IPD Project Details

Project ID: IPD4039

Project Title: Chlorella sp. FC2 IITG temporal nitrogen-starvation LC-MSMS

Description: Proteomics study in non-model alga Chlorella FC2 IITG (FC2) challenged with nitrogen starvation for 120 h. Three different nitrogen starvation time-points 40 h, 88 h and 120 h was compared with 0 h (nitrogen sufficient condition). Comparative proteomics analysis was performed using 4-plex isobaric tags for relative and absolute quantification (iTRAQ) labelling strategy, followed by chromatographic separation and final generation of tandem mass spectrometry (MS/MS) spectra of tryptic digested peptides on Q-TOF mass spectrometer.

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Sample Preparation: FC2 samples were cultured under photoautotrophic condition in a 5.0 L automated bioreactor (Biostat B plus, Sartorius, Gettingen, Germany) containing 4.0 L of BG11 medium (slightly modified). The reactor conditions were agitator speed of 400 rpm, aeration 1 vvm with 1% (v/v) CO2, light intensity 250 µE m-2 s-1 with a light: dark cycle of 16:8 h, temperature 28°C and pH 7.4. FC2 cells Samples were collected at regular intervals to assess growth dynamics, substrate utilization profiles and biochemical composition of the cells. Two-stage cultivation strategy was employed: In the first stage, FC2 cells were grown under photoautotrophic condition in the nutrient sufficient BG11 medium to obtain biomass concentration of 3 g L-1 (equivalent to absorbance 13.0 at 690 nm). The nutrient sufficient condition was maintained by intermittent feeding of the necessary limiting nutrients when their concentration reduces more than 10% from their optimal concentrations. Once the desired absorbance was reached, FC2 cells were harvested aseptically via centrifugation at 8,000 g for 10 minutes at 4°C and washed with BG11 media devoid of urea. FC2 cells were then re-suspended in BG11 media devoid of urea to grow cell under N deprived condition. All the other cultivation parameters were kept constant. Total protein was extracted from FC2 cells grown for 40 h, 88 h and 120 h following N-starvation and 0 h (control) using TRIzol reagent following standard protocol with slight modification. Briefly algal pellet was incubated in PBS (pH 7.4) containing lysozyme (1 mg mL-1) and protease inhibitor cocktail at ambient temperature for 3 h, followed by sonication for 10 min (30 s ON and

30 s OFF cycle) and centrifuged at 12,000 ×g for 15 min. The supernatant was mixed with equal volume of TRIzol reagent and processed to obtain the protein precipitates in acetone. Protein pellets were dissolved in rehydration buffer containing 8 M urea, 2 M thiourea, 2-4% CHAPS, 40 mM 13 DTT and 0.002% bromophenol blue. Algal proteins contain several secondary metabolites, salt and pigments, so 2DE clean-up was performed using the commercially available kit from GE health care. Protein in rehydration buffer was exchanged with 0.5 M TEAM buffer (compatible for iTRAQ labelling) using Amicon Ultra 0.5 mL centrifugal 3 kDa filters (Millipore, Watford, UK). Following buffer-exchange protein concentrations were determined using QuickStart Bradford reagent (BioRad, USA) and quality was checked on a 12% SDS gel.

Peptide Separation: In-solution digestion of respective protein samples (100 ?g each); control (0 h) and treatments (40 h, 88 h and 120 h) were performed using Trypsin (Trypsin Gold, mass spectrometry grade, Promega, Madison, WI, USA) at a ratio 1: 30 (trypsin: protein) ratio, following the manufacturer's instructions. Four-plex iTRAQ labelling kit (AB Sciex UK Limited, UK) having labels 114, 115, 116 and 117 was used to label trypsin-digested peptides of 0 h, 40 h, 88 h and 120 h FC2 samples respectively, following the manufacturer's instructions. All the labelled peptides were pooled and proceeded for OFFGEL fractionation using a 3100 OFFGEL Fractionator (Agilent Technologies, Santa Clara, CA) with high resolution (pH 3–10, 24 cm) IPG strips. Fractions were collected and enriched using Zip-Tip C18 pipette tips (Millipore, USA). Peptides bound with the C18 tips were eluted with acetonitrile and then dried completely using speed vac. Samples were reconstituted in 15?l of 0.1% formic acid. These samples were then subjected to MS analysis. Identified MS/MS peaks were processed using flex analysis version 3.4 (software). Spectrum mill 258 search engine was used for protein identification. The data analysis was carried out with 1% FDR and 50 SPI.

Protein Characterization: MS and MS/MS run of OFFGEL fractionated labelled peptide samples were performed following the above mentioned protocol. Chip-Cube controlled by the Mass hunter acquisition software was set to perform data acquisition in a positive ion mode. MS was scanned from 300-3000 and MS/MS from 50–3000. The instrument was operated in a data-dependent manner using Auto MS/MS, selecting max 15 precursors with intensity over 1000 for each cycle. MS/MS was done with a gas pressure of 2 X 10-2 bar in the collision cell. Data files (in .d format) were processed by Spectrum Mill Protein Identification software (Agilent Technologies, USA). The Paragon algorithm was used as the default method for search with trypsin as a digesting agent with up to two allowed miss cleavages. Protein identification was executed against the uniprot_Chlorella sp. (dated 30th April 2016; containing 9831 sequence for C. variabilis and 7001 sequence for C. protothecoides). Data was extracted between MH+ 600 and 4000, precursor mass tolerance 20 ppm and fragment mass error tolerance 50 ppm. Only peptides identified with confidence interval (C.I.) values above 95% were used for protein identification and quantification. The iTRAQ report peak areas (RPAs) corresponding to quantification ions m/z 114-117 were extracted from the raw spectra and corrected for isotopic carryover using GPS Explorer. A decoy database search was

used for calculation of the false discovery rate (FDR) and a cut-off of 1% was used to report identifications. Pathway reconstruction of the differentially regulated FC2 proteins ($p<0.05$) identified during the progression of N starvation period was achieved using the KEGG PATHWAY tool available from the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/tool/map_pathway2.html). The list of UniProt Accession IDs were uploaded and matched against reference Chlorella variabilis to map the proteins in the metabolic pathway based on their functional annotation. Mev software was used for the generation of heat-map.

Experiment Type: Shotgun proteomics

Species: Chlorella sp. FC2 IITG -121807

Tissue:

Cell Type: Photosynthetic cell (cl:0000628)

Disease: Unknown

Instrument Details: Agilent instrument model (MS:1000490)

Protein Modifications: iodoacetamide derivatized residue

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