IPD Project Details

Project ID: IPD5484

Project Title: “Trading virulence forsurvival”:Metabolic pathway switch is a key mechanism for survival of Macrophomina phaseolina upon Burkholderia contaminans challenge

Description: Macrophominaphaseolina is attributed to infect a wide range of plants. There are not a successful strategies to eradicate the fungus from the soil. In this study, a jute endophytic bacteria, Burkholderiacontaminans has been found to have a promising effect in controlling M. phaseolinain in vitro culture. Using the iTRAQ LC-MS/MS method for quantitative proteomics study, a comparative analysis of the whole proteome of M.phaseolinaunder both Burkholderiachallenged and unchallenged conditions were made and analyzed. A tough battle appears to ensue between the fungus and the bacterium, where Burkholderia manages to arrest the growth of the fungus and decrease its pathogenicity but, the latter apparently survives under ‘hibernating’ conditions by up-regulating its energy metabolism.

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Sample Preparation: Macrophomina cells was cultured with Burkholderia. The fungal mycelia were collected after 2 days of bacterial contact. Pure and co-cultured fungal mycelia were filtered and washed Fungal cell sample were homogenized in RIPA buffer containing 1 x concentration of protease inhibitor cocktail. Total soluble protein was precipitated with 6 volumes of (vol/vol) ice-cold acetone overnight at 4°C. Semi dried pellet was dissolved using 8 M urea (Sigma-Aldrich). Total protein concentration was determined.

Peptide Separation: A total of 100 µg of proteins from each sample were used for trypsin digestion followed by iTRAQ peptide labeling. Peptides from control and stressed samples were labelled with iTRAQ Except the iTRAQ labeling, similar digestion protocol was followed to prepare fungal protein samples for total protein identification. Labeled peptides were subjected for high-pH reverse-phase fractionation. For LC-MS/MS data was acquired using 5600 TripleTOF+ (ABSciex, Concord, Canada). The instrument was coupled with an Eksigent NanoLC-2DPlus system (Eksigent, Dublin, CA, USA), and the

samples were loaded at a flow rate of 2 µl/ min for 10 min and eluted from the analytical column at a flow rate of 300 nl/min in a linear gradient of 5% solvent B to 35% solvent B in 60 min. Solvent A being composed of 0.1% (v/v) formic acid in water and solvent B comprising 95% (v/v) acetonitrile with 0.1% (v/v) formic acid. The TripleTOF 5600 system was run on an information dependent acquisition (IDA) mode with a TOF/MS survey scan (350–1250 m/z) where the accumulation time was 250 ms. For fragmentation, maximum of 10 precursor ions per cycle were selected, with a total cycle time of roughly 2.3 seconds and each MS/MS spectrum was collected for 100 ms (100–1500 m/z). The parent ions with a charge state from +2 to +5 were included for the MS/MS fragmentation. The threshold precursor ion intensity was set at more than 120 cps and was not present on the dynamic exclusion list. After fragmentation of an ion by MS/MS, its mass and isotopes were excluded for 10 seconds. The MS/MS spectra were operated in high sensitivity mode with ‘adjust collision energy when using iTRAQ reagent’ settings.

Protein Characterization: All the. wiff files containing MS and MS/MS spectra generated from Triple TOF 5600 were submitted for database searching and quantitative analysis using the ProteinPilot™ V 4.5 software (ABSciex, Concord, Canada). ProteinPilot search engine was used for iTRAQ based quantitation in a data dependent mode. This search engine uses a sequence tag method plus protein database searching. Each MS/MS spectrum was searched against Macrophomina species from Uniprot/swissprot database (November 28, 2012; 14056 entries). The searching parameters were set as iTRAQ peptide label, cysteine alkylation with methyl methanethiosulfonate, trypsin digestion and identification focus for biological modifications. The resulting data set was auto bias-corrected to normalize any variations arising from unequal mixing of the differently labeled samples. False discovery rate (FDR) was estimated using a target-decoy based strategy. The proteins and peptides were filtered with 1% global protein level FDR. For quantitation the ratio threshold was set to >1.3 (equivalent to 95% confidence or better) and p-value <0.05 to ensure that quantitation was based on at least two unique peptides. Proteins were considered only if they were significant in all independent biological and technical triplicate experiments. The average values of replicates were used to indicate the final protein abundance at a given time point. A statistical analysis to compare the groups was performed using t test (Sigma Stat, Jandel Scientific, USA). For functional annotation and cellular location, the protein lists were analyzed according to the Blast2GO tool (https://www.blast2go.com/), Kyoto Encyclopedia of Genes and Genomes (KEGG). Predicted interacting partners were analyzed using STRING v10 database.

Experiment Type: Shotgun proteomics

Species: Macrophomina phaseolina

Tissue: Fungus (bto:0001494)

Cell Type: Fungus

Disease: Unknown

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: iTRAQ4plex-116 reporter+balance reagent acylated residue

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