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Effects of Salinity and Light on Growth of *Dunaliella* Isolates

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Abstract Dunaliella salina, halotorelant unicellular green algae, is the main natural source of beta-carotene. Several strains of local Dunaliella salina were isolated. Together with Dunaliella bardawil DCCBC 15 and Dunaliella salina CCAP 19/18, the strains were examined for their growth under the effects of salinities (1 M, 1.5 M and 2 M) and light intensities (50, 100 and 150 µmol photon/m^2/s). The result showed optimal growth for Dunaliella was obtained at 1.5 M and 2 M salinities and 50 µmol photon /m^2/s light intensity. Data of this study will be further applied for carotenogenic induction experiments using salinity and light stresses on these Dunaliella salina strains.

Keywords: *Dunaliella, growth, salinity, light intensity*

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1. Introduction

The unicellular motile green algae of the genus *Dunaliella* are among the most widespread eukaryotic organism in hyper saline environments, which shows a remarkable degree of adaptation to a variety of salt concentrations from 0.2% to saturation (around 35%) (Ben-Amotz and Avron 1983, 1990). *Dunaliella* was first described by Teodoresco in 1905 (Oren 1999). Species of the genus lack a rigid cell wall and have a single large cupshaped chloroplast that fills the posterior part of the cell (Nader et al. 2011).

Among Dunaliella species, Dunaliella salina is able to accumulate large amounts 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, are 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-Amotzet al. 1988; Ben-Amotz & Avron 1990; Borowitzkaet al. 1990; El-Baky et al., 2004; Çelekli and Dönmez, 2006). Carotene content is different among D. salina strains and under different culture conditions. The objective of this study was to determine growth of Dunaliella salina strains under different conditions of salinity and light intensity as basis for further experiments of carotenoids induction using salinity and light stresses.

2. Materials and Methods

2.1. The Microalgae

The experiments were carried out on 10 *Dunaliella salina* strains including 8 local *Dunaliella salina* isolates (J, K, M, N, O, P, Q and R) and 2 imported *Dunaliella bardawil* DCCBC 15 *Dunaliella salina* CCAP 19/18 kindly provided by Dr. E.W. Polle, Department of Biology, Brooklyn College of CUNY Brooklyn, NY (USA).

2.2. Experiments

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, and was added with NPK 0.1 g/l, $MgSO_4$ 1.86 g/l, EDTA 0.00876 g/l, $FeCl_3$ 0.00049 g/l, $MnCl_2$ 0.00189 g/l, $NaHCO_3$ 50mM, pH = 7.5.

Dunaliella strains was cultivated at three salinities (1, 1.5 and 2 M) and three different light intensity (50, 100 and 150 µmol photon/m^2/sec) in 50 ml flasks at 25°C temperature. Each strain was triplicate in each experiment, and all experiments were repeated at least twice.

2.3. Growth Estimation

Cell density was estimated by optical density, cell number, every three days. Briefly, optical density (OD) was measured at 750 nm (A_{750}) by Microplate Reader (Biotek) and cell number was counted using a light microscope with 0.1 mm deep counting chamber (Neubauer Haemocytometer). Lugol solution (5% iodine and 10% potassium iodide in distilled water) was used to stop cell movement.

Cell number was calculated by following formula: Number of cells/ml = total cells counted x 10^4 x dilution factor.

Specific growth rate (G: divisions/day) and cell growth productivity (P: cells/ml/day) were determined using equations according to Levasseur et al. (1993):

$$G = \ln(N_t / N_0) / t; P = (N_t - N_0) / t$$

Where: N_t and N_0 are cell density at time t and time 0 respectively.

2.4. Statistical Analysis

Data was analysed by one-way ANOVA using SPSS software. All significant levels were set at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Different Salinities on Dunaliella Growth

Growth of *Dunaliella* strains at different salinities was shown in Figure 1 (a,b,c). *Dunaliella* cells number reached the stationary growth phase after 12 days. The specific growth rate of *Dunaliella* was highest at 1M salinity for *D. salina* J; 1.5 M for *D. salina* N, O, P, Q, R and *bardawil*; and 2M for *D. salina* K, M and *D. salina* CCAP (Figure 1d). Generally specific growth rate and cell growth productivity revealed growth of all Dunaliella salina were better at 1.5 M and 2M salinities (Figure 1d, Figure 1e).

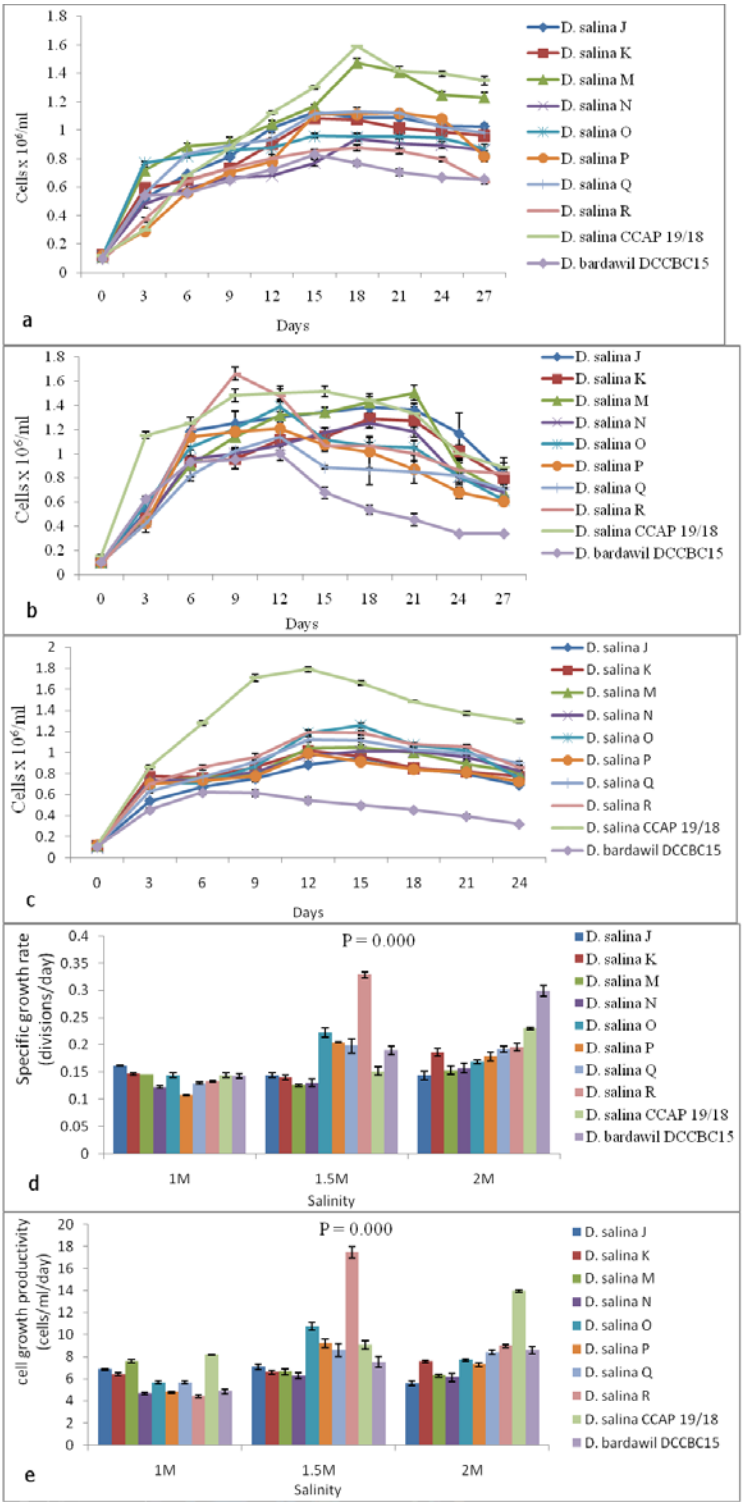

Figure 1. The growth curve of Dunaliella salina strains at 1M (a), 1.5 M(b), 2M (c); and their specific growth rate (d), growth productivity (e) at different salinities with significant statistic value (P)

3.2. Effect of Light Density on Dunaliella Growth

Figure 2 showed the average number of *Dunaliella* per ml grown at different light intensities. Growth was observed highest at 50 µmol/m^2/s and decreased with increasing light intensity up to 100 µmol/m^2/s. In particular, growth rate and productivity up to 0.36 div./day and 12.6 cells x 10^4/ml/day at 50 µmol/m^2/s were obtained (Figure 2d, Figure 2e).

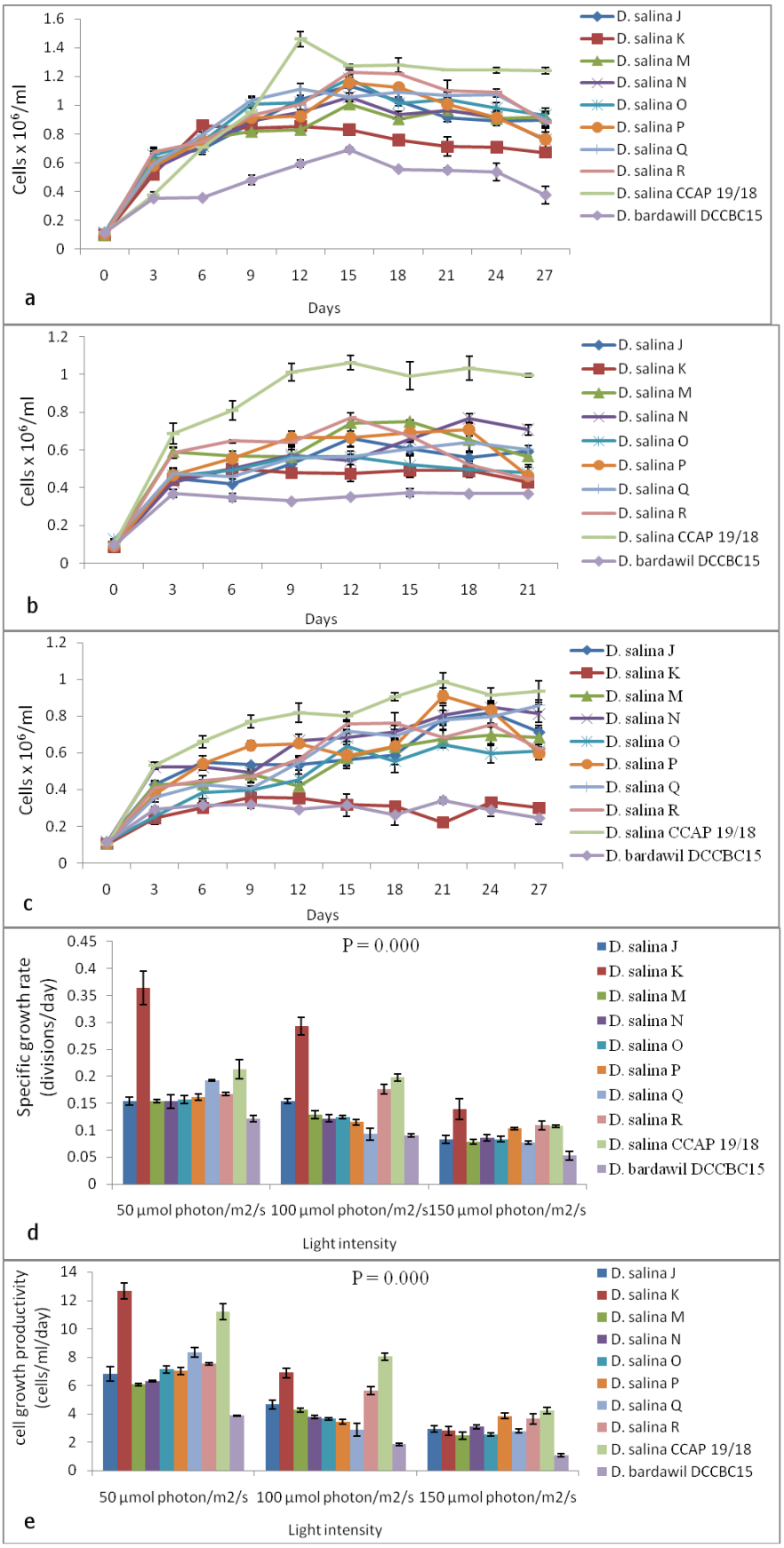

Figure 2. The growth curve of Dunaliella salina strains at 50 µmol/m^2/s (a), 100 µmol/m^2/s (b), 150 µmol/m^2/s (c); and their specific growth rate (d), growth productivity (e) at different light intensities with significant statistic value (P)

4. Conclusion

The results of the experiments showed that all *Dunaliella salina* strains referred salinity ranging from 1.5 M to 2 M for optimal growth. Also, the data suggested that growth performance for these *Dunaliella* was better at light intensity of 50 µmol photon/m^2/s. Based on these optimal salinity and light intensity, we will apply stress conditions for carotene induction on these strain in our next experiments.

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