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Exploring Nordic microalgae as a potential novel source of antioxidant and bioactive compounds

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ABSTRACT

Nordic microalgae are a group of photosynthetic organisms acclimated to growth at low temperature and in varying light conditions; the subarctic climate offers bright days with moderate temperatures during summer and cold and dark winter months. The robustness to these natural stress conditions makes the species interesting for large-scale cultivation in harsh environments and for the production of high-value compounds. The aim of this study was to explore the ability of nineteen species of Nordic microalgae to produce different bioactive compounds, such as carotenoids or polyphenols. The results showed that some of these strains are able to produce high amounts of carotenoids (over 12 $mg \cdot g^{-1}$ dry weight) and phenolic compounds (over 20 mg $GAE \cdot g^{-1}$ dry weight). Based on these profiles, six species were selected for cultivation under high light and cold stress (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 10 ℃). The strains *Chlorococcum* sp. (MC1) and *Scenedesmus* sp. (B2–2) exhibited similar values of biomass productivity under standard or stress conditions, but produced higher concentrations of carotenoids (an increase of 40% and 25%, respectively), phenolic compounds (an increase of 40% and 30%, respectively), and showed higher antioxidant capacity (an increase of 15% and 20%, respectively) during stress. The results highlight the ability of these Nordic microalgae as outstanding producers of bioactive compounds, justifying their cultivation at large scale in Nordic environments.

Introduction

Due to their ability to function in BECCS (Bioenergy with carbon capture and storage) processes, and their ability to produce different high-value compounds, photosynthetic microalgae have raised the interest of researchers and biotechnological industries [1]. Intermediates of microalgal metabolism can be used in a wide variety of applications, including cosmetics, pharmaceutical and human or animal nutrition industries [2,3]. In recent years, the economically feasible production of high value compounds, i.e. carotenoids, polyunsaturated fatty acids (PUFAs), vitamins or polyphenols, has become one of the main tasks in biotechnological research [4]. Microalgae native at latitudes up to 60N are able to grow under extreme temperatures and light conditions. Sub-arctic climate is recognized by long day length (about 20 h of sunlight) in summer with moderate average temperatures of 15 ℃ and only about 3 h of daylight in winter with an average temperature of − 4 ℃ [5]. Their environmental adaptations to a broad range of light intensities or temperatures make these algae interesting organisms for large-scale cultivation in challenging environments, and the production of biomass and high-value compounds [6].

Carotenoids belong to the main bioactive compounds produced by microalgae with high commercial interest. They are a wide group of lipophilic isoprenoids able to reduce reactive oxygen species and therefore confer high antioxidant capacity [7]. The antioxidant, colorant and provitamin properties of, e.g. β-carotene, phytoene, astaxanthin, or lutein, make them highly desirable in the cosmetic, pharmaceutical or feed industries. Different carotenoids provided by microalgae, i.e. β-carotene from *Dunaliella salina* and astaxanthin from *Haematococcus pluvialis*, are widely commercialized for pharmaceutical and aquaculture applications [4].

Phenolic compounds likewise have high commercial interest because of their antioxidant and biomedical capacities. More than 8000 phenolic compounds have been described in higher plants and microalgae with varying antioxidant activities dependent on their chemical structure [8].

Abbreviations: HL/C, high light/cold; DAD, Diode-array detection; GAE, gallic acid equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TC, total carotenoids; TPC, total phenolic compounds.

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In microalgae, these compounds are not well investigated [9], although high amounts of phenolic compounds were observed in *Chlorella pyrenoidosa* or *Spirulina platensis* [10]. Although carotenoids and phenolic compounds are the main contributors to antioxidant activity in microalgae [11], knowledge about these compounds and their antioxidant properties is scarce. To establish a correlation between the amount of these compounds and their antioxidant capacity is further complicated by the difficulties of placing microalgae in a phylogenetic classification [12] and the wide range of extraction systems used in various studies [13]. Investigating algal species of the same phylum and cultivated under the same conditions should lead to better understanding of the bioactive compounds and their antioxidant properties.

Different cultivation techniques, including exposure to stresses such as high light, cold or nitrogen starvation, have been described to increase the production of bioactive compounds [14,15]. Nordic microalgae experience these stresses in their natural environment and therefore should be primed for the production of bioactive compounds. The subartic climate experienced in Umeå, Sweden, with moderate or cold temperatures accompanied by bright sunlight [6] could provide an excellent environment for the production of bioactive compounds by Nordic microalgae. The application of these abiotic stresses to Nordic microalgal cultures provides an outstanding research opportunity with the aim of their future cultivation in large-scale bioreactors to produce bioactive compounds. Here the carotenoid profile as well as the total amount of phenolic compounds in the biomass of 19 Nordic microalgal species was determined. Based on their free radical scavenging properties, the antioxidant activity of the microalgal extracts was investigated. The six best-performing microalgal species were cultivated under high light (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and cold (10 ℃) stress, simulating spring conditions in Nordic environments, to investigate their production of biomass, carotenoids and phenolic compounds, as well as their antioxidant capacity.

Materials and methods

Algal strains and culture

The 19 species of Nordic microalgae characterized in this study had been isolated from different Nordic environments [5], and are shown in Table 1. All the species are chlorophytes, including 13 chlorophyceae and 6 trebouxiophyceae.

The microalgae were cultivated in 250 mL Erlenmeyer flasks filled with 100 mL BG-11 medium [16] at 20 ± 1 ℃, bubbled with air mixed with 5% of CO_2 under continuous irradiation with white light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The initial optical density (OD) of the cultures at 750 nm was 0.15, and they were grown until the end of exponential phase (between 8 and 12 days, depending on the species).

For high light/cold stress experiments, adequate light and temperature conditions were selected following previous studies performed in outdoor bioreactors in the city of Umeå (Sweden) during the period April-May [6]. The selected microalgae were cultivated in a Multi-Cultivator MC 1000-OD (PSI, Czech Republic) using 80 mL of BG-11 medium. The initial OD_{750} for these experiments was 0.2. Microalgal cells were grown for 10 d using a light/dark cycle of 16/8 h, simulating the light availability in Nordic environments during spring. The cultures were bubbled continuously with air mixed with 5% of CO_2. At standard conditions, light intensity and temperature were 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 20 ℃, and during high light/cold (HL/C) stress experiments 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 10 ℃.

Table 1
List of Nordic microalgal strains studied in this work. These species were isolated and identified as described [15].

Class	Species	Strain ID
Chlorophyceae	*Chlorococcum* sp.	MC1
	Coelastrella sp.	3–4
	Coelastrum astroideum	RW10
	Coelastrum microporum	FNY-1
	Desmodesmus opoliensis	SQ2
	Desmodesmus sp.	RUC-2
	Desmodesmus sp.	2–6
	Ettlia pseudoalveolaris	FNY-2
	Haematococcus pluvialis/lacustris	HP
	Monoraphidium sp.	B1–2
	Scenedesmus obliquus	13–8
	Scenedesmus sp.	B2–2
	Scotiellopsis reticulata	UFA-2
Trebouxiophyceae	*Chlorella saccharophila* (*Chloroidium saccarophilum*)	RNY
	Chlorella sorokiniana	2–21–1
	Chlorella sorokiniana	B1–1
	Chlorella vulgaris	13–1
	Chlorella vulgaris	LNY
	Micractinium sp.	P9–1

Extraction of bioactive compounds

To extract bioactive compounds, 10 mg of freeze-dried algal biomass were added to MN bead tubes Type A (Macherey Nagel, Düren, Germany), and mixed with 0.5 mL methanol (MetOH). The cells were disrupted by agitation with MN beads for 3 cycles of 2 min at 4 ℃. After that, the homogenate was incubated at 70 C for 15 min and centrifuged at 14,000 *g* for 5 min. The extraction process was repeated twice and the two supernatants were mixed.

Carotenoids determination

Identification and quantification of individual carotenoids was performed in a Merck Hitachi HPLC equipped with DAD detector (Hitachi High-Tech Corporation, Tokyo, Japan) as described by [16]. The column flow rate was maintained at 1 $mL \cdot min^{-1}$ using a RP-18 column. Carotenoids and chlorophylls were detected at 450 nm and the pigments were quantified using their correspondent standards (DHI, Hørsholm, Denmark).

Quantification of total phenolic compounds

Total phenolic compounds were determined by the Folin-Ciocalteu method in 96-well plates, as described in [17], with minor variations. 5 μL of methanolic extracts, at a concentration of 10 mg dry weight$\cdot mL^{-1}$, were mixed with 100 μL Folin-Ciocalteu reagent, which was previously diluted 10x in water. After 5 min of incubation at room temperature, 95 μL of sodium carbonate (70 $g \cdot L^{-1}$) were added to the mix. After incubation with slow agitation at 25 ℃ in the darkness for 90 min, the absorbance was measured at 760 nm on a Synergy™ H4 microplate reader (Biotek, Winooski, Vt, USA) and the concentration of phenolic compounds was expressed in equivalents of gallic acid per gram of extract (mg GAE$\cdot g^{-1}$ dry weight). The calibration curve was performed using gallic acid as standard with concentrations from 1 to 60 $mg \cdot L^{-1}$.

DPPH radical scavenging assay

A 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed as described in [18] with minor modifications. 20 μL of the extract solution were mixed with 180 μL of DPPH solution (0.1 mM prepared in MetOH). The mix was incubated for 30 min at 25 ℃ and the absorbance decrease was measured at 517 nm in a microplate reader. A colour control prepared with 180 μL of MetOH and 20 μL of each extract sample was included in all the assays. Ascorbic acid (0.1 $mg \cdot mL^{-1}$) was used as reference standard corresponding to 100% inhibition. The concentration of extract in the mix was 1 mg dry weight$\cdot mL^{-1}$ and the percentage of inhibition was calculated as described in [19] with minor modifications:

$$\%I = \left(1 - \frac{A - A_b}{A_0 - A_b}\right) \times 100$$

where A_0 is the absorbance of DPPH solution without sample, A_b is the absorbance of the reference standard and A is the absorbance of the extract with DPPH minus colour control absorbance.

For IC_{50} calculations the extract solution was obtained at different concentrations: 1, 0.8, 0.6, 0.4 and 0.2 mg·mL^{-1}. These samples were measured as described previously and the IC_{50} was calculated though the linear regression.

Statistical analysis

All experiments were performed as triplicate (n = 3) and the results were expressed as mean ± standard deviation (SD). Significant effects of HL/C stress were tested with a two-way ANOVA. Pearson correlation test was used to study the correlation between antioxidant activity, total phenolic compounds and total carotenoids. Significant differences were considered at $p < 0.05$. All statistical analyses were performed with IBM SPSS Statistics 27.0 (IBM, Armonk, NY, USA).

Results and discussion

Screening of bioactive compounds and antioxidant activity in Nordic microalgae

The potential of 19 Nordic green microalgae (Table 1) was investigated to produce bioactive and antioxidant compounds. Grown under standard conditions (continuous light at 20 ℃ and 100 μmol·m^{-2}·s^{-1}, Table 2) the carotenoid profile of these microalgae, measured by HPLC, indicated that their total amount of carotenoids varied from 4.40 mg·g^{-1} dry weight in *Chlorella saccharophila* (RNY) to 17.7 mg·g^{-1} dry weight in *Coelastrella* sp. (3−4). The highest carotenoid content was detected in 3–4 (17.7 mg·g^{-1} dry weight), *Desmodesmus opoliensis* (SQ-2) (15.8 mg·g^{-1} dry weight), *Chlorococcum* sp. (MC-1) (15.1 mg·g^{-1} dry weight) and *Scenedesmus* sp. (B2–2) (12.5 mg·g^{-1} dry weight), making them potential sources for carotenoids already in the absence of stress. Furthermore, most of these species displayed a high percentage of lutein content, between 33% and 56% of the total pigments. The highest percentages of lutein were observed in *Chlorella sorokiniana* (B1–1), *Chlorella saccharophila* (RNY) and B2–2. However, the highest percentages of β-carotene were detected in 3–4 and *Coelastrum astroideum* (RW10), with 23–26% of total pigments. Some species of *Coelastrella* have been observed to be high carotenoid producers under stress conditions such as high light or

Table 2
Pigment composition, amount of total carotenoids (TC) in mg·g^{-1} dry weight and total phenolic compounds (TPC) in mg GAE·g^{-1} dry weight as well as their antioxidant potential using a DPPH assay represented as IC_{50} (mg·mL^{-1}) of Nordic microalgae cultivated at continuous light (100 μmol·m^{-2}·s^{-1}) and 20 ℃. Pigments determined were neoxanthin (Neo), violaxanthin (Viola), antheraxanthin (Ant), lutein (Lut), c-lutein (c-Lut), astaxanthin (Ast), chlorophyll *b* (Chl *b*), chlorophyll *a* (Chl *a*), β,ε-carotene (βε-Car) and ββ-carotene (ββ-Car). Values are represented as mean (n = 3) ± SD.

	Neo	Viola	Ant	Lut	c-Lut	Ast	Chl *b*	Chl *a*	βε-Car	ββ-Car	TC	TPC	IC_{50}
Chlorococcum sp. (MC1)	1.93 ± 0.13	2.46 ± 0.32	0.13 ± 0.04	7.27 ± 0.58	0.75 ± 0.05	–	46.4 ± 3.94	33.7 ± 2.73	1.06 ± 0.16	1.50 ± 0.13	15.1 ± 0.82	30.3 ± 0.99	0.66 ± 0.03
Coelastrella sp. (3–4)	3.10 ± 0.22	1.49 ± 0.10	0.39 ± 0.09	8.00 ± 0.96	0.34 ± 0.02	–	22.2 ± 3.05	22.9 ± 1.55	2.12 ± 0.14	2.22 ± 0.10	17.7 ± 1.59	11.1 ± 0.58	0.69 ± 0.02
Coelastrum astroideum (RW10)	1.82 ± 0.08	1.44 ± 0.08	0.13 ± 0.01	5.14 ± 0.63	0.51 ± 0.06	–	25.4 ± 1.02	22.4 ± 2.16	1.97 ± 0.17	1.29 ± 0.05	12.3 ± 0.84	24.1 ± 2.68	0.64 ± 0.05
Coelastrum microporum (FNY1)	2.97 ± 0.17	1.44 ± 0.28	0.06 ± 0.00	3.56 ± 0.17	0.17 ± 0.01	–	24.7 ± 1.55	23.5 ± 3.85	0.35 ± 001	1.94 ± 0.08	10.5 ± 0.99	19.7 ± 0.76	0.65 ± 0.05
Desmodesmus opoliensis (SQ2)	2.01 ± 0.16	2.07 ± 0.15	0.13 ± 0.03	8.25 ± 0.27	0.42 ± 0.02	–	30.9 ± 2.81	33.9 ± 4.25	–	2.92 ± 0.19	15.8 ± 0.24	16.4 ± 0.46	0.58 ± 0.03
Desmodesmus sp. (RUC-2)	0.99 ± 0.07	1.03 ± 0.10	0.14 ± 0.00	3.00 ± 0.15	0.17 ± 0.01	–	19.4 ± 2.53	17.4 ± 0.75	0.14 ± 0.02	0.67 ± 0.04	6.14 ± 0.71	18.8 ± 1.07	0.82 ± 0.09
Desmodesmus sp. (2–6)	2.02 ± 0.15	1.35 ± 0.12	0.09 ± 0.02	3.72 ± 0.29	–	–	25.9 ± 1.79	24.7 ± 3.42	–	1.45 ± 0.12	8.63 ± 0.74	9.90 ± 0.29	0.73 ± 0.06
Ettlia pseudoalveolaris (FNY2)	2.24 ± 0.09	0.08 ± 0.01	–	3.16 ± 0.29	0.10 ± 0.01	–	9.57 ± 0.82	8.76 ± 0.49	0.46 ± 0.04	0.71 ± 0.04	6.75 ± 0.39	21.2 ± 0.83	0.62 ± 0.05
Haematococcus pluvialis (HP)	0.92 ± 0.02	0.92 ± 0.07	0.26 ± 0.01	4.13 ± 0.16	0.21 ± 0.00	0.29 ± 0.04	20.8 ± 3.46	19.9 ± 1.82	0.60 ± 0.09	0.87 ± 0.06	8.20 ± 0.78	14.1 ± 1.03	0.63 ± 0.04
Monoraphidium sp. (B1–2)	0.75 ± 0.07	1.66 ± 0.14	0.47 ± 0.03	3.94 ± 0.28	0.16 ± 0.03	–	15.3 ± 2.11	15.1 ± 1.62	0.10 ± 0.00	1.00 ± 0.15	8.08 ± 0.57	11.4 ± 0.10	0.67 ± 0.05
Scenedesmus obliquus (13–8)	1.18 ± 0.03	0.90 ± 0.06	0.31 ± 0.05	3.26 ± 0.10	0.12 ± 0.01	–	16.0 ± 0.84	15.4 ± 1.09	0.18 ± 0.03	0.68 ± 0.07	6.63 ± 0.82	7.53 ± 0.30	0.68 ± 0.06
Scenedesmus sp. (B2–2)	1.46 ± 0.10	1.38 ± 0.21	0.10 ± 0.01	6.70 ± 0.40	0.41 ± 0.03	–	34.7 ± 3.06	35.6 ± 2.34	0.22 ± 0.03	2.21 ± 0.09	12.5 ± 1.28	20.4 ± 1.71	0.59 ± 0.03
Scotiellopsis reticulata (UFA-2)	1.05 ± 0.11	0.84 ± 0.06	0.12 ± 0.02	2.52 ± 0.34	0.20 ± 0.05	–	13.7 ± 1.28	15.3 ± 0.76	0.24 ± 0.03	0.71 ± 0.05	5.67 ± 0.65	11.6 ± 0.50	0.70 ± 0.04
Chlorella saccharophila (RNY)	0.53 ± 0.04	0.60 ± 0.08	0.16 ± 0.02	2.45 ± 0.16	0.19 ± 0.00	–	11.6 ± 1.96	9.56 ± 0.91	0.11 ± 0.03	0.37 ± 0.09	4.40 ± 0.20	11.8 ± 0.30	0.84 ± 0.07
Chlorella sorokiniana (2–21–1)	0.76 ± 0.12	0.79 ± 0.08	0.08 ± 0.05	2.99 ± 0.57	0.16 ± 0.02	–	16.8 ± 1.04	15.6 ± 0.79	0.30 ± 0.09	0.72 ± 0.03	6.27 ± 0.49	12.7 ± 0.36	0.72 ± 0.06
Chlorella sorokiniana (B1–1)	0.57 ± 0.02	0.80 ± 0.04	0.19 ± 0.04	2.98 ± 0.21	0.17 ± 0.01	–	12.5 ± 1.76	11.3 ± 0.86	0.11 ± 0.01	0.51 ± 0.02	5.32 ± 0.58	16.5 ± 0.56	0.64 ± 0.07
Chlorella vulgaris (13–1)	0.64 ± 0.05	0.73 ± 0.05	0.22 ± 0.03	2.52 ± 0.3	0.10 ± 0.04	–	13.0 ± 1.84	12.5 ± 0.78	0.33 ± 0.05	0.57 ± 0.03	5.12 ± 0.31	6.67 ± 0.21	0.75 ± 0.05
Chlorella vulgaris (LNY)	0.54 ± 0.08	0.60 ± 0.02	0.25 ± 0.07	2.84 ± 0.24	0.37 ± 0.03	–	10.1 ± 1.79	10.2 ± 1.00	0.24 ± 0.05	0.46 ± 0.04	5.32 ± 0.19	12.1 ± 0.43	0.67 ± 0.05
Micractinium sp. (P9–1)	0.81 ± 0.05	0.98 ± 0.18	0.16 ± 0.02	3.28 ± 0.09	0.29 ± 0.04	–	17.2 ± 0.97	17.0 ± 2.10	0.20 ± 0.02	0.98 ± 0.16	6.69 ± 0.28	9.71 ± 0.20	0.51 ± 0.04

osmotic stress [20]. Nevertheless, the values reported for these strains were lower than those observed here in the strain 3–4 grown under standard conditions. Nordic microalgae thus have an obvious potential to produce carotenoids at large scale. Nordic species in general attained higher amounts of carotenoids than other strains of *Chlorella*, *Scenedesmus*, *Desmodesmus* or *Micractinium* isolated from different environments [19,21–23]. The production of such high values of carotenoids may be a consequence of the constant stress Nordic species are exposed to, especially bright light accompanied by low temperatures, meteorological conditions common in Scandinavia.

The total phenolic compounds (TPC) content was also determined in the 19 species of Nordic microalgae using a Folin-Ciocalteu assay. Related to gallic acid equivalents (GAE), the total amount of these compounds varied from 6.67 in *Chlorella vulgaris* (13−1) to 30.3 mg GAE·g^{-1} dry weight in MC1. The highest values of phenolic compounds were produced by MC1, RW10 (24.1 mg GAE·g^{-1} dry weight), *Ettlia pseudoalveolaris* (FNY-2) (21.2 mg GAE·g^{-1} dry weight) and B2–2 (20.4 mg GAE·g^{-1} dry weight) with more than 20 GAE·g^{-1} dry weight (Table 2). The values of total phenolic compounds in the five species of *Chlorella* genus (13–1, B1–1, 2–21–1, LNY, RNY) were similar to those reported for *Chlorella sorokiniana* [24] (11.4 mg GAE·g^{-1} dry weight). Of the Chlorophyceae, B2–2 had a 4 times higher concentration of phenolic compounds than the *Scenedesmus* sp. strain reported in [25] and MC1 5 times higher than *Chlorococcum minutum* reported in [26]. However, it is difficult to compare directly the potential of Nordic microalgae with strains isolated from moderate or warm climate, as the amounts of total phenolic compounds published in the literature vary regarding species and also regarding different studies on the same species [9]. These differences could be related to growth phase or the culture medium used for each strain.

Carotenoids and phenolic compounds are two of the most relevant antioxidant molecules in plants and microalgae. For that reason, methanolic extracts of the 19 Nordic microalgal strains were evaluated for their antioxidant activity using a DPPH scavenging assay. The IC_{50} of the strains demonstrated that the highest antioxidant capacity was exhibited by *Micractinium* sp. (P9–1) with an IC_{50} of 0.52 mg·mL^{-1}, followed by SQ-2 and B2–2, with IC_{50} of 0.58 and 0.59 mg·mL^{-1}, respectively (Table 2). The antioxidant capacity to scavenge DPPH radicals was lower in the five *Chlorella* species than in the other species (Table 2). However, P9–1, which like *Chlorella* belongs to the class of Trebouxiophyceae, displayed the highest antioxidant capacity, making a correlation between antioxidant activity and microalgal class difficult. The high antioxidant capacity of *Micactinium* species has apparently not been reported before, nevertheless ethanolic extracts of an Antarctic freshwater *Micractinium* sp. (ETMI) strain were reported to show *in vitro* anti-inflammatory and anti-cancer activity [27]. It will be interesting to further investigate this Nordic strain with unusually high antioxidant capacity despite its low amounts of carotenoids and phenolic compounds. The antioxidant capacity of Nordic microalgae was higher than that reported in previous studies using *Chlorococcum* and *Chlorella* strains [28] or tropical microalgae [29]. The antioxidant system of Nordic microalgae therefore seems to be more effective, probably due to the hard environmental conditions the organisms are exposed to in these latitudes.

Correlation between bioactive compounds and antioxidant activity in Nordic microalgae

Due to the antioxidant properties of carotenoids and phenolic compounds, the total amount of these compounds should correlate with the antioxidant properties of Nordic microalgae to remove free radicals. A correlation analysis was therefore performed (Table 3) to investigate the antioxidant potential of carotenoids and of phenolic compounds in the Nordic microalgal strains. Total carotenoids, expressed as xanthophylls + carotenes, contributed higher ($R^2 = -0.365$) to the DPPH radical scavenging activity (expressed as IC_{50} values) than phenolic compounds ($R^2 = -0.216$). Both classes of compounds displayed a non-significant correlation against the antioxidant activity of Nordic microalgae, suggesting that other compounds also contribute in scavenging free DPPH radicals. Previously, antioxidant activities could be correlated to the amount of carotenoids, phenolic compounds and tocopherols in six species of microalgae belonging to three phyla [30]. However, other studies concluded that no correlation exists investigating nine different species belonging to three phyla using the DPPH scavenging assay [19]. The correlation between antioxidant activity and bioactive compounds therefore seems to depend on the antioxidant test performed and the taxonomic classification of the microalgal species. Using the oxygen radical absorbance capacity (ORAC), the trolox equivalent antioxidant capacity (TEAC) or the ferric reducing antioxidant potential (FRAP) let to a correlation between the total amount of carotenoids and their antioxidant activity; assays using DPPH or thiobabituric acid reactive substance (TBARS), however, failed to highlight such a correlation in twelve [29] or sixteen [31] species of microalgae. A correlation between the total amount of phenolic compounds and their antioxidant activity was established using the DDPH assay in bacillariophyte and haptophyte species, but not in chlorophytes [32]. These data are in agreement with the results of the present study using chlorophytes. Thus, the conflicting results reported in different studies show that further research is needed using more homogeneous groups of microalgae, cultivating under the same conditions, and including different antioxidant assays in order to obtain a full picture of the correlation between total amount of antioxidant molecules in microalgae and antioxidant properties of microalgal biomass.

Table 3
Multiple regression analysis using Pearson correlation and t-test to calculate the antioxidant potential of total carotenoids or total phenolic compounds based on DPPH radical scavenging. Significant correlations ($p < 0.05$) are marked by asterisk.

X variable	Y variable	N	R^2	Sig (bilateral)	t Stat
Carotenoids	Antioxidant potential (DPPH)	19	-0.365	0.124	-8.743
Phenolic compounds	Antioxidant potential (DPPH)	19	-0.216	0.375	-10.235
Carotenoids	Phenolic compounds	19	0.467 *	0.044	-4.933

A significant positive correlation ($R^2 = 0.467$) was determined between total carotenoids and phenolic compounds in Nordic microalgae (Table 3) highlighting their potential to produce these bioactive compounds at large scale. A positive correlation between carotenoids and phenolic compounds was also reported for a group of microalgae belonging to three different phyla [33]. Interestingly, the authors are unaware of any other study using a high number of microalgal species displaying a positive correlation. Thus, the best performing Nordic microalgal strains were tested further to enhance the production of these bioactive compounds.

Total biomass under HL/C stress

Abiotic stress is known to enhance the concentration of valuable products in microalgae, although often growth and biomass productivity are limited by these adverse culture conditions. Nordic microalgae are well adapted to different stress conditions, including high/low light intensities and/or cold temperatures [14]. Based on their ability to produce bioactive compounds, six species of the 19 Nordic microalgal strains were selected to be cultivated under high light (500 μmol·m^{-2}·s^{-1}) and cold (10 ℃) stress (HL/C). These culture conditions simulate the natural environmental conditions during spring in the region around Umeå (63°49'42"N, 20°15'35"E) [6]. The total biomass (g of dry weight·L^{-1}) of the six species (*Chlorococcum* sp. (MC-1), *Coelastrella* sp. (3−4), *Coelastrum astroideum* (RW10), *Desmodesmus opoliensis* (SQ-2),

Haematococcus pluvialis (HP) and *Scenedesmus* sp. (B2–2)) was compared after 10 days of cultivation under standard conditions and under HL/C stress (Fig. 1). The final biomass of SQ2, RW10 and HP exhibited a significant decrease (50% of total biomass) under HL/C stress in comparison to standard conditions. The biomass production of 3–4 decreased by 25% during exposure to stress, however, the difference was not significant (Fig. 1). Interestingly, the biomass production of MC1 and B2–2 was similar under standard conditions and HL/C stress (Fig. 1), with values of 0.4 and 0.25 g dry weight·L^{-1}, respectively. These results confirm the good adaptation abilities of some microalgae to the Nordic climate. Previous studies revealed that species of the genus *Chlorella* or *Chlamydomonas* were able to obtain similar or higher values of biomass when cultivated at low temperatures (4 or 15 ℃) [34,35]. Alterations in their carbon cycle (polysaccharide and fatty acid metabolism), amino acid metabolism, photosynthesis, lipids or carotenoid metabolism helped the adaptation to cold stress. The microalga *Dunaliella salina* and the Nordic strains 2–6 and RUC-2 were cultivated under high light stress (400 or 600 μmol·m^{-2}·s^{-1}) and produced higher values of biomass than under standard conditions [16,36]. They responded to this stress situation by increased lipid and carotenoid content.

Total carotenoids and phenolic compounds under HL/C stress

An increased production of bioactive compounds has been described previously for different species of microalgae exposed to stress conditions [7]. The amount of total carotenoids in the biomass of the six Nordic microalgal species selected was therefore determined after growth in HL/C stress conditions and compared to standard culture conditions (Fig. 2A). MC1 responded best to HL/C stress, the carotenoid concentration in its biomass increasing by 40%, up to 21.9 mg·g^{-1} dry weight followed by B2–2, with an increase in total carotenoids of 25% (15.3 mg·g^{-1} dry weight). 3–4 and SQ2 responded to HL/C stress by decreased carotenoid production of 70% and 55%, respectively (Fig. 2 A). The concentration of total carotenoids did not change significantly in RW10 and HP, independent of the growth conditions. These microalgae either are not stressed, or they have alternative ways to deal with the stress, e.g. changes in the fatty acid profile as has been reported for other microalgae [37,38]. Studies on changes in the carotenoid profile in response to cold stress are very limited. *Porphyridium cruentum* cultivated at 0 or 15 ℃ was reported to increase the production of biomass, polyunsaturated fatty acids and carotenoids (mainly zeaxanthin) [39]. The biomass of *Chlamydomonas reinhardtii* increased total carotenoids during cold stress, the optimal temperature for carotenoid production being found to be 15 ℃ [40]. Cold and/or high light stress or a combination of the two thus provide excellent approaches to increase the production of carotenoids in some microalgae.

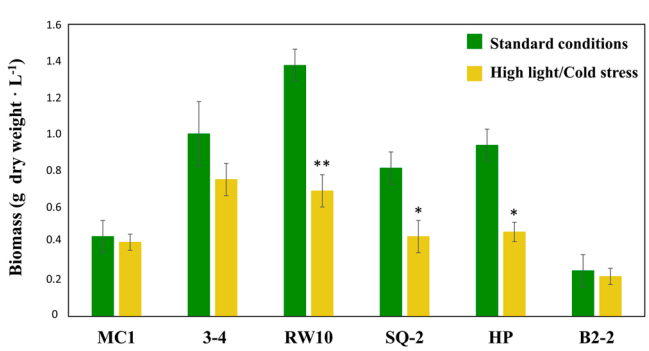

Fig. 1. Biomass production of the Nordic microalgae *Chlorococcum* sp. (MC1), *Coelastrella* sp. (3−4), *Coelastrum astroideum* (RW10), *Desmodesmus opoliensis* (SQ2), *Haematococcus pluvialis* (HP) and *Scenedesmus* sp. (B2–2) determined after 10 days of cultivation under standard conditions (20 ℃ and 100 μmol·m^{-2}·s^{-1}) (dark green) and high light/cold stress (10 ℃ and 500 μmol·m^{-2}·s^{-1}) (yellow). * Significant differences at $p < 0.05$ and * * $p < 0.01$.

The chlorophyll content and carotenoid profiles of the six Nordic microalgae cultivated either under standard conditions or HL/C stress was also determined by HPLC (Table 4). The results showed a decrease in total chlorophyll amount in all species after exposure to HL/C stress, mainly caused by a diminished chlorophyll *a* content. While in MC1 the chlorophyll *a* decreased by 20%, in SQ2 and 3–4 it decreased by over 70% and 95%, respectively (Table 4). These two strains had difficulties in coping with HL/C stress. In SQ2 and 3–4 also the chlorophyll *b* content decreased, which was different to the other four species (Table 4). These strains in general displayed low production of biomass (Fig. 1) and total carotenoids (Fig. 2 A) during HL/C stress. A low content of total chlorophyll, mainly chlorophyll *a*, during high light stress was previously reported in the Nordic strain *Desmodesmus* sp. (RUC-2) [16]. A decrease in chlorophyll was also reported for *Chlamydomonas reinhardtii* grown under cold stress (10 ℃), which produced the highest content of chlorophyll at 15 ℃ within 24 h of culturing [40].

Based on the carotenoid profile of these six species, the production of lutein seems to be the main response to HL/C stress in Nordic microalgae. During stress exposure the lutein content increased in MC1, RW10, HP and B2–2 (Table 2), the four species able to adapt to HL/C stress. Lutein and β,β-carotene increased in MC1 remarkably from 10.2 and 1.61 mg·g^{-1} to 15.5 and 3.36 mg·g^{-1}. In HP, additionally, the total astaxanthin increased from 0.41 to 1.91 mg·g^{-1}. These values for astaxanthin are lower than those reported previously for this microalga under N starvation [14]. β,ε-carotene and violaxanthin decreased in all species during HL/C stress, and these compounds seem not to be necessary during stress.

The amount of total phenolic compounds can also increase when microalgae are exposed to stress conditions [9]. Phenolic compounds might be produced as antioxidants in response to high light and cold stress, together with carotenoids and other compounds. In the Nordic microalgae MC1 and B2–2, total phenolic compounds increased during exposure to HL/C stress (40%, up to 34.5 mg GAE·g^{-1} dry weight and 30%, up to 25.0 mg GAE·g^{-1} dry weight, respectively) (Fig. 2B), while the amount remained similar or slightly lower in 3–4, SQ2, RW10 and HP compared to standard conditions. Previous studies reported lower concentrations of total phenolic compounds when *Phaeodactylum tricornutum* or *Dunalliella salina* were exposed to different stress conditions [41,42]. This decrease was accompanied by lower amounts of biomass, as observed here for 3–4, RW10, SQ2 and HP. The total phenolic compounds increased in high light conditions in *Chlamydomonas reinhardtii*, as did the final biomass [15], similar to the response observed here for MC1 and B2–2 during HL/C stress.

Antioxidant activity under HL/C stress

Antioxidant molecules neutralize radicals, which appear during various types of stress in microalgae. The IC_{50} values were determined using the DPPH scavenging assay in the six selected Nordic microalgae cultivated under standard conditions or HL/C stress (Fig. 2 C). All species showed a higher antioxidant capacity when cultivated under HL/C stress, but for 3–4 the difference in IC_{50} value was not significant. The highest increase in antioxidant capacity was observed in HP and B2–2, for which the IC_{50} values decreased by 26% and 20%, respectively, under HL/C stress compared to standard conditions (Fig. 2 C). A decrease of between 30% and 40% of IC_{50} values has previously been reported for the tropical microalgae *Neprhroselmis* sp., *Picochlorum* sp. and *Cylindroteca closterium*, when cultivated under high light stress, while other strains displayed increased IC_{50} values during the same conditions [29]. *Neprhroselmis* sp., *Picochlorum* sp. and *Cylindroteca closterium* additionally had a decreased number of total carotenoids during growth in high light stress, despite their increased antioxidant activity. These results are in agreement with the data obtained for the Nordic strains 3–4 and SQ2. Some of these Nordic microalgae, including MC1, B2–2, HP or RW10, therefore have high potential for their cultivation under HL/C stress and seem to be outstanding producers of

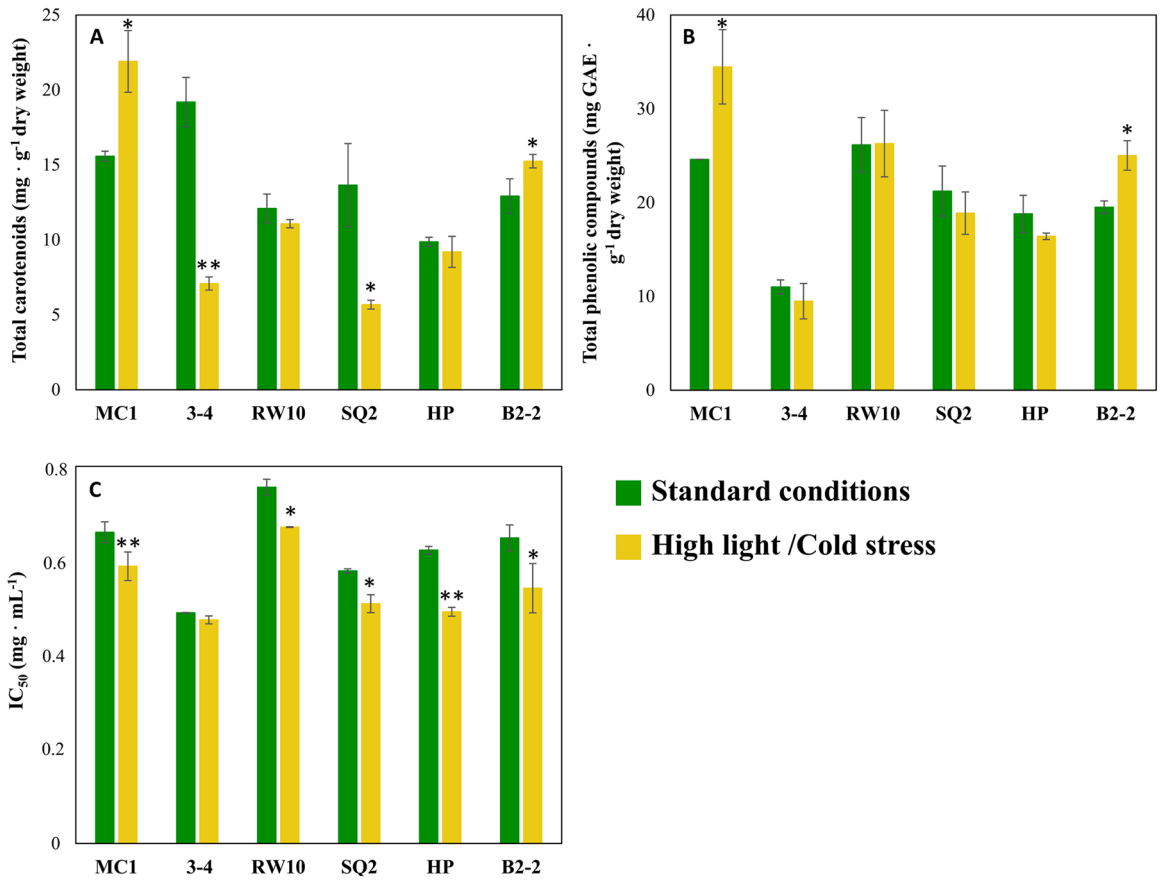

Fig. 2. Amount of total carotenoids (A) and total phenolic compounds (B) as well as IC_{50} using the DPPH scavenging assay (C) in the biomass of the Nordic microalgae *Chlorococcum* sp. (MC1), *Coelastrella* sp. (3–4), *Coelastrum astroideum* (RW10), *Desmodesmus opoliensis* (SQ2), *Haematococcus pluvialis* (HP) and *Scenedesmus* sp. (B2–2) determined after 10 days of cultivation under standard conditions (20 ℃ and 100 μmol·m^{-2}·s^{-1}) (dark green) or high light/cold stress (10 ℃ and 500 μmol·m^{-2}·s^{-1}) (yellow). * Significant differences at $p < 0.05$ and * * $p < 0.01$.

Table 4
Pigment composition (mg·g^{-1} dry weight) of six Nordic microalgae cultivated under standard conditions (SC, 20 ℃ and 100 μmol·m^{-2}·s^{-1}) or high light/cold stress (HL/C, 10 ℃ and 500 μmol·m^{-2}·s^{-1}). Pigments determined were neoxanthin (Neo), violaxanthin (Viola), antheraxanthin (Ant), lutein (Lut), c-lutein (c-Lut), astaxanthin (Ast), chlorophyll *b* (Chl *b*), chlorophyll *a* (Chl *a*), β,ε-carotene (βε-Car) and ββ-carotene (ββ-Car). Values are represented as mean (n = 3) ± SD.

		Neo	Viola	Ant	Lut	c-Lut	Ast	Chl *b*	Chl *a*	βε-Car	ββ-Car
Chlorococcum sp. (MC1)	SC	1.72 ± 0.15	0.52 ± 0.04	0.30 ± 0.01	10.2 ± 0.35	0.57 ± 0.07	–	15.5 ± 2.15	15.4 ± 2.03	0.63 ± 0.07	1.61 ± 0.06
	HL/C	1.64 ± 0.20	0.38 ± 0.09	0.23 ± 0.07	15.5 ± 1.24	0.84 ± 0.12	–	14.6 ± 0.63	12.5 ± 1.29	–	3.36 ± 0.33
Coelastrella sp. (3–4)	SC	2.25 ± 0.19	0.68 ± 0.14	0.16 ± 0.02	11.0 ± 0.74	0.55 ± 0.15	–	18.5 ± 1.63	20.1 ± 0.24	1.36 ± 0.10	3.15 ± 0.25
	HL/C	0.18 ± 0.01	0.08 ± 0.01	0.24 ± 0.03	5.48 ± 0.49	0.63 ± 0.07	–	3.25 ± 0.58	1.54 ± 0.39	–	0.48 ± 0.07
Coelastrum astroideum (RW10)	SC	1.64 ± 0.26	0.52 ± 0.05	0.13 ± 0.02	5.93 ± 0.28	0.51 ± 0.06	–	9.08 ± 0.74	9.82 ± 0.60	1.54 ± 0.10	1.82 ± 0.19
	HL/C	0.40 ± 0.00	0.28 ± 0.02	0.57 ± 0.01	7.81 ± 0.25	1.02 ± 0.07	–	9.25 ± 0.53	4.71 ± 0.20	0.12 ± 0.04	0.88 ± 0.07
Desmodesmus opoliensis (SQ2)	SC	1.13 ± 0.17	0.39 ± 0.09	0.18 ± 0.03	8.86 ± 0.76	0.49 ± 0.14	–	15.3 ± 1.79	17.5 ± 0.57	0.10 ± 0.01	2.50 ± 0.22
	HL/C	0.42 ± 0.02	0.13 ± 0.01	0.07 ± 0.03	4.72 ± 0.38	0.22 ± 0.03	–	9.75 ± 0.49	5.60 ± 0.54	–	0.12 ± 0.01
Haematococcus pluvialis (HP)	SC	1.26 ± 0.03	0.56 ± 0.04	0.08 ± 0.01	4.81 ± 0.63	0.59 ± 0.03	0.41 ± 0.03	5.17 ± 0.22	6.35 ± 0.32	1.08 ± 0.23	1.08 ± 0.11
	HL/C	0.12 ± 0.02	0.04 ± 0.00	0.08 ± 0.01	6.50 ± 0.65	0.08 ± 0.01	1.91 ± 0.27	4.34 ± 0.33	3.08 ± 0.39	–	0.48 ± 0.12
Scenedesmus sp. (B2–2)	SC	0.54 ± 0.06	0.22 ± 0.01	–	8.02 ± 0.60	0.46 ± 0.10	–	23.0 ± 2.09	17.2 ± 1.17	–	3.67 ± 0.30
	HL/C	1.23 ± 0.15	0.77 ± 0.03	–	10.7 ± 1.23	–	–	26.2 ± 3.92	11.9 ± 1.96	–	2.59 ± 0.38

bioactive compounds.

Conclusions

Nordic microalgae provide a highly promising source of bioactive compounds such as carotenoids or phenolic compounds. Moreover, these species have high antioxidant potential based on their capacity to scavenge free radicals. The most promising strains for cultivation under high light and cold stress conditions, simulating Scandinavian environments, were *Chlorococcum* sp. (MC1), *Coelastrella* sp. (3−4), *Coelastrum astroideum* (RW10), *Desmodesmus opoliensis* (SQ-2), *Haematococcus pluvialis* (HP) and *Scenedesmus* sp. (B2–2). The species *Chlorococcum* sp. (MC1) and *Scenedesmus* sp. (B2–2) showed the best stress tolerance, they produced the most biomass and bioactive compounds and can potentially be cultivated in large-scale processes in a Nordic environment.

CRediT authorship contribution statement

Conceptualization, A.L-V., and C.F.; methodology, A.L-V.; resources, C.F., R.L., and J.V.; writing—original draft preparation, A.L-V.; writing—review and editing, C.F., R.L., and J.V.; supervision, C.F.

Declaration of Competing Interest

The authors declare no conflict of interest, and the funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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