IPD Project Details

Project ID: IPD6660

Project Title: Elevated carbon dioxide levels lead to proteome-wide alterations for optimal growth of a fast-growing cyanobacterium, Synechococcus elongatus PCC 11801

Description: Biological CO2 mitigation by photosynthetic microorganisms has emerged as a promising approach for generating biomass-based energy during the course of CO2 fixation. Additionally, cyanobacteria-based biofuels have also garnered immense attention lately because of the depleting fossil fuel reserves and the growing energy demands. Synechococcus elongatus PCC 11801, a fast-growing, high CO2 tolerant, novel isolate of cyanobacteria, is an interesting candidate for metabolic engineering applications. Since under laboratory conditions, it exhibited a high level of tolerance to different environmental stresses (CO2, light, temperature, salts and butanol), it seemed like an encouraging prospect for the production of fuels and other industrially relevant chemicals. This is the first-ever functional global proteomics investigation of Synechococcus elongatus PCC 11801 isolate grown at elevated CO2 levels using high-throughput iTRAQ approach. Three independent biological replicates were analyzed and a total of 861 proteins were identified, out of which 492 proteins were found to be present in all the three replicates. Of these, 248 proteins showing the same trend across the three replicates (?1.5 fold up-regulation or ?0.66 fold down-regulation) were chosen for pathway analysis. The metabolic responses were marked with a down-regulation of inorganic carbon transporters alongside an induction of nitrogen transport and absorption proteins in order to uphold the apposite carbon-nitrogen equilibrium. Further acclimation progression exposed an increased expression of proteins taking part in photosynthesis and generation of light harvesting pigments such as chlorophyll. Similarly, a downshift of proteins involved in photoprotection and defense against ROS (reactive oxygen species) was observed. Another principal discovery was the perturbation in expression of proteins belonging to central metabolic pathways like glycolysis, TCA cycle, pentose phosphate pathway as a coping mechanism to high CO2 stress. Further, validation studies were carried out using MRM assays and western blotting of key altered proteins.

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Sample Preparation: The bacterial culture was grown in a CO2 incubator shaker at different CO2 concentrations (0.04%, 0.5%, 1% and 10% CO2). Three independent biological replicates for each CO2 condition were taken for this study. The cells were harvested by centrifugation and the bacterial pellets were stored at -80°C till use. Cell lysis was carried by subjecting the pellet to several freeze-thaw cycles followed by sonication in a buffer containing 8 M Urea and 50 mM ammonium bicarbonate. The supernatant was collected after centrifugation and was quantified using QuickStart Bradford reagent (Bio-Rad, USA) for further proteomics experiments.

Peptide Separation: In-solution protein digestion using trypsin enzyme (Trypsin Gold, Mass spectrometry grade, Promega, Madison, WI, USA) was performed followed by iTRAQ labeling of digested peptides as per the manufacturer's instructions (AB Sciex, USA). The following labeling strategy was followed throughout; 114- 0.04% CO2, 115-0.5% CO2, 116-1% CO2 and 117-10% CO2. The labeled peptides were fractionated using Agilent 3100 OFFGEL fractionator and further sample clean-up was performed using zip-tips having C-18 columns. These zip-tipped fractions were reconstituted in 0.1% formic acid and analyzed on a Triple-TOF 5600 instrument in a positive ion mode. The MS and MS/MS spectra were acquired in the range of 350-1250 and 100-1800 m/z, respectively. Additionally, the instrument was operated in DDA (data-dependent acquisition) mode wherein a maximum of 20 precursors per cycle were chosen for fragmentation from each MS spectrum.

Protein Characterization: The raw .wiff files obtained from Triple-TOF 5600 instrument were analyzed using the ProteinPilot software (5.0.1, Applied Biosystems Sciex). For protein identification UniProt fasta file of a close homolog, Synechococcus elongatus PCC 7942, was taken and the ParagonTM search algorithm was used. The sample type chosen was iTRAQ 4plex (peptide labeled) and Triple TOF 5600 was chosen as the instrument. Further, MMTS and Trypsin was chosen for cysteine alkylation and enzyme, respectively. The search was carried out with a Thorough effort and the detected protein threshold confidence was set at 99%.

Experiment Type: Shotgun proteomics

Species: Synechococcus elongatus -32046

Tissue:

Cell Type: Photosynthetic cell (cl:0000628)

Disease: Unknown

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: No PTMs

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