a gradient of 2% solvent B to 10% in 5 min followed by gradient increase to 45% and sharp increase to 95%, then retention of 95% for 10 min. Solvent A was aqueous solution in 0.1% formic acid, and solvent B was 95% acetonitrile in 0.1% formic acid. The eluted peptides were injected into the mass spectrometer and the MS1 data were acquired in full scan mode at 120000 orbitrap resolution with mass range from 375-2000 Da. Data was acquired using the Thermo Xcalibur software setup version 4.3.73.11 (Thermo Fischer Scientific, Inc 2019). Precursors were allowed to fragment using Higher-energy C-trap dissociation (HCD) in ion trap (IT) detector with collision energy of 28 in a data dependent MSnScan acquisition. Charge state screening of precursor ions and monoisotopic precursor selection was enabled. The parent ions once fragmented were excluded for 40 sec with exclusion mass width of +/- 10ppm. The lock mass option (polydimethylcyclosiloxane; m/z 445.120025) enabled accurate mass measurement in the MS Mode. For data analysis, raw LC-MS/MS data files obtained from the mass spectrometer were processed with Proteome DiscovererTM (Version 2.4.1.15, Thermo FisherTM Scientific Inc). The Proteome Discoverer processing workflow was employed in the label free quantitation (LFQ) of relative protein abundance across the samples and controls. For the search process, Mascot and Sequest HT tools were used. Peak lists obtained from MS/MS spectra were identified using the MSF files. Protein identification was conducted against a concatenated target/decoy version of the in-house predicted proteins or in-house database (11213 target sequences) obtained from the draft genome sequence of Penicillium funiculosum along with Proteome Discoverercontaminant database. The identification settings were as follows trypsin digestion with maximum of 2 missed cleavages; minimum peptide length 6; precursor tolerance 10ppm; mass tolerance 0.6 Da; fixed modifications; carbamidomethyl c

(+57.021464 Da), variable modifications; oxidation of m (+15.994915 Da), acetylation of protein n-term (+42.010565 Da). Peptides and proteins inferred from the spectrum results using SwissProt and Uniprot database. Peptide Spectrum Matches (PSM's), peptides and proteins were validated at a target False Discovery Rate (FDR) strict to 0.01 and relaxed to 0.05. The relative protein abundance estimation including normalization, hierarchical cluster analysis, scatter plots were performed using Proteome Discover LFQ workflow.Venn representations of protein were done using http://bioinformatics.psb.ugent.be/webtools/Venn/. Function assignment and annotation by gene ontology terms (GO; www.geneontology.org), InterPro terms (InterProScan, EBI) and enzyme classification codes (EC) were determined using the Blast2GO suite(16). Annotations were made with default parameters and were augmented by using the Annotation Expander (ANNEX) and by the addition of the GO terms associated with functional domains derived from scanning the InterPro database. Carbohydrate and auxiliary-active enzyme families were assigned using the CAZy database - http://www.cazy.org. Normalized protein abundance values of all the secretomes containing SCB (5 g/L – 45 g/L) were compared to the secretome without SCB by ANOVA ($p < 0.05$) and student's t test ($p < 0.05$).

**Experiment Type:** Top-down

**Species:** Data in species_details  No Data

**Tissue:** Unknown  No Data

**Cell Type:** Unknown  No Data

**Disease:** Unknown  No Data

**Instrument Details:** Orbitrap Fusion Lumos (MS:1002732)  Data in instrument_details

**Protein Modifications:** No PTMs

**PubMed ID:** 34446097