IPD Project Details

Project ID: IPD2854

Project Title: Intelligent versus Blind response”- Unveiling a Hypercellulolytic Fungus Strategy for Efficient Polymeric Carbon Deconstruction via Multiplexed Quantitative Proteomics

Description: Fungi are ubiquitous and are often confronted with the need to secure utilisable carbon from their external growth milieu through the use of extracellular proteins to scavenge for carbon from a vast array of complex polymeric carbon sources. This attribute is conserved across evolution in fungi. To understand how filamentous fungi extracellular proteins are modulated in response to the presence polymeric carbons in the environment, we have typed the array of the main extracellular proteins involved and their dynamics using a known hypercellulolytic fungus â€“ Penicillium funiculosum (NCIM 1228), through multiplexed quantitative proteomics

Principal Investigator: Dr Syed Shams Yazdani

PI Affiliation: International Centre for Genetic Engineering and Biotechnology Aruna Asaf Ali Marg 110 067 New Delhi, India

Sample Preparation: Penicillium funiculosum NCIM 1228 previously identified as a hypercellulolytic strain was maintained on potato dextrose agar plates. Conidial suspensions were prepared by growing P. funiculosum NCIM 1228 potato dextrose agar for seven days at 30 oC. Spores were collected from lawns of fungi culture in sterile distilled water, filtered through a glass wool plug to remove hyphal fragments and counted on a hemocytometer. To culture in liquid suspensions for enzyme production, conidia at 106 spores.mL-1 were inoculated in a base medium - KH2PO4 2.0 g.L-1; (NH4)2SO4 1.4 g.L-1; Urea 0.3 g.L-1 ; MgSO4.7H2O 0.3 g.L-1; FeSO4.7H2O 5.0 mg.L-1; MnSO4.H2O 1.6 mg.L-1 and ZnSO4.7H2O 1.4 mg.L-1, containing 2% carbon. Submerged culture experiments were carried out in 100 mL shake flasks containing 20 mL cultures in duplicates. The flasks were kept at 30 ?C for 3 and 6 days (corresponding to the early phase and late log phases of the fungus) respectively with orbital shaking at 150 rpm (Innova 44, Eppendorf AG – Germany). Induced cultures were centrifuged at 7,000 rpm for 10 min at 4 °C, and the supernatants were filtered using syringe filters with a 0.45-?m PVDF membrane (Millipore, Germany). The obtained secretome was concentrated using Vivaspin columns with a 5 kDa MWCO (GE Healthcare, USA).

Peptide Separation: Sequencing grade trypsin (2.5 ?L of 1 ?g/?L; Pierce Biotechnology, USA) was added, and the proteins were digested overnight at 37 °C.

Protein Characterization: The raw MS data files were processed by MaxQuant 1.5.2.8 (27) for peak detection and quantification. MS/MS spectra were searched against in-house predicted proteins (11213 target sequences) obtained from the draft genome sequence of Penicillium funiculosum NCIM 1228 (unpublished work), using the Andromeda search engine. Known contaminants were included for protein identification in MaxQuant. The identification settings were kept at default by setting the “type” parameter under the group-specific parameter to “reporter ion MS2” and selecting the TMT 6-plex option. Trypsin as a protease with two missed cleavage sites was allowed, carbamidomethyl cysteine was specified as a fixed modification, and oxidation of methionine was specified as variable modifications. The instrument-specific parameter was kept at the software default for Orbitrap machine (a peptide precursor mass tolerance of 20 ppm and a fragment mass tolerance of 4.5 ppm). Match-between-runs was allowed using default parameters. The decoy database search option was enabled, and Peptide-Spectrum Matches (PSMs), peptides and proteins were validated at a 1.0% False Discovery Rate (FDR). The relative protein abundance estimation including normalisation, hierarchical cluster analysis (Euclidian distance), scatter plots were performed in Perseus version 1.5.3.2 available with MaxQuant. Protein identification and quantification were only accepted when corrected reporter ions were valid in technical replicates. The “reverse”, “only identified by site” and “common contaminant” hits were removed from the MaxQuant output using Perseus filters. The Box and Whisker plots were made in GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, http://www.graphpad.com. Venn representations of protein was 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. 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://ww.cazy.org.

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: TMT6plex-126 reporter+balance reagent acylated residue, monohydroxylated residue, iodoacetamide derivatized residue

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