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Lipidomic profiling reveals lipid regulation in the snow alga *Chlamydomonas nivalis* in response to nitrate or phosphate deprivation

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ABSTRACT

The snow alga *Chlamydomonas nivalis* in the exponential phase was subjected to nitrate or phosphate deprivation for 0–72 h to study its stress responses in lipid profiles analyzed by UPLC/Q-TOF-MS (ultra performance liquid chromatography/quadrupole-time of flight-mass spectrometry). Three clusters were distinguished as the control, nitrate-deprived and phosphate-deprived groups in OPLS-DA (orthogonal projection on latent structure discriminant analysis) score plots based on their lipidome data. Altogether, the lipidomic approach identified twenty-two ions in nitrate-deprived group including nine l, 2-diacylglyceryl-3-O-4′-(N, N, N-trimethyl)-homoserine (DGTSs), one phosphatidylethanolamine (PE), two monogalactosyldiacylglycerols (MGDGs), four digalactosyldiacylglycerols (DGDGs), three phosphatidylglycerols (PGs), two sulfoquinovosyl-diacylglycerols (SQDGs) and one phosphatidylinositiol (PI), and nineteen ions in phosphate-deprived group including four DGTSs, one PE, one MGDG, seven DGDGs, three PGs, two SQDGs and one PG as "differentiating lipid biomarkers". Moreover, the common and specific biomarkers were found in the two nutrient deprived groups by SUS (shared and unique structure) plot. Biomarkers-based *z*-score plot and heat map further showed how lipid biomarker expressions deviate from the control. The up- or down-regulation of these lipid biomarkers provided new insights into the lipid metabolism of the snow alga in response to nitrate or phosphate deprivation stress condition.

1. Introduction

Chlamydomonas nivalis attracts attention because it is a potential bioresource with inherent capacity of adapting to extreme environments such as low temperature, poor nutrition, permanent freeze-thaw cycles and high irradiation [1]. This unicellular organism has a distinguished history as a model for investigating the mechanism of cell response and adaptation to environmental stress [2,3]. Nutrient depletion is common in harsh habitats that the algae must adapt to survive [1,4]. Nitrogen and phosphorus availability have significant impacts and broad effects on algae because they represent a major constituent of numerous cellular compounds and involve in generating and transforming metabolic energy [5,6]. The responses of *Chlamydomonas* to nutrient-limitation might include the biosynthesis of alternative transport systems that facilitated efficient import of the limiting nutrient into the cell and the induction of hydrolytic enzymes that enabled cells to access alternative sources of the limiting nutrient [7]. However, the other acclimation and regulatory responses such as the metabolic changes, the mechanisms by which the changes are regulated, and the signaling system enabling the algae to perceive the environmental nutrient status are not well understood.

The focus of this work was to study the lipidome change in response to nutrient deprivation using *C. nivalis* as a model organism. Lipids play a pivotal role in transcriptional and translational control, signal transduction, cell–cell interactions, and environmental response [8]. Variations of lipid class composition in algae caused by culture nutrient status of the medium have been reported [9,10]. However, the strategy regarding how the functional lipids change at the lipidomic level in algae in response to the nutrient stress conditions have not been studied. Lipidomics,

Abbreviations: DGDG, digalactosyl-diacylglycerol; DGTS, l, 2-diacylglyceryl-3-O-4′-(N, N, N-trimethyl)-homoserine; ESI, electrospray ionization; MGDG, monogalactosyl-diacylglycerol; OPLS-DA, orthogonal projection on latent structure discriminant analysis; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositiol; PUFA, polyunsaturated fatty acids; SQDG, sulfoquinovosyl-diacylglycerol; SUS, shared and unique structure; UPLC/Q-TOF-MS, ultra performance liquid chromatography/quadrupole-time of flight-mass spectrometry; VIP, very important in the projection.

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firstly defined in the literature in 2003 [11], is a new platform for biosystem-based study that allows unbiased representation and quantitative determination of the suite of lipids and that helps to understand lipids interacting function within a biological sample in a holistic context [12–15]. It has already been applied in the scientific fields, including pharmaceuticals, nutrition, and food-agriculture [16–19]. However, there are limited reports on the molecular mechanism at the lipidomic level for the identification of lipid biomarkers from microalgae in response to stress conditions except for our recent studies [20,21]. Ultra-performance liquid chromatography (UPLC) integrated with Q-TOF-MS have greatly improved the resolution, sensitivity and analytical speed in lipidomic/metabonomic research [22,23]. Data collection by LC-MS followed by a multivariate statistical data analysis is the most straightforward steps in a lipidomic/metabonomic study [24].

In the present work, the lipidome in *C. nivalis* cultured under nitrate or phosphate deprivation was investigated by coupling UPLC/Q-TOF-MS with multivariate statistical analysis (OPLS-DA) to reveal the effect of nutrient availability on the regulation of lipid biosynthesis. The potential biomarkers were selected and identified by elucidating their structures, and their biological functions related to acclimation to nutrient status of nitrate or phosphate deprivation are also discussed in this paper.

2. Materials and methods

2.1. Materials

The snow alga *Chlamydomonas nivalis* UTEX LB2824 was purchased from the Culture Collection of Algae at University of Texas at Austin (UTEX), USA. HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt Germany). Formic acid was purchased from Dikma (Lake Forest, CA, USA). BHT (butylated hydroxytoluene) was purchased from Sigma (St. Louis, MO, USA). Glycolipids (MGDG, DGDG and SQDG) and phospholipids (PI, PG and PE) standards were purchased from Lipid Products (Redhill, UK). The other chemicals and solvents were of analytical grade. Distilled water was purified in-house using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Cell culture and biomass harvest

The seed culture of the snow alga *C. nivalis* (UTEX LB2824) with original concentration of 5×10^5 cells/mL started to grow in 100-mL Bold 1NV medium (UTEX formula) in 250-mL shake flasks under continuous illumination of 2000 ± 200 lx at $20 \pm 0.5\,^{\circ}C$ for 6 days to reach the late log-phase (1.6×10^6 cells/mL). Then the cells were harvested by centrifugation and re-suspended in Bold 1NV medium deprived of nitrate or phosphate, followed by growth for 6, 12, 24, 48, and 72 h, respectively. To monitor cell growth, cell number was counted with a haemocytometer under a microscope. Biomass was harvested by centrifugation and washed twice by distilled water prior to freeze drying. The medium was autoclaved at 121 °C for 30 min before use.

2.3. Extraction, liquid chromatographic separation and mass spectrometry of total lipids

The methods for extraction, liquid chromatographic separation and mass spectrometry of total lipids used in this study were the same as described previously [20]. In summary, $CHCl_3$:CH_3OH:H_2O (1:1:0.5, v/v/v) containing 0.025% BHT was used to extract total lipids from dried algal biomass. Then, lipid separation was performed by optimal elution program in UPLC and lipid detection by Q-TOF Micromass Spectrometer.

2.4. Data analysis

The original data from both ESI positive and negative modes were acquired, extracted and processed using MassLynx 4.1 data processing system (Waters, Milford, MA, USA) as previously described [20]. The ion intensities for each peak detected were normalized, within each sample, to the sum of the peak intensities in that sample. The resulting normalized peak intensities were then normalized to the cell number and multiplied by 10,000. Then the normalized data were $\log_{10}$ transformed to establish a normal distribution across all the lipid metabolites within each sample [25].

2.5. Statistical analysis

The three-dimensional datasets (m/z, retention time, normalized ion intensity) from both ESI modes were exported for OPLS-DA analysis using SIMCA-P+ V12.0 software (Umetrics AB, Umea, Sweden). Pareto scaling (scaled to square root of standard deviation) was used in all models to avoid chemical noise and artifacts.

Potential biomarkers were selected according to VIP value, S-plot and column loading plot [21]. The procedures for structural elucidation of potential lipid biomarkers have been described in our recent study [20]. SUS plot was constructed to find the shared as well as the unique potential biomarkers between the nitrate- and phosphate-deprived groups.

The transformed data were used to generate *z*-score plots and heat-map [25]. The *z*-score of individual lipid metabolites was calculated based on a control mean and its standard deviation using the formula: *z*-score = [(treatment metabolite abundance − control mean)/standard deviation of control)]. Heat-map was displayed by MultiExperiment Viewer in hierarchical clustering based on Pearson's correlation as a distance metric.

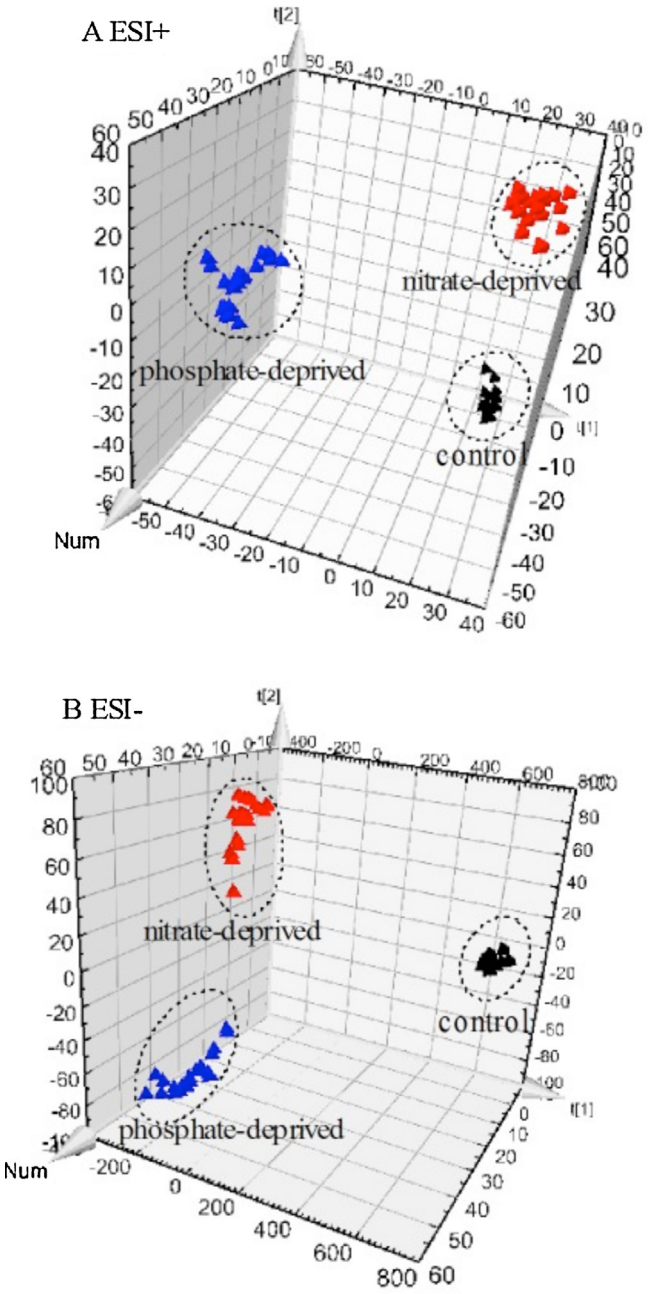

Fig. 1. Three-dimensional OPLS-DA score plot of the control, nitrate-deprived and phosphate-deprived groups in ESI positive-ion mode (A, mode performance parameters: $R^2X = 0.504$, $R^2Y = 0.987$, $Q^2 = 0.965$) and ESI negative-ion mode (B, mode performance parameters: $R^2X = 0.997$, $R^2Y = 0.978$, $Q^2 = 0.968$). ▲, the control group; ▲, nitrate-deprived group; ▲, phosphate-deprived group. The samples of each cluster are from the five different time treatments (6, 12, 24, 48, and 72 h).