

Carotene and Antioxidant Capacity of *Dunaliella Salina* Strains

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Abstract Beta-carotene is a terpenoid pigment that is highly valuable due to its nutritional benefit as a precursor of vitamin A and its antioxidant properties. A marine green alga *Dunaliella salina* is well known for high carotene, with above 95% β -carotene, under growth-limiting conditions. Carotene contents are different among *D. salina* strains and under different culture conditions. Selecting a *Dunaliella salina* strain with high carotene amount for mass cultivation is crucial. Hence, this study aimed to select a candidate *Dunaliella salina* for carotene production. Analysis of total carotene contents and antioxidant capacities from 8 different local isolated *D. salina* strains (A9, A10, A11, A12, A13, D, E and G) and 2 imported strains (*D. salina* CCAP 19/18 and *D. bardawil* DCCBC 15) revealed that *D. bardawil* DCCBC 15 excelled than other strains basing on total carotene and antioxidant capacity per cell and per volume of culture.

Keywords: *Dunaliella salina*, *Dunaliella bardawil*, carotene, Antioxidant activity

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1. Introduction

Dunaliella is a unicellular chlorophyte, which can grow in a wide range of harsh environmental conditions such as subzero temperatures, extreme salinities, pH, and lights (Oren 2005, Ben-Amotz et al., 2009). Among *Dunaliella* species, *Dunaliella salina* is able to accumulate large amount of β -carotene (more than 10% of dry weight) under proper inductive conditions. Most of the accumulated β -carotene, mainly consisting of the 9-*cis* and all-*trans* isomer, is currently being used as a food coloring agent and pro-vitamin A in animal food; additive to cosmetics; multivitamin preparations and health food products (antioxidant and anti-cancer agent), and in the medical treatment of diseases (Ben-Amotz et al. 1982; Ben-Amotz & Avron 1983; Ben-Amotz et al. 1988; Ben-Amotz & Avron 1990; Borowitzka et al. 1990; El-Baky et al., 2004; Çelekli and Dönmez, 2006). Carotene contents are different among *D. salina* strains and under different culture conditions. Therefore selecting a *Dunaliella salina* strain with high carotene amount for mass cultivation is crucial. This is our partial report in an effort of continuous searching a candidate *Dunaliella salina* for carotene production.

2. Materials and Methods

2.1. *Dunaliella* Strains and Culture Conditions

Ten *Dunaliella salina* strains including *Dunaliella salina* var. *bardawil* DCCBC 15 (*D. bardawil*) and

Dunaliella salina CCAP 19/18 which were kindly provided by Dr. E.W. Polle, Department of Biology, Brooklyn College of CUNY Brooklyn, NY (USA), and 8 local *Dunaliella salina* isolates from Viet Nam (A9, A10, A11, A12, A13, D, E and G) were used. The algae were grown and maintained in the low cost modified natural seawater medium 1.5M (MD4) according to Tran *et al.* (2014). Briefly, the medium contained natural seawater, enriched with NPK 0.1 g/l, MgSO₄ 1.86 g/l, EDTA 0.00876 g/l, FeCl₃ 0.00049 g/l, MnCl₂ 0.00189 g/l, NaHCO₃ 50 mM, pH = 7.5. All cultures were maintained at 25°C and continuous light of 50 μ mol photons/m²/s.

2.2. Experimental Design

Dunaliella strains were grown in 1.5 M medium (MD4) for 12 days and subjected to salinity stress by adding NaCl to the cultures to obtain a salinity of 4M. Analysis of growth was performed every 3 days by cell counting. Carotene contents and antioxidant capacities were analyzed on day 12 (before salinity stress), day 15 and 36 (after salinity stress).

2.3. Growth Determination

Cell number was counted using a light microscope with 0.1 mm deep counting chamber (Neubauer Haemocytometer) every three days. Cell number was calculated using the following formula: Number of cells/ml = total counted cells x 10⁴ x dilution factor.

2.4. Total Carotene

One ml of algal suspension was centrifuged at 1000xg for 5 min and the pellet was extracted with 3 ml of ethanol:

hexane 2:1 (v/v). Two ml of water and 4 ml of hexane were added and the mixture was vigorously shaken and centrifuged again at 1000×g for 5 min. The hexane layer was separated and its absorbance was determined at 450 nm. Total carotene was calculated: $A_{450} \times 25.2$, equal the micrograms of carotene in 1 ml of sample (Shaish et al., 1992; Prieto et al., 2011).

2.5. Antioxidant Activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) solution was prepared by dissolving 0.004 g of DPPH in 100 ml of methanol (Romeilah et al., 2010; Das et al., 2011). One milliliter of algae was centrifuged in 10000 rpm at 4°C for 15 min and pellet was extracted with 1ml ethanol absolute and gently vortex. The extract was let stand at 4°C for 4 hours and added with 2ml DPPH solution. The mixture was incubated for 30 min in dark at room temperature. A blank sample (absolute ethanol) was also taken as control. Absorbance at 517 nm of the extract was determined spectrophotometrically. Antioxidant activity was calculated based on the inhibition of free radical DPPH in percent according to the formula: $I\% = (A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}} \times 100$ (Yaltirak et al., 2009; Albayrak et al., 2010; Tran et al., 2014).

2.6. Data Analysis

Data was processed in Excel and analyzed by one-way ANOVA using SPSS software. All significant levels were set at $p < 0.05$.

3. Results and Discussion

Growth varied depending on different *D. salina* strains (Figure 1), a common characteristic of strain specificity (Phadwal and Singh, 2003). Of the 10 strains investigated, *D. salina* CCAP 19/18 and *D. salina* D produced higher cell number than others. Generally these strains were transiently stressed after being subjected to higher salinity, but they re-adapted following 3 days and maintained stable growth phase for a long period (Figure 1). Exceptionally *D. bardawil* and *D. salina* D didn't reveal recovery after salinity stress induction, and *D. salina* D experienced the most severe stress based on cell decrease. This sensitivity to salinity and recovery capacity under stress is one of attributes probably involving to carotene genesis.

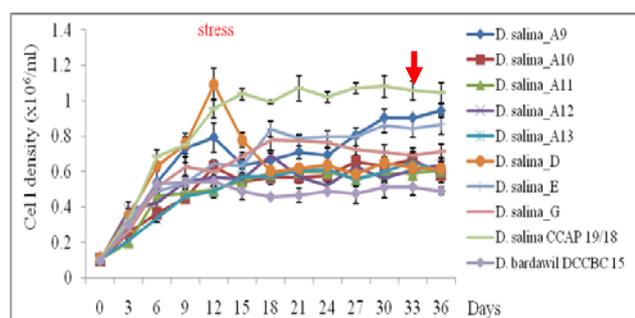


Figure 1: Growth of *Dunaliella salina* strains in MD4 medium at a salinity of 1.5M, and then were subjected to salinity stress at a salinity of 4M on day12 for carotene induction

Carotene amounts and antioxidant capacities of extracts from *Dunaliella* strains increased after salt stress, and *D. bardawil* showed the first candidate in this selection regarding carotene content and antioxidant capacity (Figure 2 and Figure 3). All local *D. salina* isolates produced more carotene than the imported *D. salina* CCAP19/18; this characteristic could probably be due to *Dunaliella* CCAP19/18 experienced less salinity stress under current culture conditions (Figure 1) which inversely related to its ability of carotenogenesis.

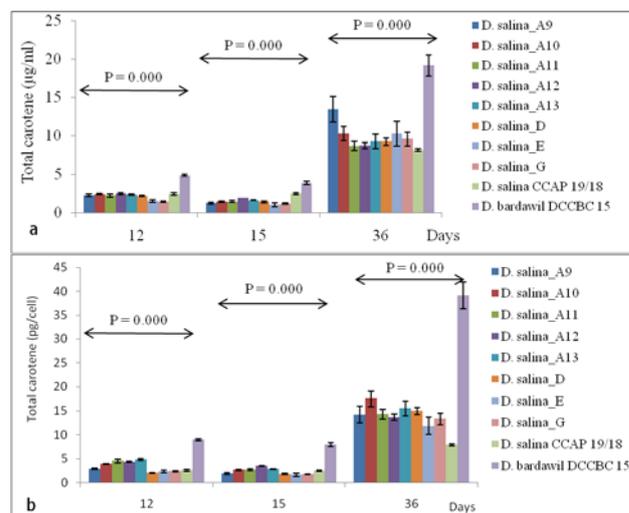


Figure 2. Total carotene contents of *Dunaliella salina* strains per culture volume (a) and per cell (b) with significant statistic value (P)

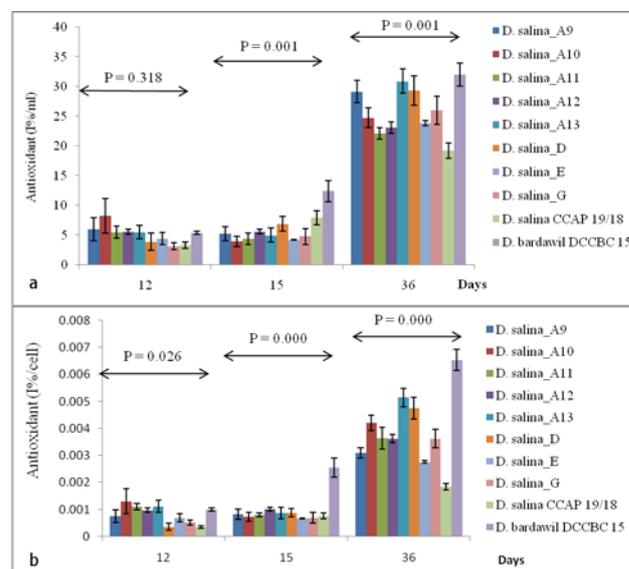


Figure 3. Antioxidant capacity of extracts from *Dunaliella salina* strains based on culture volume (a) and per cell (b) with significant statistic value (P)

Regarding carotene productivity (per volume of culture), the local *D. salina* A9 provided higher potential following *D. badawil* (Figure 2a). In contrast, *D. salina* A10 revealed better carotene induction per cell. Both these characteristics are ideal in strain selection for carotene exploitation or any other specific compounds, which were attained from *D. bardawil*. Carotenenes are valuable compounds with high antioxidant capacity (Ben-Amotz 1982, Borowitzka 1990, Tran et al. 2014). The carotene content from extract of *D. bardawil* supported its

antioxidant capacity based on both per volume of culture and per cell (Figure 2 and Figure 3); this trend seemed to be maintained from extracts of *D. salina* A9 and *D. salina* A10. However, it was not well supported by local isolates *D. salina* A13 and *D. salina* D, which could be due to other antioxidants involved such as phenolic compounds, vitamins etc (Balsano and Alisi 2009).

4. Conclusion

All *D. salina* strains investigated could resist to salinity stress at 4M. A local isolate *D. salina* A9 revealed its out-competence than other local strains regarding carotene productivity and antioxidant exploitation capability. Together with *D. salina* A9, the imported strain *D. bardawil* deserves to be considered for further pilot culture under climate conditions in Vietnam. Other local isolates are potential and need further investigation of proper conditions for higher carotene induction and antioxidant activity.

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