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Nitrogen availability modulates CO_2 tolerance in a symbiotic chlorophyte

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ABSTRACT

We studied acclimation to nitrogen starvation, a very high (20%) CO_2 level, and combination thereof in a symbiotic chlorophyte *Desmodesmus* sp. 3Dp86E-1 to reveal potential mechanisms of its outstanding CO_2 tolerance. The changes in growth rate, photosynthetic activity, cell pigment (chlorophyll and carotenoid), and elemental N and C contents were followed with special attention to rearrangement of the cell ultrastructure in the cultures grown with sparging by air or 20% CO_2 in complete or N-free medium. Transcriptome of the microalga was screened for the presence of putative components of carbon concentrating mechanisms (CCM). The elevated CO_2 was beneficial for the microalga whereas N-starvation impaired the buffer capacity, growth, and photosynthetic performance of the culture and promoted the accumulation of C-rich reserve compounds, degradation of the photosynthetic apparatus and pyrenoid. These effects were exacerbated under 20% CO_2 in comparison with the air-grown cells. It is concluded that the exceptional CO_2 tolerance in *Desmodesmus* sp. 3Dp86E-1 might have been acquired during co-evolution with the animal host. This trait is obviously conveyed to the symbiotic alga by concerted and swift responses of different mechanisms (CCM, photosynthetic apparatus, biosynthesis of reserve compounds) maintaining the pH homeostasis and carbon fixation and sink balance in the cell.

1. Introduction

Contemporary phototrophs including microalgae are characterized by a low CO_2 affinity of Rubisco, the key enzyme of photosynthetic carbon fixation, probably inherited from their ancestors that lived in the CO_2-enriched atmosphere [1–4]. To cope with relatively low CO_2 levels (0.04%[1]) typical of current atmosphere, microalgae evolved sophisticated carbon concentrating mechanisms (CCM) increasing CO_2 concentration near the active site of Rubisco approximately by 10^3 [4]. Accordingly, the overwhelming majority of modern microalgae are adapted to the atmospheric CO_2 whereas high CO_2 levels are often deleterious for them [5]. Although species withstanding high CO_2 levels are relatively widespread, the organisms naturally capable of growth and efficient photosynthesis under very high CO_2 concentrations (>10%) are scarce. The phenomenon of high CO_2 tolerance has been vigorously studied for several decades in the context of the phytoplankton ecology and the environmental impact of technogenic CO_2 emission [1,6–8] but its understanding remains limited. Recently, the interest to the mechanisms of high CO_2 tolerance was re-fueled by potential application of microalgae for mitigation of the greenhouse effect and boosting microalgal culture productivity [9].

A new extremely CO_2-tolerant [10] *Desmodesmus* strain 3Dp86E-1 was isolated from the association with a hydroid from a subarctic sea [11,12] and partially characterized [10,13,14] in our laboratory. We hypothesized that this trait was acquired during co-evolution with the respiring animal host under long dark periods of polar nights [10] but the mechanistic insight into the high CO_2 tolerance of the symbiotic chlorophytes and knowledge of the oligotrophic condition impact on this trait are largely lacking. Interactive effects of nitrogen starvation and elevated CO_2 are of special interest since the former stress is known to modify considerably the efficiency of photosynthetic carbon fixation by and its allocation in microalgal cells enhancing the accumulation of carbon-rich reserve compounds e.g. carbohydrates and lipids [13,15,16]. Moreover, nitrogen deprivation is commonly used in biotechnology to foster the accumulation of the value-added compounds in cultivated microalgae [17–19]. At the same time, the effect of nitrogen limitation on the ability of microalgae to cope with very high CO_2 levels remains unknown. We propose the novel *Desmodesmus* sp. 3Dp86E-1 as a useful model organism to bridge, at least in part, this gap.

Abbreviations: C, carbon; CA, carbonic anhydrase; CCM, carbon concentrating mechanism; Chl, chlorophyll; Car, carotenoid; DIC, dissolved inorganic carbon; DW, dry weight; N, nitrogen; PS, photosystem; STM, stroma and thylakoid membranes.

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[1] Here and below, volumetric CO_2 percentages in the air or air–gas mixture used for the culture sparging are specified.

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Here, we report for the first time on the interactive effects of a very high (20%) CO_2 level and nitrogen deprivation on the growth, photosynthesis, and ultrastructure of a symbiotic microalga cultivated as monoculture under laboratory conditions. This is also the first, to the best of our knowledge, report on the presence of putative CCM components in a symbiotic chlorophyte. Basing on the findings obtained in this and our previous works, we tried to infer a comprehensive framework of the mechanisms conferring the studied symbiotic chlorophyte its outstanding CO_2 tolerance.

2. Materials and methods

2.1. Microalgae and cultivation conditions

Desmodesmus sp. strain 3Dp86E-1 isolated from the association with the hydroid *Dynamena pumila* as described by Gorelova et al. [20] and deposited to the microalgal culture collection of Timiryazev Institute of Plant Physiology (IPPAS) under ID IPPAS S-2014 served as the object for this work.

The pre-cultures grown in 0.75 L flasks in 0.3 L of BG-11 medium [21] at 40 μmol PAR photons·m^{-2}·s^{-1} and the atmospheric CO_2 level were kept at the exponential phase by daily dilution with the medium. At the beginning of each experiment, cells were harvested by centrifugation (1200 ×*g* for 5 min), washed twice in fresh BG-11 medium (or, in the experiments with N starvation, in N-free BG-11_0 medium [21]), and resuspended in the same medium without pH adjustment. For batch-cultivation experiments, the cultures were started at an initial chlorophyll (Chl) concentration and biomass content of 25 mg·L^{-1} and 0.4 g·L^{-1}, respectively.

The cells were grown in 1.5 L glass columns (6.6 cm internal diameter) in a temperature-controlled water bath at 27 °C and constant bubbling with air or 20% CO_2:80% air mixture prepared and delivered at a rate of 300 mL·min^{-1} (STP) using a PC-controlled gas mixer UFPGS-4 (Sovlab, Novosibirsk, Russia). Air passed through 0.22 μm bacterial filter (Merck-Millipore, Billerica, MA, USA) and pure (99.999%) CO_2 from cylinders were used. A continuous illumination of 480 μmol PAR photons·m^{-2}·s^{-1} by a white light-emitting diode source as measured with a LiCor 850 quantum sensor (LiCor, Lincoln NE, USA) in the center of an empty column was used. Culture pH was measured with a bench-top pH-meter (Hanna Instruments, Ann Arbor MI, USA).

Growth estimation was based on Chl and dry weight contents [22]. The residual nitrate and orthophosphate contents in the culture liquid were checked using ion-exchange HPLC [23].

Under the specified conditions, at least three independent experiments were carried out for each treatment repeated in duplicate columns. The average values ($n = 6$) and corresponding standard errors are shown unless stated otherwise.

2.2. Determination of C and N contents in biomass

Carbon (C) and nitrogen (N) contents in the biomass were determined using a Vario EL Cube CNS (carbon, nitrogen, sulfur) element analyzer (Elementar, Hanau, Germany) calibrated with a certified acetanilide standard (Elementar) according to the manufacturer's specifications [14].

2.2.1. Electron microscopy and micromorphometric analysis

The microalgae samples for TEM were fixed in 2% (w/v) glutaraldehyde solution in 0.1 M sodium cacodylate buffer at room temperature for 0.5 h and then post-fixed for 4 h in 1% (w/v) OsO_4 in the same buffer. The samples, after dehydration through graded ethanol series including anhydrous ethanol saturated with uranylacetate, were embedded in araldite. Ultrathin sections were made with an LKB-8800 (LKB, Sweden) ultratome, stained with lead citrate according to Reynolds [24] and examined under JEM-100B or JEM-1011 (JEOL, Tokyo, Japan) microscopes.

All quantitative morphometric analyses were done on sections through the cell equator or sub-equator; at least 10 samples from each treatment were examined. Frequency of the pyrenoid occurrence was calculated as total section number percentage of cell sections containing this structure. Linear sizes as well as subcellular structure area were measured on the TEM micrographs of the cell ultrathin sections ($n = 20$) using ImageJ software (NIH, Bethesda MA, USA). The significance of the difference of the means was tested by Student's *t*-test using Origin software (OriginLab, Northampton MA, USA). For the sake of clarity, the area of chloroplast excepting the starch grains and pyrenoid is referred to below as the 'stroma and thylakoid membranes' (STM).

2.3. Pigment and fatty acid extraction and analysis

Cells were pelleted by centrifugation, transferred to a glass-glass homogenizer with a chloroform-methanol (10 mL, 2:1, v/v) mixture and extracted to remove all pigment. The lipid fraction including the pigments was separated according to Folch et al. [25]. The chloroform phase was used for further pigment and lipid analysis. Chl *a* and *b* and total carotenoids (Car) were quantified using absorption coefficients for chloroform [26]. The Car profile was resolved with HPLC as described elsewhere [27]. The chloroform phase of the extract was also used for the GC/MS analysis of the cell lipid fatty acid composition [27].

2.4. Variable chlorophyll fluorescence measurements

The induction curves of Chl fluorescence were recorded and analyzed using a Fluorpen FP100s portable Pulse Amplitude Modulated fluorometer (Photon Systems Instruments, Drasov, Czech Republic) as described earlier [28,29].

2.5. mRNA isolation and cDNA library synthesis

To maximize the representation of different genes in the assembly, including those expressed under specific cultivation conditions, cells from all experimental variants were sampled for total RNA isolation and transcriptome sequencing. Total RNA was isolated using RNEasy kit (Qiagen, Hilden, Germany). Cells were disrupted in RLT buffer using a TissueLyser II homogenizer (Qiagen, Hilden, Germany). After extraction, RNA quality was checked using capillary electrophoresis on a Bioanalyzer 2100 (Agilent, Santa-Clara, USA). Prior to cDNA library construction, polyA–mRNA fraction was selected using oligo-dT magnetic beads (Illumina, San-Diego, CA, USA) and processed using a NextFlex Rapid Directional RNA-Seq Kit (Bioo Scientific, Austin, TX, USA).

2.6. Transcriptome sequencing, assembly, and screening

cDNA libraries were quantified using a Qubit 1.0 fluorometer (Invitrogen, Carlsbad, CA, USA) and quantitative PCR, diluted to 10 pM and sequenced using a HiSeq2000 instrument from both ends of the fragment (read length = 100 nt) with TruSeq v.3 sequencing chemistry (Illumina, San-Diego, CA, USA). Raw data were processed with CASAVA v. 1.8.2. Assembly was performed using CLC genomics Workbench with following parameters: word size = 64, bubble size = 50, minimum contig length = 300.

The contigs were annotated according to molecular function, biological process, and cellular component by Blast2GO (www.blast2go.com) v3.0 InterPro [30] scan with Nr and Pfam annotation [31] in Gene Ontology (GO) database (http://geneontology.org/), and at Kyoto Encyclopedia of Genes and Genomes Pathway database (KEGG, http://www.genome.jp/kegg/) Automatic Annotation Server.

To reveal protein structures evolutionary related to the putative sequences of the CCM-related proteins found at the previous step, the SWISS-MODEL template library (SMTL version 2015-09-09, PDB release 2015-09-04) [32] was searched with BLAST [33] and HHBlits [34].