IPD Project Details

Project ID: IPD3211

Project Title: A Data-Independent-Acquisition-based proteomic approach towards understanding the acclimation strategy of Microchloropsis gaditana CCMP526 in hypersaline conditions

Description: Salinity is one of the significant factors that affect growth and cellular metabolism, including photosynthesis and lipid accumulation, in microalgae and higher plants. Microchloropsis gaditana CCMP526 can acclimatize to different salinity levels by accumulating compatible solutes, carbohydrates, and lipid as an energy storage molecule. We used proteomics to understand the molecular basis for acclimation of M. gaditana to increased salinity levels (55 and 100 PSU). Correspondence analysis (CA) was used for identification of salinity-responsive proteins (SRPs). The highest number of altered proteins was observed in 100 PSU. Gene Ontology (GO) enrichment analysis revealed a separate path of acclimation for cells exposed to 55 and 100 PSU. Osmolyte and lipid biosynthesis was up-regulated in high saline conditions. However, concomitantly lipid oxidation pathways were also up-regulated at high saline conditions, providing acetyl-CoA for energy metabolism through the TCA cycle. Carbon fixation and photosynthesis were tightly regulated, while chlorophyll biosynthesis was affected under high salinity conditions. Importantly, temporal proteome analysis of salinity-challenged M. gaditana revealed vital salinity-responsive proteins which could be used for strain engineering for improved salinity resistance.

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Sample Preparation: Protein was isolated using a Bioline II DNA/RNA/Protein extraction kit (Bioline, Australia) following the manufacturer’s protocol. The isolated protein was quantified using the absorbance at 280 nm (Thermo Scientific, Australia). The samples were stored in -80°C until further processing. The pH of the sample was checked using a pH strip before processing. The optimum for reduction, alkylation, and trypsinization should be pH 8. Approximately 50 mg of protein was denatured using TCEP (Thermo Scientific, Cat. #77720) to a final concentration of 10 mM. T The solution was then incubated at 50-65°C for 30 min. Chloroacetamide (CAA) was added to a final concentration of 40 mM to alkylate the reduced protein. The mixture was incubated at room temperature in the dark for 20 min.

Peptide Separation: Trypsin at a dilution of 1:100 was added and incubated overnight at 37°C with shaking. The reaction was stopped by adding 1% formic acid (FA). The pH of the solution should be ~3. The samples were then desalted using Ziptips (Agilent, OMIX-Mini Bed 96 C18, A57003MBK). The residual acetonitrile (ACN) was removed using a vacuum concentrator. The sample was dissolved into 20 ml of 0.1% FA and sonicated in a water bath for 10 min. Any insoluble precipitates were removed by centrifuging at high speed for 5 min. iRT (internal retention time reference) peptides were added before transferring the sample into an MS vial. Total of 45 samples (3 conditions at 5 time points and triplicates for each) were processed.

Protein Characterization: Acquired DDA .raw files were searched against the N. gaditana UniProtKB/SwissProt database (v2017_07) using Byonic (Protein Metrics) embedded into Proteome Discoverer (Thermo Scientific) to obtain peptide sequence information. Only peptides identified at a false discovery rate (FDR) of 1% based on a decoy database were considered for further analysis. Spectronaut Orion (Biognosys) was used to create the corresponding spectral library as well as to evaluate all DIA data using in-house parameters. To correct for differences in sample density and loading, the peak areas for each peptide were normalized based on the assumption that on average, a similar number of peptides are up- and down-regulated. Two-sided t-tests were used to calculate p-values athe nd FDR for each time point (0, 1, 6, 24 and 72 h) and salinity level (55 and 100 PSU) against control (38 PSU), based on multiple hypotheses testing corrections by Benjamini-Hochberg method (implemented in R). GO terms were enriched using a Fisher exact test with p-value <0.05.

Experiment Type: SWATH MS

Species: Data in species_details No Data

Tissue: Unknown No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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