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Enhancement of Carotenoids in Green Alga-*Botryococcus braunii* in Various Autotrophic Media under Stress Conditions

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ABSTRACT

Botryococcus braunii is a green colonial micro alga that is used mainly for the production of hydrocarbons, exopolysaccharides, and carotenoids. Growth and carotenoid production of *B. braunii* culture were studied by using autotrophic media such as CHU13, Z8, BBM and BG11 under stress conditions at high light intensity (3.5 ± 0.2 klux). The cultures were grown under low light (1.5 ± 0.2 klux) intensity at 25 ± 1°C temperature for a period of 14 days followed by exposure to stress conditions (0.1% sodium chloride, 0.1% sodium chloride with 0.1% sodium bicarbonate and 0.1% sodium chloride with 4 mM sodium acetate) and incubated further for a period of 21 days under high light intensity (3.5 ± 0.2 klux). Among the different autotrophic media used, BG11 and Z8 media were found to be the best for biomass and carotenoid production. The data in the table shows 3.6 and 3.2 (g/L) biomass with a carotenoid content of 0.28 and 0.27% (w/w) in BG11 and Z8 media with 0.1% sodium chloride, respectively. Under these conditions, the carotenoid profile indicated violaxanthin (6-9%), lutein (79-84%), astaxanthin (3-8%), zeaxanthin (0.32-0.78%), and β-carotene (1.75-2.14%), which are identified by mass spectrum. These results indicate that *B. braunii* culture can grow in a wide range of media and produce lutein as major carotenoid which can be enhanced under stress conditions.

Keywords: *B. braunii*, microalga, carotenoids, autotrophic media, stress, HPLC, LC-MS

INTRODUCTION

Micro algal production of high value added products, particularly carotenoids for human health and nutrition, are gaining importance during the recent years, but its broad industrial application still requires studies to improve the methods in order to be economically competitive in the market (Spolaore 2006). Lutein is one of the carotenoids recommended as a dietary supplement for humans that protects the macula against oxidation and, in general against the age-related macular deseases. The best known commercial micro algae, such as *Chlorella*, *Chlamydomonas*, and *Haematococcus* are unicellular green algae. Some species accumulate high concentrations of carotenoids under certain culture conditions. The extraction of β-carotene from *Dunaliella salina* has already reached large-scale production. Another promising carotenoid is astaxanthin, a high-value keto carotenoid extensively used as pigmentation source in aquaculture, especially for salmon and trout. Efforts have been made to produce astaxanthin in commercially viable manner from *H. pluvialis* (Lorenz and Cysewski 2000). *B. braunii* is a green colonial microalga belonging to the family *Chlorophyceae* and is grouped into three different races A, B and L depending on the type of hydrocarbons they synthesize (Dayananda *et al.* 2005; Zhang *et al*. 2007; Dayananda *et al.* 2010). Race A produces C_{23} to C_{33} odd numbered *n*-alkadienes, mono-, tri-, tetra-, and pentaenes, which are derived from fatty acids (Metzger *et al.* 1985, 1990). Race B produces C_{30} to C_{37} unsaturated hydrocarbons known as botryococcenes and small amounts of methyl branched squalenes (Sato *et al*. 2003; Metzger and Largeau 2005; Achitouv *et al.* 2004; Barupal *et al*. 2010; Weiss *et al*. 2010). Whereas race L, produces a single tetraterpenoid hydrocarbon known as lycopadiene (Metzger *et al*. 1990). Tetramethylsqualene can also be combined with long chain polyaldehydes and carotenoids to produce polyacetals and botryoxanthins (Metzger *et al*. 2007; Zhang *et al*. 2007). This alga is mainly known for the production of hydrocarbons, exopolysaccharides and carotenoids (Samori *et al*. 2010). *B. braunii* undergoes a color change because of the accumulation of secondary carotenoids in the matrix. The presence of carotenoids is more pronounced in races B and L (Grung *et al.* 1989). In the linear stage of growth, both the races produce almost equal amounts of β-carotene, echinenone, canthaxanthin, lutein, violaxanthin, loroxanthin, and neoxanthin. However, lutein is the major carotenoid (22-29%) reported in the linear phase of these races. Canthaxanthin (46%) together with echinenone (20-28%) are the dominating carotenoids in the stationary phase (Grung *et al*. 1989). Grung *et al*. (1994) reported the presence of adonixanthin in L race in stationary phase. Some newly identified carotenoids such as botryoxanthin-A (Okada *et al*. 1996), botryoxanthin-B, and α-botryoxanthin (Okada *et al.* 1998), braunixanthin 1 and 2 (Okada *et al.* 1997) isolated from the B race may contribute to the color of the algal colonies. The present study focused on the carotenoid enhancement in *B. braunii* in various autotrophic media under stress conditions.

MATERIALS AND METHODS

Chemicals

All the chemicals and solvents were of analytical and HPLC grade obtained from Ranbaxy Fine Chemicals Ltd. (Mumbai, India). Standard astaxanthin, violaxanthin, lutein, zeaxanthin, α-carotene and β-carotene were obtained from Sigma Chemicals Co. (St. Louis, MO, USA).

Algal culture, media and cultivation conditions

The sample was collected from Indian fresh water bodies of Kodaikanal (latitude 10.31N and longitude 77.32E), India (Dayananda *et al.* 2007) and cultured in modified CHU13 medium (Lar-

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Original Research Paper

geau *et al.* 1980). This strain was identified as 'A' race based on hydrocarbon profile. Stock cultures of *B. braunii* were maintained routinely on agar slants of CHU13 and BG11 media (Richmond 1986) as well as in liquid media. The cultures were incubated at 25 ± 1°C under 1.5 ± 0.2 klux light intensity with a 16-h photoperiod.

1. Growth and carotenoid production in different media

Growth and carotenoid production of *B. braunii* culture was studied in different autotrophic media such as BBM (Kanz and Bold 1969) CHU13 (Largeau *et al.* 1980), Z8 (Renstrom *et al.* 1981), BG11 (Richmond 1986), and with different stress conditions under high light (3.5 ± 0.2 klux) intensity. A set of Erlenmeyer flasks of 150 mL capacity containing 40 mL medium were maintained for each autotrophic medium studied. The culture flasks were inoculated at 20% (v/v) and incubated at 25 ± 1°C under 1.5 ± 0.2 klux light intensity with a 16-h photoperiod for a period of two weeks. Later the culture flasks were divided into two groups, one group was incubated at 25 ± 1°C with low light (1.5 ± 0.2 klux) and another group was incubated at high light (3.5 ± 0.2 klux) intensity with different stress conditions as follows. (1) Control (standard medium), (2) 0.1% sodium chloride, (3) 0.1% sodium chloride plus 0.1% sodium bicarbonate, (4) 0.1% sodium chloride plus 4 mM sodium acetate for a period of three weeks. The culture were harvested and analysed for biomass yield and carotenoid content. All the experiments were carried out in triplicate in two repeated experiments.

2. Biomass estimation

The cultures were harvested by centrifugation at 3,000 × *g* for 10 min and the cells were washed with distilled water. Then the pellet was freeze dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight (g/L).

3. Carotenoid estimation

Known amount of freeze-dried algal biomass was extracted with 90% acetone and absorbance was measured at 450 nm and the concentration of carotenoid was determined using the Davies (1976) method.

4. High performance liquid chromatography (HPLC) analysis of carotenoids

The carotenoid profile of stress-induced *B. braunii* cultures was analyzed by HPLC (LC-10A; Shimadzu) using a reversed phase (Wakosil 11 5C 18RS; Melbourne, Australia) 25 cm × 4.6 mm column with an isocratic solvent system consisting of acetonitrile/methanol/dichloromethane (70: 10: 20) at a flow rate of 1.0 mL/min and detected at 450 nm (Ranga Rao *et al.* 2006)

5. Liquid chromatography mass spectrometry in atmospheric pressure chemical ionization (LC-MS-APCI)

The carotenoids were identified in *B. braunii* culture by using a Waters 2996 modular HPLC system (auto-sampler, gradient pump, thermo-regulator and DAD), coupled to a Q-Tof Ultima (UK) mass spectrometer. In brief, APCI source was heated at 130°C and the probe was kept at 500°C. The corona (5 kv), HV lens (0.5 kv) and cone (30 v) voltages were optimized. Nitrogen was used as sheath and drying gas at 100 and 300/h, respectively. The spectrometer was calibrated in the positive mode, $[M+2H]^+$ and $[M+H]^+$ ions were recorded. Mass spectra of carotenoids were acquired with an m/z 400-600 scan range at 450 nm by a diode array detector and confirmed with respective standards (Ranga Rao *et al.* 2009).

Statistical analysis

All experiments were done in triplicates and the data presented are the averages of mean of three independent experiments with standard deviation. The data were analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel XP (Microsoft Corp., Redmond, WA), and the mean separations were performed by Duncan's multiple range test (DMRT) at $P < 0.05$.

Table 1 Influence of media on biomass and carotenoid production in *B. braunii*.

Media	Biomass yield g/L	Carotenoid (%)
CHU13		
Control (without addition)	1.7 ± 0.16 b	0.17 ± 0.04 a
0.1% NaCl	2.9 ± 0.03 a	0.18 ± 0.05 a
0.1% NaCl + 0.1% $NaHCO_3$	3.1 ± 0.07 a	0.23 ± 0.05 b
0.1% NaCl + 4 mM CH_3COONa	3.0 ± 0.27 a	0.20 ± 0.23 b
Z8		
Control (without addition)	3.1 ± 0.09 a	0.18 ± 0.02 c
0.1% NaCl	3.2 ± 0.03 a	0.27 ± 0.08 a
0.1% NaCl + 0.1% $NaHCO_3$	3.0 ± 0.01 a	0.23 ± 0.06 b
0.1% NaCl + 4 mM CH_3COONa	3.2 ± 0.02 a	0.25 ± 0.02 a
BBM		
Control (without addition)	2.4 ± 0.04 b	0.19 ± 0.02 b
0.1% NaCl	2.4 ± 0.08 b	0.16 ± 0.03 c
0.1% NaCl + 0.1% $NaHCO_3$	2.9 ± 0.02 a	0.22 ± 0.05 a
0.1% NaCl + 4 mM CH_3COONa	2.1 ± 0.10 c	0.14 ± 0.07 c
BG11		
Control (without addition)	3.2 ± 0.05 b	0.25 ± 0.05 b
0.1% NaCl	3.6 ± 0.02 a	0.28 ± 0.03 a
0.1% NaCl + 0.1% $NaHCO_3$	3.3 ± 0.04 b	0.23 ± 0.08 b
0.1% NaCl + 4 mM CH_3COONa	3.1 ± 0.08 c	0.23 ± 0.07 b

Data recorded on 21 days old culture. Values are mean ± SD. Values are not sharing a similar superscript with in the same column are significantly different ($P < 0.05$) as determined by DMRT.

RESULTS AND DISCUSSION

Growth pattern of *B. braunii* in various media

The growth profile of *B. braunii* culture as evaluated in CHU13, Z8, BBM and BG11 media is shown in **Table 1**. Among the media studied, BG11 and Z8 supported high biomass yields followed by CHU13 and BBM. *B. braunii* culture showed (**Table 1**) 3.6 and 3.2 (g/L) biomass with a carotenoid content of 0.28 and 0.27% (w/w) in BG11 and Z8 media with 0.1% sodium chloride, respectively. Dayananda *et al*. 2007 reported the biomass yield of 2.0 and 2.8 g/L in *B. braunii* culture (SAG 30.81 and LB-572) treated with different levels of BG11 media (Dayananda *et al*. 2007). Increase in biomass yield of *B. braunii* under light and dark conditions was reported by Tanio *et al*. (2010). Modifications of autotrophic or heterotrophic culture media was reported to improve productivities of biomass and carotenoid content in *Haematococcus pluvialis* (Tripathi *et al*. 1999). Fazeli *et al*. (2006) reported that 0.1 to 0.5 M salinity enchanced the total carotenoid production in *Dunaliella tertiolecta*.

Carotenoid composition of *B. braunii* analysed by HPLC

The carotenoid profile of *B. braunii* culture grown in various autotrophic media were analysed by HPLC. The order of carotenoids elution through a C_{18} column was as follows xanthophylls, chlorophylls, and carotenoids. Violaxanthin, astaxanthin, lutein, zeaxanthin and α- and β-carotene were identified using authentic standards. The xanthophylls and carotenoid samples were eluted under isocratic conditions within 21 min. The carotenoids were identified in *B. braunii* culture by mass spectra as shown in **Fig. 1** with positive-ion APCI, protonated molecules were observed at cone voltages ranging from 10 to 30 v. Spectra were generated from 10 µl injections of 50 ng/µL solutions. All these spectra are characterized by $[M+H]^+$ ions. Additional ions corresponding to $[M+H-H_20]^+$ was observed for astaxanthin, violaxanthin, lutein and zeaxanthin. Astaxanthin and zeaxanthin both showed significant $[M+H-H_20]^+$ ions in addition to $[M+H]^+$ ions. α- and β-carotene did not show $[M+H-H_20]^+$ ions pre-

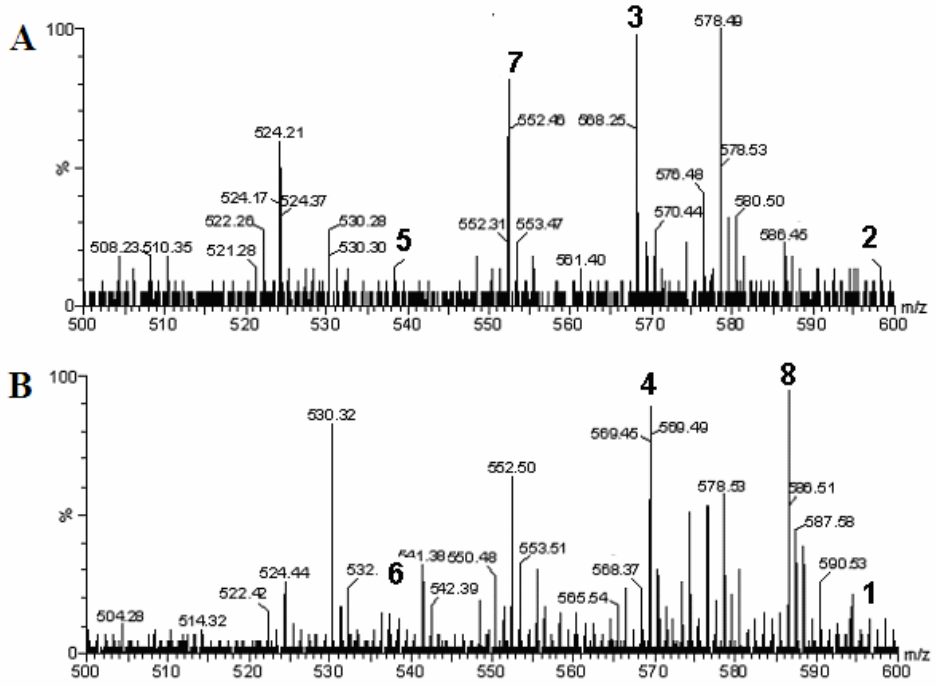

Fig. 1 LC-MS (APCI) profile of carotenoids from *B. braunii* culture. (**A**) BG11 media and (**B**) Z8 media with 0.1% NaCl, 1. Violaxanthin, 2. Astaxanthin, 3. Lutein, 4. Zeaxanthin, 5. α-Carotene, 6. β-Carotene, 7. β-Cryptoxanthin and 8. Antheraxanthin. Refer to materials and methods for LC-MS (APCI) conditions.

Table 2 Carotenoid profile of *B. braunii* grown in different media.

Media	Vio %	Ast %	Lut %	Zea %	α-car %	β-car %
CHU13						
1	6.47 ± 0.27 b	11.35 ± 0.15 b	59.46 ± 0.09 c	0.99 ± 0.08 b	1.88 ± 0.06 c	19.82 ± 0.02 b
2	4.70 ± 0.14 c	0.23 ± 0.03 d	69.70 ± 0.12 b	0.71 ± 0.06 bc	2.75 ± 0.12 b	22.11 ± 0.09 a
3	9.05 ± 0.10 a	4.79 ± 0.04 c	79.47 ± 0.11 a	0.55 ± 0.13 c	5.16 ± 0.16 a	0.96 ± 0.08 d
4	6.14 ± 0.05 d	15.14 ± 0.14 a	68.52 ± 0.19 b	1.26 ± 0.05 a	4.90 ± 0.09 a	3.73 ± 0.05 c
Z8						
1	6.16 ± 0.19 a	15.47 ± 0.38 b	68.57 ± 0.54 a	0.94 ± 0.03 b	4.91 ± 0.50 b	3.71 ± 0.12 a
2	6.25 ± 0.83 a	8.54 ± 0.22 d	79.10 ± 0.12 a	0.78 ± 0.05 c	3.15 ± 0.31 c	2.14 ± 0.06 b
3	7.33 ± 0.13 b	11.83 ± 0.10 c	75.22 ± 0.16 b	0.97 ± 0.08 b	3.35 ± 0.28 c	2.87 ± 0.07 b
4	5.12 ± 0.18 a	18.36 ± 0.12 a	69.59 ± 0.15 b	1.27 ± 0.10 a	5.64 ± 0.11 a	----
BBM						
1	4.68 ± 0.15 c	12.70 ± 0.10 a	54.81 ± 0.10 c	1.00 ± 0.13 a	4.45 ± 0.13 b	22.33 ± 0.11 a
2	9.09 ± 0.23 a	11.88 ± 0.12 b	64.38 ± 0.07 b	0.80 ± 0.18 b	2.55 ± 0.17 c	11.27 ± 0.17 c
3	6.00 ± 0.26 b	5.14 ± 0.31 c	70.74 ± 0.24 a	0.72 ± 0.11 c	14.53 ± 0.24 a	1.96 ± 0.08 d
4	4.56 ± 0.07 c	11.39 ± 0.15 b	59.22 ± 0.55 c	0.84 ± 0.14 b	4.13 ± 0.09 b	19.83 ± 0.31 b
BG11						
1	5.26 ± 0.19 b	13.14 ± 0.07 b	51.91 ± 0.11 c	1.65 ± 0.05 a	3.86 ± 0.03 b	2.16 ± 0.05 a
2	8.82 ± 0.10 a	3.01 ± 0.12 c	84.37 ± 0.08 a	0.32 ± 0.03 b	1.62 ± 0.07 c	1.75 ± 0.08 b
3	8.16 ± 0.09 a	5.03 ± 0.14 d	83.12 ± 0.24 a	0.48 ± 0.04 b	1.65 ± 0.05 c	1.52 ± 0.10 b
4	6.84 ± 0.08 b	16.28 ± 0.05 a	66.40 ± 0.20 b	1.47 ± 0.07 a	6.46 ± 0.08 a	2.52 ± 0.10 a

1. Control (without addition), **2.** 0.1% NaCl, **3.** 0.1% NaCl + 0.1% $NaHCO_3$, **4.** 0.1% NaCl + 4 mM CH_3COONa. Vio-violaxanthin, Ast-astaxanthin, Lut-lutein, Zea-zeaxanthin, α-car-α-carotene, β-car-β-carotene. Represented as relative % of total carotenoids. Data recorded on 21 days old culture. Values are mean ± SD. Values are not sharing a similar superscript with in the same column are significantly different ($P < 0.05$) as determined by DMRT.

sumably because these compounds do not contain hydroxyl or epoxy functional groups. These two compounds showed $[M+H]^+$ ions. The individual peaks were quantified using authentic standards and details were presented in **Table 2**. The carotenoid levels in BG11 and Z8 media with 1% NaCl was as follows violaxanthin (6-9%), lutein (79-84%), astaxanthin (3-8%), zeaxanthin (0.32-0.78%), and β-carotene (1.75-2.14%). Culture grown in CHU13 media, at 0.1% sodium chloride plus 0.1% sodium bicarbonate showed lutein content (79%), violaxanthin (9%), zeaxanthin (0.55%), and β-carotene (0.96%). In contrast, lutein (70%), astaxanthin (5.14%), violaxanthin (6%), zeaxanthin (0.72%), α-carotene (14.53%) and β-carotene (1.96%) were found in BBM media at 0.1% sodium chloride plus 0.1% sodium bicarbonate. The levels of lutein in the case of the B and L races were reported to be significantly lower than that of race A (Grung *et al.* 1989, 1994). Further, hydroxylation of hydrocarbon carotenoids is known to be responsible for the formation of 3-hydroxy cyclic carotenoids and epoxy carotenoids. The presence of traces of β-carotene in *B. braunii* may therefore be related to the conversion of these compounds to lutein. This may be a reason for the higher content of lutein in this alga. Lutein is the major carotenoid among the total carotenoids from *B. braunii* as reported by Ranga Rao *et al.* (2006).

Influence of sodium chloride and sodium bicarbonate in CHU13 medium

The growth profile of *B. braunii* culture in CHU13 medium supplemented with different concentration of sodium chloride and sodium bicarbonate was evaluated and the data is

Table 3 Influence of sodium bicarbonate and sodium chloride on biomass and carotenoid production in *B. braunii* grown in CHU13 medium.

Stress conditions	Biomass yield g/l	Carotenoid (%)
Control (without addition)	1.6 ± 0.04 d	0.150 ± 0.01 d
0.2% $NaHCO_3$	1.8 ± 0.08 d	0.155 ± 0.04 d
0.4% $NaHCO_3$	2.0 ± 0.23 c	0.162 ± 0.06 d
0.6% $NaHCO_3$	2.4 ± 0.05 c	0.167 ± 0.12 c
0.8% $NaHCO_3$	2.7 ± 0.13 b	0.174 ± 0.15 c
1% $NaHCO_3$	2.9 ± 0.06 a	0.194 ± 0.09 b
0.1% NaCl	3.0 ± 0.10 a	0.256 ± 0.14 a
0.2% $NaHCO_3$ + 0.1% NaCl	2.0 ± 0.18 c	0.148 ± 0.08 d
0.4% $NaHCO_3$ + 0.1% NaCl	2.2 ± 0.05 c	0.158 ± 0.13 d
0.6% $NaHCO_3$ + 0.1% NaCl	2.3 ± 0.07 c	0.170 ± 0.07 c
0.8% $NaHCO_3$ + 0.1% NaCl	2.6 ± 0.04 b	0.185 ± 0.01 b
1% $NaHCO_3$ + 0.1% NaCl	2.1 ± 0.03 c	0.165 ± 0.04 c

Data recorded on 21 days old culture. Values are mean ± SD. Values are not sharing a similar superscript with in the same column are significantly different ($P < 0.05$) as determined by DMRT.

Table 4 Effect of light on biomass yield and carotenoid production in *B. braunii* grown in CHU13 medium.

Light intensity	Biomass yield g/L	Carotenoid (%)
Low light (1.5 ± 0.2 klux)		
Control (without addition)	1.9 ± 0.05 c	0.22 ± 0.05 b
0.1% NaCl	2.3 ± 0.09 b	0.23 ± 0.02 a
0.1% NaCl + 0.1% $NaHCO_3$	1.6 ± 0.06 c	0.19 ± 0.06 c
0.1% NaCl + 4 mM CH_3COONa	1.8 ± 0.05 c	0.20 ± 0.01 b
High light (3.5 ± 0.2 klux)		
Control (without addition)	2.8 ± 0.09 a	0.21 ± 0.03 b
0.1% NaCl	2.6 ± 0.11 a	0.27 ± 0.08 a
0.1% NaCl + 0.1% $NaHCO_3$	2.1 ± 0.16 b	0.24 ± 0.06 a
0.1% NaCl + 4 mM CH_3COONa	2.5 ± 0.08 a	0.16 ± 0.04 d
0.2% NaCl + 0.1% $NaHCO_3$	2.2 ± 0.11 b	0.15 ± 0.02 d
0.4% NaCl + 0.1% $NaHCO_3$	2.7 ± 0.05 a	0.20 ± 0.01 b
0.5% NaCl + 0.1% $NaHCO_3$	2.4 ± 0.10 a	0.17 ± 0.05 c
1% NaCl + 0.1% $NaHCO_3$	2.8 ± 0.04 a	0.17 ± 0.03 c

Data recorded on 21 days old culture. Values are mean ± SD. Values are not sharing a similar superscript with in the same column are significantly different ($P < 0.05$) as determined by DMRT.

presented in **Table 3** in terms of biomass yield and total carotenoid production. The biomass yields and carotenoid content obtained was 3 g/L and 0.25% (w/w) and 2.9 g/L, 0.19% (w/w) respectively in cultures treated independently with 0.1% sodium chloride and 1% sodium bicarbonate. Optimum conditions for achieving high astaxanthin content (>1.4% w/w) and astaxanthin production (13 mg/L) were found to be with 31-50 mM sodium acetate and 0.55-0.63% (w/v) of sodium chloride as reported by Sarada *et al*. (2002). Increased biomass yields and carotenoid content in *B. braunii* when treated with sodium chloride (17 mM to 85 mM) was reported by Ranga Rao *et al*. (2007). The effect of different concentrations of NaCl (0.3M to 2M) and light intensities of 50 and 150 µmol/m²s on total carotenoids accumulation was 2.4, 2.1 and 1.4 folds high in *D. salina* (CCAP), *D. salina* (WT) and *D. tertiolecta*, respectively (Fazeli *et al*. 2006). Exposure of culture to high salt levels also increased the accumulation of carotenoids when compared to non-stressed cells (Pelah *et al*. 2004). *Haematococcus lacustris* accumulates astaxanthin and its intermediates under various stress conditions such as high irradiance, high temperature, nutrient deficiency and high salinity (Dong *et al.* 2007). As salinity increased, carotenoid production enhanced in *D. salina* (Hadi *et al*. 2008).

Effect of light intensity on growth and carotenoid production in *B. braunii* culture in CHU13 medium

The growth of *B. braunii* was observed in CHU13 medium at high (3.5 ± 0.2 klux) and low light (1.5 ± 0.2 klux) incubation conditions. The biomass and carotenoid contents were estimated in *B. braunii* culture. Under the high light conditions and 0.1% NaCl biomass obtained was 2.6 g/L with carotenoid content of 0.27% (w/w) which was comparable to or slightly higher than in control (**Table 4**). The biomass yield of 2.3 g/L and carotenoid content of 0.23% (w/w) were observed in low light conditions with 0.1% NaCl. Exposure of *B. braunii* culture to low or high light conditions significantly influenced the level of lutein accumulation (**Table 5**). Light is one of the most important factors responsible for carotenoid production in *H. pluvialis* (Boussiba *et al.* 1992; Kobayashi *et al.* 1992). A high light intensity of 3.5 klux caused relatively large quantities of lutein accumulation in the cells of *B. braunii.* Even though exposure to high light intensities resulted in cell death, those which survived contained large quantities of lutein. On the contrary, in lower light intensities the amount of lutein accumulated was comparatively low but the survival rates of the cells were higher. Ben-Amotz *et al.* (1985) found low contents of pigments except lipid in *B. braunii* cells when grown in 2.9% NaCl. The algae produce some metabolites to protect from salt injury and also to balance as per the surroundings osmotic (Richmond 1986). The effect of light intensity and its effect on β-carotene content of cells of *Dunaliella salina* and the volumetric production as well as the extraction rate was reported by Hejazi and Wijffels (2003). Canthaxanthin content was achieved in *Chlorella zofingiensis* under stress conditions and light intensity reported by Pelah *et al*. 2004. The light induced changes in β-carotene production and fatty acid content in *D. salina*

Table 5 Influence of light on carotenoid composition in *B. braunii* grown in CHU13 medium.

Media	Vio %	Ast %	Lut %	Zea %	α-car %	β-car %
Low light (1.5 ± 0.2 klux)						
1	4.32 ± 0.21 b	9.15 ± 0.12 a	56. 31± 0.05 b	1.32 ± 0.04 b	8.12 ± 0.62 a	19.82 ± 0.03 a
2	3.52 ± 0.18 b	8.23 ± 0.08 a	71.45 ± 0.18 a	1.89 ± 0.18 b	5.76 ± 0.23 b	8.61± 0.19 c
3	7.15 ± 0.15 a	6.72 ± 0.16 b	60.21 ± 0.16 b	3.81 ± 0.21 a	6.45 ± 0.18 b	15.34 ± 0.21 b
4	5.89 ± 0.17 b	9.34 ± 0.78 a	66.76 ± 0.12 a	1.67 ± 0.15 b	4.94 ± 0.14 c	12.36 ± 0.05 b
High light (3.5 ± 0.2 klux)						
1	5.12 ± 0.18 b	10.47 ± 0.25 a	67.14 ± 0.54 b	1.45 ± 0.23 c	5.89 ± 0.23 c	8.95 ± 0.37 c
2	4.25 ± 0.83 b	7.54 ± 0.22 c	80.56 ± 0.24 a	0.56 ± 0.43 c	2.14 ± 0.15 d	4.14 ± 0.56 d
3	6.21 ± 0.65 a	6.51 ± 0.36 c	75.22 ± 0.16 a	0.98 ± 0.54 c	1.38 ± 0.22 d	8.28 ± 0.86 c
4	5.12 ± 0.18 b	9.34 ± 0.12 b	59.22 ± 0.55 b	3.27 ± 0.10 b	10.64 ± 0.17 b	12.23 ± 0.98 b
5	3.68 ± 0.15 c	8.79 ± 0.15 b	50.91 ± 0.11 c	4.00 ± 0.13 a	10.23 ± 0.13 b	20.86 ± 0.11 a
6	3.12 ± 0.23 c	10.81 ± 0.12 a	62.21 ± 0.07 b	2.76 ± 0.54 b	4.55 ± 0.17 c	15.27 ± 0.17 b
7	6.00 ± 0.26 a	8.04 ± 0.31 b	53.43 ± 0.15 c	3.74 ± 0.21a	10.53 ± 0.24 b	17.85 ± 0.24 a
8	3.56 ± 0.07 c	10.32 ± 0.15 a	52.56 ± 0.18 c	4.82 ± 0.3 a	12.13 ± 0.09 a	14.83 ± 0.31 b

1. Control (without addition), **2.** 0.1% NaCl, **3.** 0.1% NaCl + 0.1% $NaHCO_3$, **4.** 0.1% NaCl + 4 mM CH_3COONa, **5.** 0.2% NaCl + 0.1% $NaHCO_3$, **6.** 0.4% NaCl + 0.1% $NaHCO_3$ **7.** 0.5% NaCl + 0.1% $NaHCO_3$, **8.** 1% NaCl + 0.1% $NaHCO_3$. Data represent mean ± SD of three replicates. Represented as relative % of total carotenoid. Data recorded on 21 days old culture. Values are mean ± SD. Values are not sharing a similar superscript with in the same column are significantly different ($P < 0.05$) as determined by DMRT.

reported by (Lamers *et al*. 2010). *Botryococcus* is known for hydrocarbon production and throughout the world efforts are continuing to improve its cultivation methodologies. In this context the present study has focused on an indigenous strain for its adaptability in different autotrophic media for growth and carotenoid production which could be a value addition to spent biomass after extraction of hydrocarbon. This alga represents a potential source of lutein, a commercially interesting carotenoid of application in aquaculture and poultry farming, as well as in the prevention of cancer and diseases related to retinal degeneration.

CONCLUSION

The present study has demonstrated that treatment of sodium chloride, sodium chloride with sodium bicarbonate to CHU13, Z8, BBM and BG11 media could enhance the biomass yield and carotenoid production in *B. braunii*. Moreover, high light intensity influenced lutein accumulation in *B. braunii*. These results indicate that the organism has the ability to grow in wide range of media and produces lutein as major carotenoid. *B. braunii* could be possibly a good source of lutein for uses in nutraceutical applications.

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REFERENCES

Achitouv E, Metzger P, Rager MN, Largeau C (2004) C_{31}–C_{34} methylated squalenes from a bolivian strain of *Botryococcus braunii*. *Phytochemisty* **65**, 3159-3165

Barupal DK, Kind T, Kothari SL, Lee DY, Fiehn O (2010) Hydrocarbon phenotyping of algal species using pyrolysis gas chromatography mass spectrometry. *BMC Biotechnology* **10**, 40

Ben-Amotz A, Tornabene TG, Thomas WH (1985) Chemical profile of selected species of micro algae with special emphasis on lipids. *Journal of Phycology* **21**, 72-81

Boussiba S, Fan L, Vonshak A (1992) Enhancement and determination of astaxanthin accumulation in green alga *Haematococcus pluvialis*. *Methods in Enzymology* **213**, 386-391

Davies BH (1976) Carotenoids. In: Goodwin TW (Ed) *Chemistry and Biochemistry of Plant Pigments*, Academic Press, London, pp 38-166

Dayananda C, Sarada R, Bhattacharya S, Ravishankar GA (2005) Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*. *Process Biochemistry* **40**, 3125-3131

Dayananda C, Sarada R, Usha Rani M, Shamala TR, Ravishankar GA (2007) Autotrophic cultivation of *Botryococcus braunii* for the production of hydrocarbons and exopolysaccharides in various media. *Biomass and Bioenergy* **31**, 87-93

Dayananda C, Sarada R, Vinod Kumar, Ravishankar GA (2007) Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies. *Electronic Journal of Biotechnol*ogy **10**, 1-14.

Dayananda C, Kumudha K, Sarada R, Ravishankar GA (2010) Isolation, characterization and outdoor cultivation of green microalgae *Botryococcus* sp. *Scientific Research and Essays* **5 (17)**, 2497-2505

Dong QL, Zhao XM, Xing XY, Hu JZ, Gong JX (2007) Mechanism of salt stress inducing astaxanthin synthesis in *Haematococcus pluvialis*. *Huaxue Gongcheng/Chemical Engineering* **35**, 45-47

Fazeli MR, Fighi H, Samadi N, Jamalifar H (2006) Effects of salinity on β-carotene production by *Dunaliella tertiolecta* DCCBC26 isolated from the Urmia salt lake, north of Iran. *Bioresource Technol*ogy **96**, 2453-2456

Fazeli MR, Tofighi H, Samadi N, Jamalifar H, Fazeli A (2006) Carotenoids accumulation by *Dunaliella tertiolecta* (Lake Urmia isolate) and D*unaliella salina* (ccap 19/18 & wt) under stress conditions. *DARU* **14**, 146-150

Grung M, Metzger P, Liaaen Jensen S (1989) Primary and secondary carotenoids in two races of green *Botryococcus braunii*. *Biochemistry System Ecology* **17**, 263-269

Grung M, Metzger P, Liaaen Jensen S (1994) Algal carotenoids 53; secondary carotenoids of algae 4; secondary carotenoids in the green alga *Botryococcus braunii*, race L, new strain. *Biochemistry System Ecology* **22**, 25-29

Hadi MR, Shariati M, Afsharzadeh S (2008) Microalgal biotechnology: Carotenoid and glycerol production by the green algae *Dunaliella* isolated from the Gave-khooni salt marsh, Iran. *Biotechnology and Bioprocess Engineering* **13**, 540-544

Hejazi MA, Wijffels RH (2003) Effect of light intensity on β-carotene production and extraction by *Dunaliella salina* in two-phase bioreactors. *Biomolecular Engineering* **20**, 171-175

Kanz T, Bold HC (1969) Morphological and taxonomical investigation of *Nostoc* and *Anabaena* in culture. In: *Physiological Studies*, University of Texas, Austin, TX, Publication No. 6924

Kobayashi M, Kakizono T, Nishio S, Nagai S (1992) Effects of light intensity, light quality and illumination cycle on astaxanthin formation in the green alga *Haematococcus pluvialis*. *Journal of Fermentation Bioengineering* **74**, 61-63

Lamers PP, Van de Laak CCW, Kaasenbrood PS, Lorier J, Janssen M, De Vos RCH, Bino RJ, Wijffels RH (2010) Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. *Biotechnology and Bioengineering* **106**, 638-648

Largeau C, Caradevall E, Berkaloff C, Dhamliencourt P (1980) Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. *Phytochemisty* **19**, 1043-1051

Lorenz T, Cysewski GR (2000) Commercial potential for *Haematococcus* micro algae as a natural source of astaxanthin. *Trends in Biotechnol*ogy **18**, 160-167

Metzger P, Allard B, Casadevall E, Berkaloff C, Couté A (1990) Structure and chemistry of a new chemical race of *Botryococcus braunii* that produces lycopadiene, a tetraterpenoid hydrocarbon. *Journal of Phycology* **26**, 258-266

Metzger P, Berkaloff C, Casadevall E, Coute A (1985) Alkadiene and botryococcene producing races of wild strains of *Botryococcus braunii*. *Phytochemisty* **24**, 2305-2312

Metzger P, Largeau C (2005) *Botryococcus braunii*: A rich source for hydrocarbons and related ether lipids. *Applied Microbiology and Biotechnol*ogy **66**, 486-496

Metzger P, Rager MN, Largeau C (2007) Polyacetals based on polymethylsqualene diols, precursors of algaenan in *Botryococcus braunii* race B. *Organic Geochemistry* **38**, 566-581

Okada S, Mastuda M, Yamaguchi K (1996) Botryoxanthin-A a new member of the new class of carotenoids from green microalga *Botryococcus braunii*, Berkeley. *Tetrahedron Letters* **37**, 1065-1068

Okada S, Tonegawa I, Mastuda M, Murakami M, Yamaguchi K (1997) Braunixanthins 1 and 2, new carotenoids from the green microalga *Botryococcus braunii*. *Tetrahedron* **53**, 11307-11316

Okada S, Tonegawa I, Mastuda M, Murakami M, Yamaguchi K (1998) Botryoxanthin-B and alpha botryoxanthin-A from green microalga *Botryococcus braunii*. *Phytochemisty* **47**, 1111-1115

Pelah D, Sintov A, Cohen E (2004) The effect of salt stress on the production of canthaxanthin and astaxanthin by *Chlorella zofingiensis* grown under limited light intensity. *World Journal of Microbiology and Biotechnology* **20**, 483-486

Ranga Rao A, Dayananda C, Sarada R, Shamala TR, Ravishankar GA (2007) Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresource Technology* **98**, 560-564

Ranga Rao A, Sarada R, Baskaran V, Ravishanakar GA (2009) Identification of carotenoids from green alga *Haematococcus pluvialis* by HPLC and LC-MS (APCI) and their antioxidant properties. *Journal of Microbiology and Biotechnology* **19**, 1333-1341

Ranga Rao A, Sarada R, Baskaran V, Ravishankar GA (2006) Antioxidant activity of *Botryococcus braunii* extract elucidated *in vitro* models. *Journal of Agricultural and Food Chemistry* **54**, 4593-4599

Renstrom B, Borch G, Skulberg OM, Jensen SL (1981) Optical purity of (3S, 3'S) astaxanthin from *Haematococcus pluvialis*. *Phytochemisty* **20**, 2561-2564

Richmond A (1986) *CRC Handbook of Micro Algal Mass Culture*, CRC Press, Inc. Boca Raton, Florida, USA, 127 pp

Samori C, Torr C, Samorì G, Fabbri D, Galletti P, Guerrini F, Pistocchi R, Tagliavini E (2010) Extraction of hydrocarbons from microalga *Botryococcus braunii* with switchable solvents. *Bioresource Technology* **101**, 3274-3279

Sarada R, Sila Bhattacharya, Bhattacharya S, Ravishankar GA (2002) A response surface approach for the production of natural pigment astaxanthin from green alga, *Haematococcus pluvialis* effect of sodium acetate, culture age, and sodium chloride. *Food Biotechnology* **16**, 107-120

Sato Y, Ito Y, Okada S, Murakami M, Abe H (2003) Biosynthesis of the triterpenoids, botryococcenes and tetramethylsqualene in the B race of *Botryococcus braunii* via the non-mevalonate pathway. *Tetrahedron Letters* **44**, 7035-7037

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. *Journal of Biosicence Bioengineering* **101**, 87-96

Tanoi T, Kawachi M, Watanabe MM (2010) Effects of carbon source on growth and morphology of *Botryococcus braunii*. *Journal of Applied Phycology* in press

Tripathi U, Sarada R, Ramachandra Rao S, Ravishankar GA (1999) Production of astaxanthin in *Haematococcus pluvialis* cultured in various media.

Bioresource Technology **68**, 197-199

Weiss TL, Chun HJ, Okada S, Vitha S, Holzenburg A, Laane J, Devarenne TP (2010) Raman spectroscopy analysis of botryococcene hydrocarbons from the green microalga *Botryococcus braunii*. *Journal of Biological Chemistry* **285**, 32458-32466

Zhang Z, Metzger P, Sachs JP (2007) Biomarker evidence for the co-occurrence of three races (A, B and L) of *Botryococcus braunii* in El Junco Lake, Galápagos. *Organic Geochemistry* **38**, 1459-1478