IPD Project Details

Project ID: IPD6285

Project Title: Temporal proteome profiling of Botrytis cinerea reveals proteins involved in plant invasion and survival

Description: Botrytis cinerea is a necrotrophic fungal pathogen that poses a significant threat to many crops. Understanding the proteome dynamics of phytopathogens during infection can help combat plant diseases. However, most proteomics studies in phytopathogens face interference from abundant host proteins. Here, we optimized a solid media that better mimics in-planta conditions and used it to perform the temporal protein dynamics in Botrytis cinerea. An agar media with 20% tomato fruit extract and 2% deproteinised leaf extract was utilized for label-free quantitative proteomics at 12, 36, 72 and 120 hpi. Out of 3244 quantified proteins, 2045 showed differential regulation. Glycosyl hydrolases, pectin esterases, stress protein DDR48, RhoGEF and essential transcription factors were found to be upregulated during the early phase, highlighting their role in fungal virulence. Meanwhile, pathways such as macromolecule synthesis, purine, and carbohydrate metabolism were upregulated in the late-growth phase. Overall, the study provides a comprehensive understanding of proteome dynamics during Botrytis infection.

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Sample Preparation: Protein extraction from the samples was done using TCA-acetone method as described by Fernández R.G. et al (2014) with minor modifications. The extracted proteins were quantified using BCA assay (Sigma, Germany) and run on SDS-PAGE. The total protein extracts were subjected to trypsin digestion and subsequent LC-MS/MS analysis.

Peptide Separation: A total of 50 µg of protein extract from each time point was first reduced with five mM TCEP and alkylated with 50 mM iodoacetamide and then digested with Trypsin (1:50) for 16 h at 37 °C. Digests were cleaned using a C18 silica cartridge and dried using a speed vac. The dried peptides were resuspended in buffer A (2% acetonitrile, 0.1% formic acid).

Protein Characterization: The peptide mixtures were subjected to label-free quantitative proteomics analysis on an Easy-nlc-1000 system coupled with an Orbitrap Exploris mass spectrometer. From each time point, 1µg of peptide mixture was loaded on a 15 cm long C18 column, 3.0?m Acclaim PepMap (Thermo Fisher Scientific) and separated with a 0–40% gradient of buffer B (80% acetonitrile, 0.1% formic acid) at a flow rate of 300 nl/min) over a period of 60 minutes. The MS1 spectra were acquired in the Orbitrap in profile peak mode at a resolution of 60000 in the mass range of 375-1500 Da with maximum injection time of 25ms and AGC target set to 300%. Dynamic exclusion was employed for 30s excluding all charge states for a given precursor. Top 12 precursors were subjected for MS2 fragmentation using HCD at a resoltuion of 15000 with maximum injection time of 22 ms and AGC target of 200%

Experiment Type: Bottom-up

Species: Botrytis cinerea-40559

Tissue: Fungus (bto:0001494)

Cell Type: Fungal spore (cl:0002369)

Disease: Other

Instrument Details: Orbitrap Exploris 120 (MS:1003095) Array

Protein Modifications:

PubMed ID: