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SHORT COMMUNICATION

Desmodesmus sp. 3Dp86E-1—a Novel Symbiotic Chlorophyte Capable of Growth on Pure CO_2

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Abstract A novel chlorophyte *Desmodesmus* sp. 3Dp86E-1 isolated from a White Sea hydroid *Dynamena pumila* was cultivated at CO_2 levels from atmospheric (the 'low-CO_2' conditions) to pure carbon dioxide (the 5, 20, and 100 % CO_2 conditions) under high (480 μE/(m^2 s) PAR) light. After 7 days of cultivation, the '100 % CO_2' (but not 5 or 20 % CO_2) cells possessed ca. four times higher chlorophyll content per dry weight (DW) unit than the low-CO_2 culture. The rate of CO_2 fixation under 100 % CO_2 comprised ca. 1.5 L/day per L culture volume. After a lag period which depended on the CO_2 level, biomass accumulation and volumetric fatty acid (FA) content of the *Desmodesmus* sp. 3Dp86E-1 bubbled with CO_2-enriched gas mixtures increased and was comparable to that of the culture continuously bubbled with air. Under the low-to-moderate CO_2 conditions, the FA percentage of the algal cells increased (to 40 % DW) whereas under high-CO_2 conditions, FA percentage did not exceed 15 % DW. A strong increase in oleate (18:1) proportion of total FA at the expense of linolenate (18:3) was recorded in the '100 % CO_2' cells. Electron microscopy and pulse–amplitude-modulated chlorophyll fluorescence investigation revealed no damage to or significant downregulation of the photosynthetic apparatus in '100 % CO_2' cells grown at the high-PAR irradiance. Possible mechanisms of high-CO_2 tolerance of *Desmodesmus* sp. 3Dp86E-1 are discussed in view of its symbiotic origin and possible application for CO_2 biomitigation.

Keywords Biomitigation · *Desmodesmus* · Extreme CO_2 · Fatty acids · Ultrastructure

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Introduction

Though microalgae are tolerant to extremely high (up to 100 %) levels of CO_2 and are known for a long time, the species of outstanding CO_2 tolerance are rarely encountered in nature (Seckbach et al. 1970; Miyachi et al. 2003) and represented mainly by thermoacidophile species from Rhodophyta or, even more scarcely, from Chlorophyta (Sakai et al. 1995). All the extremely CO_2-tolerant microalgae described so far are, as far as we know, free-living species.

The advent of the conception of CO_2 biomitigation with the use of microalgae reinvigorated the interest to CO_2-tolerant microalgae and mechanisms of CO_2 tolerance (Baba and Shiraiwa 2012; Wang et al. 2008). In the present work, we demonstrated that the chlorophyte *Desmodesmus* sp. 3Dp86E-1 (further referred to as *Desmodesmus* sp.) recently isolated from the marine hydroid *Dynamena pumila* (Gorelova et al. 2012) is tolerant to extremely high-CO_2 levels. Previously, we found that it is capable of growth at high-PAR irradiances and, under stressful conditions, accumulates considerable amounts of lipids (Solovchenko et al. 2013b). We believe that the findings present in this paper suggest that the *Desmodesmus* sp. is a promising candidate for application in biomitigation of CO_2 as well. To the best of our knowledge, this is the first report on high-CO_2 tolerance of symbiotic microalgae.

Materials and Methods

The strain 3Dp86E-1 was isolated from association with the hydroid *D. pumila* from Rugozerskaya Guba at Kandalaksha Bay of White Sea (66° 34′ N, 33° 08′ E) as described by Gorelova et al. (2009, 2012) and identified as *Desmodesmus* sp. (GenBank accession nos. JQ313132 and KJ463405) and deposited to the microalgal culture collection of Timiryazev

Institute of Plant Physiology, Moscow, Russia (IPPAS) under accession number IPPAS S-2014. The microalga was routinely cultivated on BG11 medium (Stanier et al. 1971) in glass columns (40 mm ID) at continuous illumination with 480 μE/(m^2 s) PAR (Solovchenko et al. 2013b) bubbled, at the rate of 300 mL/min, with filter-sterilized air (0.035 % CO_2; designated as 'low-CO_2' cells), 5 and 20 % ('moderate CO_2' cells) or pure CO_2 from a cylinder ('100 % CO_2' cells). Cultures were initiated at 0.8 g/L DW (25 mg/L chlorophyll, Chl). Samples were collected aseptically at 72-h intervals, and pH was measured with a Hanna HI 100 digital pH meter (Hanna, UK). Carbon content in the biomass was determined using an Vario EL Cube CNS (carbon, nitrogen, and sulfur) element analyzer (Elementar, Germany) calibrated with a certified acetanilide standard (Elementar, Germany). The rate of CO_2 fixation was calculated according to de Morais and Costa (2007). The maximum quantum yield of photosystem II (Fv/Fm) was measured using a Fluorpen FP100s portable pulse amplitude modulated (PAM) fluorometer (Photon Systems Instruments, Drasov, Czech Republic) according to the manufacturer's protocol (Solovchenko et al. 2013a). The microalgal samples for transmission electron microscopy (TEM) were prepared and examined as described by Gorelova et al. (2009). Size and area of chloroplast, oil bodies, vacuoles, and starch grains were measured on TEM micrographs of the cell ultrathin sections (n=20) using ImageJ software (NIH, USA); the significance of the difference of the means was tested by Student's t test using Origin software (Microlab, USA). DW, total Chl, and FA contents were assayed according to Solovchenko et al. (2013a, b). The results of two independent experiments, each carried out in duplicate (means ± SE), are presented in the figures.

Results and Discussion

The low-CO_2 cultures of *Desmodesmus* sp. reached stationary phase within 3–4 days (Fig. 1a). Bubbling of the *Desmodesmus* sp. with CO_2-enriched gas mixtures resulted in a slight decrease in DW accumulation apparent as lag depended on CO_2 level, typically followed by a steady increase. As a result, by the 11th day of cultivation, the DW of the CO_2-enriched cultures was only slightly lower than that of the low-CO_2 culture (Fig. 1a). By contrast, Chl content per unit (g) DW in the low-to-moderate CO_2 culture under high-PAR irradiance did not exceed 30 mg, whereas under 100 % CO_2, it was three to four times higher (Fig. 1b).

Notably, only a slight decrease in pH was observed in the CO_2-enriched cultures in comparison with the low-CO_2 cultures (9±1 vs. 10.5±1 pH at the seventh day of cultivation), suggesting high-buffering capacity of the culture and a minor effect of pH on the balance of inorganic carbon forms available to the microalga. Average CO_2 fixation rate estimated from the carbon content of the biomass was ca. three times higher under 20 and 100 % CO_2 than under atmospheric CO_2 (1.5–2.0 vs. 0.5 L CO_2/day per L culture, respectively). The CO_2 fixation rate under 100 % CO_2, without optimization carried out, was comparable to or higher than the rates recorded in dedicated photobioreactors (López et al. 2013).

The total fatty acid (TFA) content increased after a 3-day lag until the seventh day of cultivation regardless of the CO_2 level (Fig. 2). Low-CO_2 cultures displayed a further increase in TFA content; at the same time, their FA composition did not change considerably (cf. Fig. 3a, b). A similar TFA trend was recorded in the 5 % CO_2 culture. By contrast, a lower TFA content was recorded in the 20 and 100 % CO_2 cultures (Fig. 2) accompanied by profound changes in their FA composition comprised mainly by an increase in oleate (18:1) and linoleate (18:2) proportions at the expense of linolenate (18:3) and 16:4 (Fig. 3c). It is known that CO_2-sensitive algal species (mainly free-living) respond even to short-term elevated CO_2 exposure by a decline in the FA unsaturation index, suggesting inhibition of FA desaturation and C16-to-C18 elongation (Muradyan et al. 2004) and channeling of the excess fixed carbon into the biosynthesis of reserve lipids (e.g., triacylglycerols) as reviewed recently by Solovchenko and Khozin-Goldberg (2013). By contrast, the symbiotic *Desmodesmus* sp. retained high photosynthetic activity even under extremely high CO_2. Accordingly, the limited decrease of unsaturation characteristic of the *Desmodesmus* sp. cell lipids grown under 20 or 100 % CO_2 may reflect the retention of chloroplast thylakoid membrane lipids (incorporating mainly unsaturated fatty acids).

Interestingly, after 11 days of bubbling with pure CO_2 ultrastructure of the algal cells remained essentially unchanged as compared with that of the cells grown under low CO_2 (Fig. 4; for detailed ultrastructure of 'low-CO_2' cells of the *Desmodesmus* sp., see Gorelova et al. (2012)). A considerable part of the cell was occupied by chloroplasts (Fig. 4). A small (<20 %) decline in the chloroplast area was recorded under high-CO_2 conditions, whereas relative area occupied by thylakoids and stroma (calculated by subtracting the area of the starch grains and pyrenoid from the total area of the chloroplast) did not change significantly (0.52±0.03 vs. 0.55±0.02). Collectively, the changes observed suggested relative ultrastructural intactness of the photosynthetic apparatus (Fig. 5); this suggestion was confirmed by the high photosynthetic activity of the cells as estimated via chlorophyll fluorescence measurements: average Fv/Fm value was as high as 0.71 vs. 0.73 in the CO_2-enriched cultures and those grown at the atmospheric CO_2 level respectively. In the cells grown under 100 % CO_2, pyrenoids were not uniform in size and structure and were encountered ca. 3.5 times less frequently in the sections of the '100 % CO_2' cells in comparison to that of the 'low-CO_2' cells.

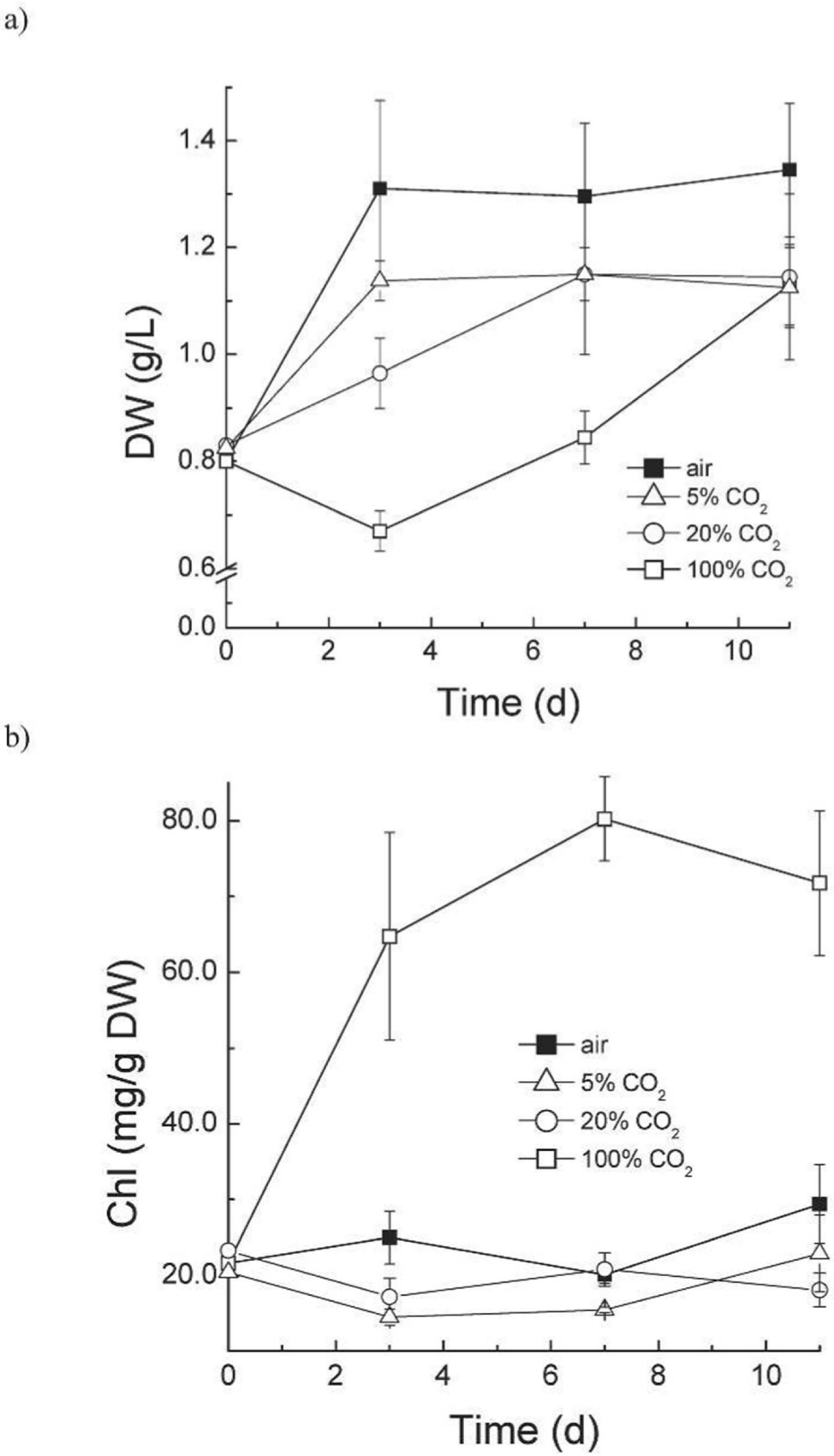

Fig. 1 **a** Growth and **b** changes in chlorophyll content of *Desmodesmus* sp. 3Dp86E-1 cultures bubbled with air : CO_2 mixtures of different composition

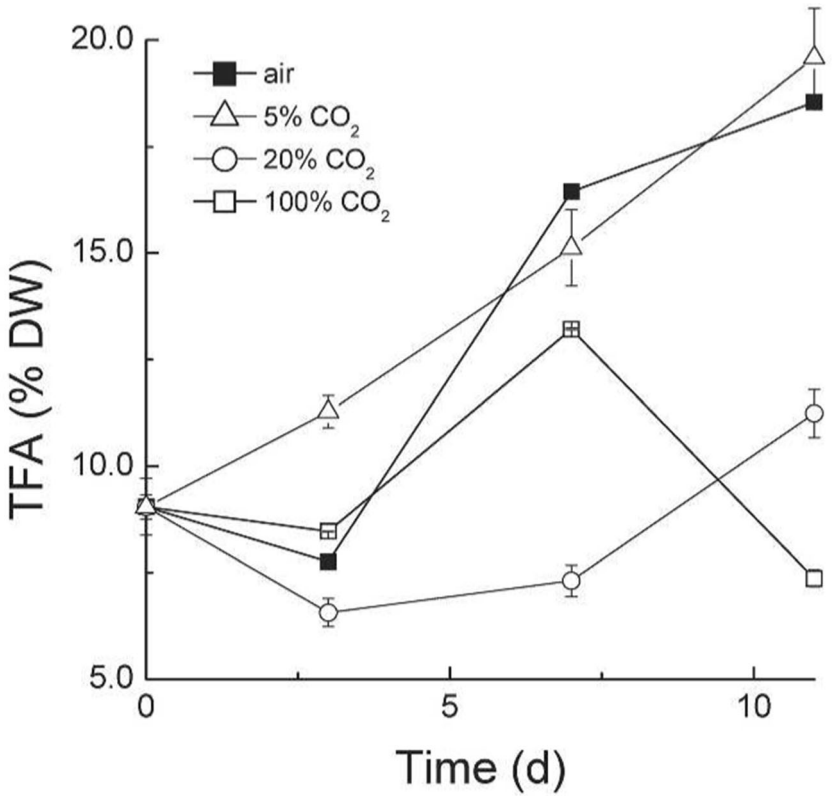

Fig. 2 Changes in total fatty acid percentage of *Desmodesmus* sp. 3Dp86E-1 cells from the cultures bubbled with air : CO_2 mixtures of different composition

The chloroplast envelope of the '100 % CO_2' cells retained its electron density; occasionally, it formed invaginations, sometimes curled, into the cytoplasm. The chloroplasts of the '100 % CO_2' cells were filled with thylakoids, mostly intact in terms of ultrastructure. Elongated grana-like stacks were observed (Fig. 4b, c). At the same time, the regions with loosely packed, sometimes fan-shaped, thylakoids increased (Fig. 4d). The latter probably reflects the enrichment of chloroplast membranes with photosystem I (Bumba and Vácha 2003), characteristic of high-CO_2-acclimated microalgal cells (Satoh et al. 2002; Miyachi et al. 2003). The alterations in thylakoid topology may reflect the rearrangement(s) in their structure related to the conspicuous increase in Chl content in the '100 % CO_2' cells (Fig. 1b). On the other hand, elevated Chl content could, to a certain extent, augment the efficient assimilation of CO_2 in the '100 % CO_2' cells contributing to their high-CO_2 tolerance.

One should also note a considerable high-CO_2-induced decline in size and number (not shown) of the structures involved in carbon storage in form of carbohydrates (starch grains) and lipids (oil bodies). Accordingly, the area of these inclusions decreased 3.7 times under 100 % CO_2 (Fig. 5), mainly due to a reduction in size of the inter-thylakoid starch grains, which shrank 12 times. Taking into account the rise in Chl content (Fig. 1b), as well as well-formed granal–lamellar system of the chloroplasts, it is possible to suggest that the

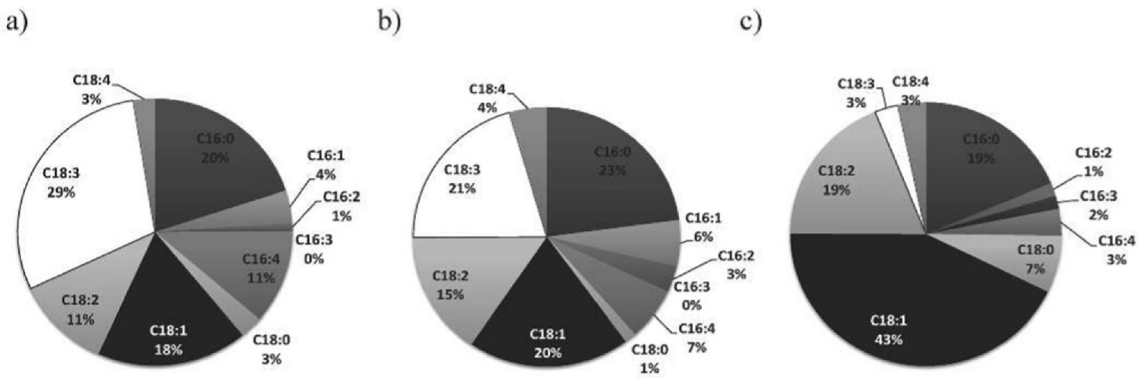

Fig. 3 Fatty acid composition of *Desmodesmus* sp. 3Dp86E-1 cells **a** at day 0 and after 11 days of cultivation, **b** with air, or **c** 100 % CO_2. The fatty acid composition of the cells grown at 5 or 20 % CO_2 was not significantly different from that shown in **b** or **c**, respectively

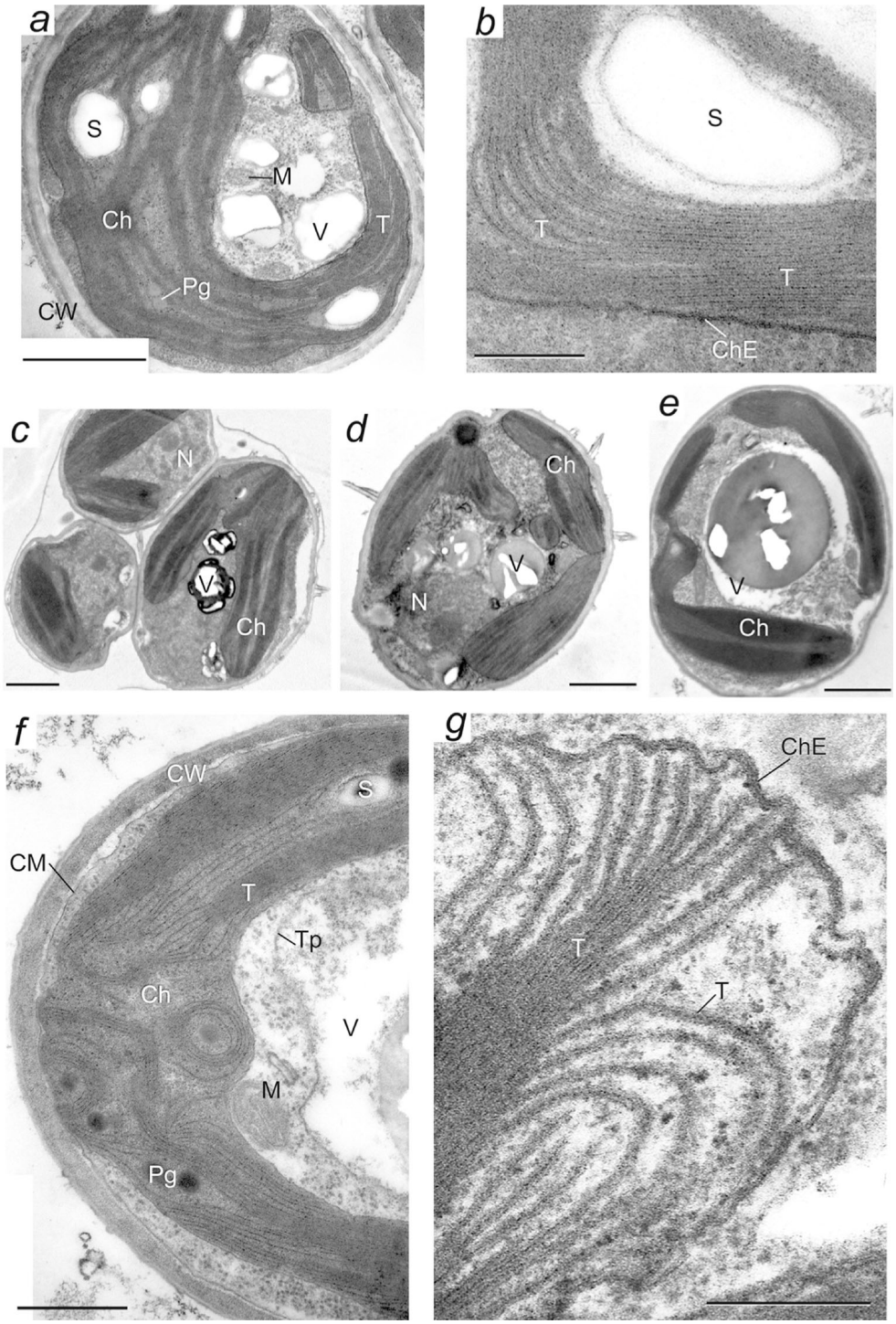

Fig. 4 The ultrastructure of *Desmodesmus* sp. 3Dp86E-1 cells (**a**, **c**–**f**) and chloroplasts (**b**, **g**) cultivated for 11 days at (**a**, **b**) the atmospheric (0.03 %) CO_2 level or (**c**–**g**) 100 % CO_2. Note the fan-shaped thylakoids in **g**. *Ch* chloroplast, *CM* cytoplasmic membrane, *CW* cell wall, *ChE* chloroplast envelope, *M* mitochondrion, *N* nucleus, *Pg* plastoglobule, *S* starch grain, *T* thylakoid(s), *Tp* tonoplast, *V* vacuole with granules constituted by a matter of inhomogeneous electron density. Scale bars, 1 μm (**a**, **c**–**e**), 0.5 μm (**f**), 0.2 μm (**b**, **g**)

increase in TFA content (Fig. 2) was due to elevated synthesis of chloroplastic lipids.

Notably, a significant increase in vacuole size was detected in the '100 % CO_2' cells (by 4.2 times in comparison with the 'low-CO_2' cells; Fig. 5), whereas a number of vacuoles did not differ significantly. These vacuoles contained granules of inhomogeneous electron density (Fig. 4). A similar increase in the vacuole size was recorded in other CO_2-tolerant microalga *Chlorococcum littorale* and associated with a buildup of a tonoplast-associated H^+ pump supporting the pH homeostasis of the algal cells, thereby facilitating the recovery from the high-CO_2 stress and contributing to its high-CO_2 tolerance (Miyachi et al. 2003). Additional studies (e.g., investigation of the enzymes involved in carbon uptake and fixation) are required to reveal the precise mechanisms of high-CO_2 tolerance of *Desmodesmus* sp.

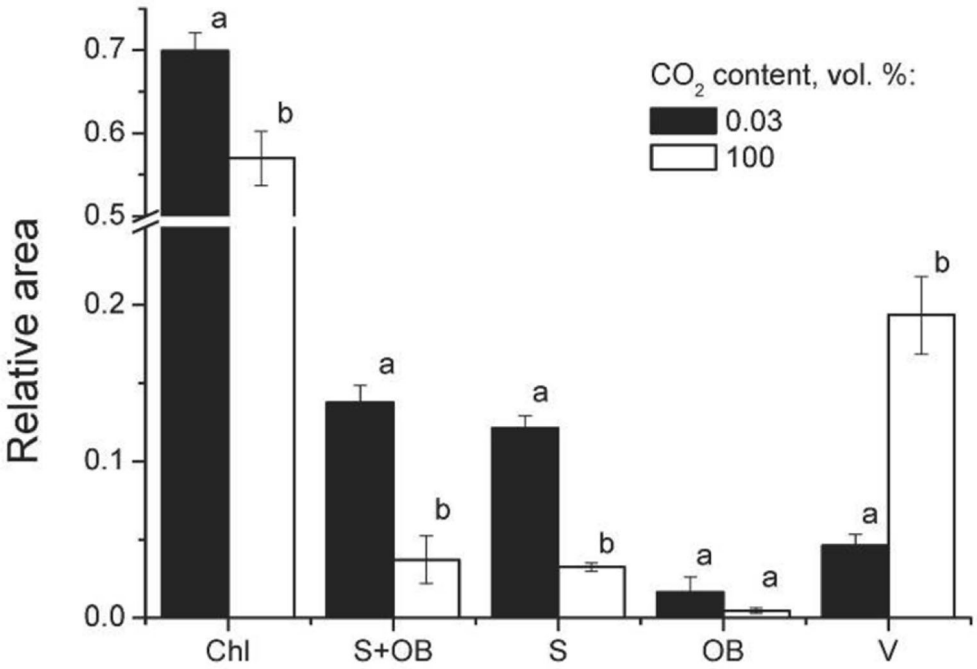

Fig. 5 Changes in relative area of chloroplast (*Chl*), total (*S+OB*) and individual area of starch grains (*S*) and oil bodies (*OB*), and the area of vacuoles (*V*) induced by 11 days of cultivation at 100 % CO_2. The relative area was calculated as a proportion of the protoplast area. *Different letters* denote the data significantly differing at the α= 0.05 level (n=20)

Thus, the microalga *Desmodesmus* sp. 3Dp86E-1 is characterized by the outstanding CO_2 tolerance in a wide range of its concentrations. One may speculate that it stems, at least in part, from its symbiotic origin. In this case, the microalgal photobiont should be continuously affected by elevated CO_2 levels due to respiration of its animal host and the heterotrophic bacteria from the same association (Gorelova et al. 2013). Moreover, at the latitudes of the White Sea, this effect may be exacerbated during long polar nights when photosynthesis is hardly possible. It is possible to suggest that the CO_2 concentrating mechanisms (CCM; see, e.g., Kupriyanova and Pronina 2011) of symbiotic microalgae may become, during co-evolution with their animal hosts, considerably more versatile in comparison to those of their free-living counterparts; hence, the CCM of the symbiotic microalgae could be, depending on the CO_2 level, swiftly shut down or redeployed to prevent the damage to the photosynthetic apparatus.

Our findings strongly suggest that *Desmodesmus* sp. 3Dp86E-1 is a promising candidate for CO_2 biomitigation applications performing well not only when grown on pure CO_2 but also during cultivation at the CO_2 levels typical of flue gases (ca. 20 %; López et al. 2013). Still, the optimal rate(s) of CO_2 feeding to the culture, as well as specific cultivation conditions providing the highest CO_2 fixation rates, remain to be investigated.

Finally, the associations and symbioses of microalgae and invertebrates, especially those living at high altitudes, deserve close attention as a potential source of highly CO_2-tolerant microalgal strains.

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