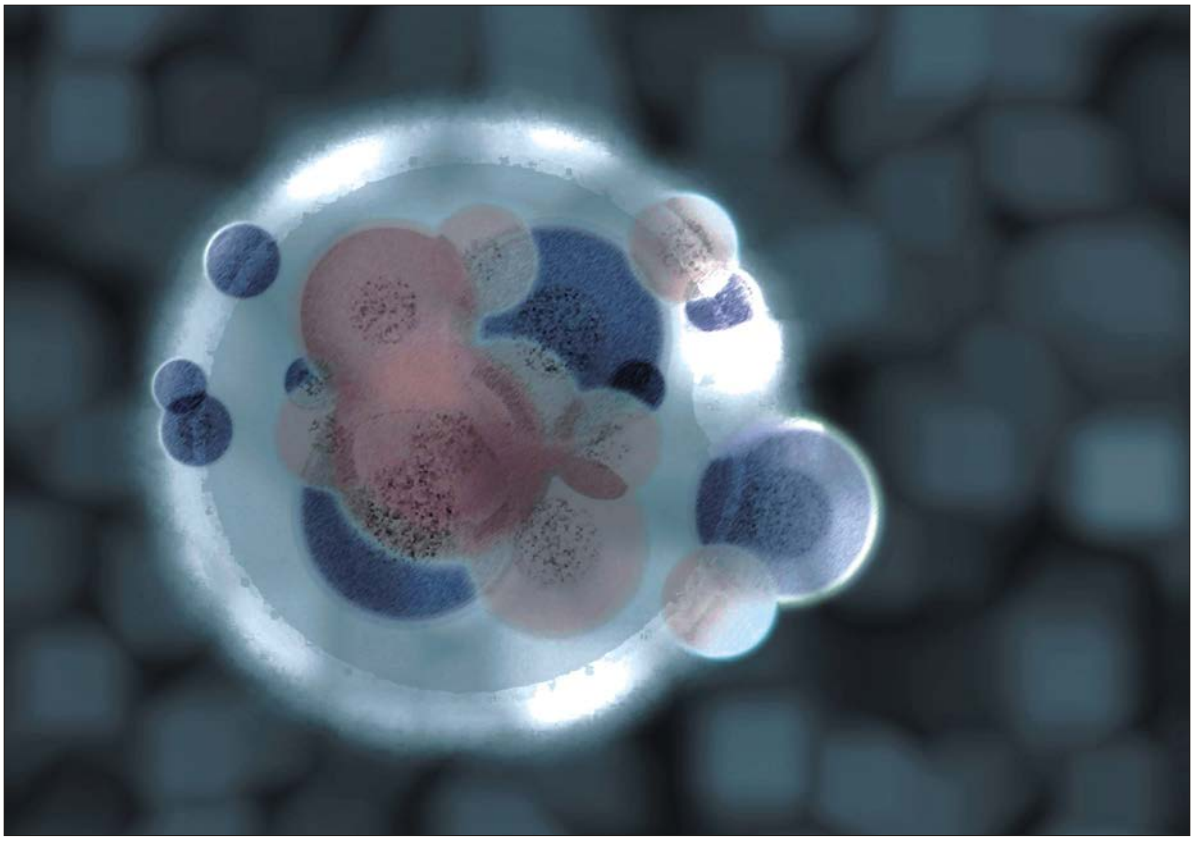

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Effects of Light Intensity, Salinity and Temperature on Growth in Çamaltı Strain of *Dunaliella viridis* Teodoresco from Turkey

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Abstract: In this study, *Dunaliella viridis* was isolated from Çamaltı salt work and its growth rate, cell density, chlorophyll-a and total carotenoids content were studied in a batch system. This strain was cultured at different NaCl concentrations (1, 2 and 3M), different temperatures (25 and 28°C) and different light intensities (50 and 75 μmol photon/m^2/sec). In this experiment, maximum growth rate was at 2 M salinity with 28°C temperature and 50 μmol photon/m^2/sec light intensity. Maximum cell density for *D. viridis* was obtained at 25°C, 50 μmol photon/m^2/sec, 2M cultures. The highest chlorophyll-a and total carotenoids were calculated as 2.84±0.50 and 1.11±0.05 pg $cell^{-1}$, respectively. The optimum temperature and salinity for growth of *D. viridis* strain were around 25°C and 2 M NaCl. The present study shows that cell densities and pigment yields of *D. viridis* Çamaltı strain are strongly dependant on salinity, temperature and light intensity.

Key words: *Dunaliella viridis*, salt works, growth parameters, salinity, pigment yield

INTRODUCTION

The unicellular green algae of the genus *Dunaliella* are among the most widespread eukaryotic organisms in hyper saline environments, and shows a remarkable degree of adaptation to a variety of salt concentrations from as low as 0.2%, to about 35% (Ben-Amotz and Avron, 1983, 1990). It is an obligatory phototrophic, oxygenic, aerobic, unicellular organism (Javor, 1989). *Dunaliella* species are lack of a rigid cell wall, ovoid in shape and contain large cup-shaped chloroplast with two equal flagella (Borowitzka and Borowitzka, 1992).

Dunaliella salina (Dunal) Teodoresco and *D. viridis* Teodoresco are predominant microalgae species in solar salt works (Davis, 1990). Under stress conditions such as lack of nitrogen sources, high salinities and high levels of irradiance, *D. salina* stores large amounts of β-carotene, a pigment which is used as pro-vitamin A in animal food, as a food coloring agent and as an additive to health food products (anti-cancer and antioxidant agent) (Ben-Amotz and Avron, 1990). Because of this ability, the emphasis of research was placed on the mass culture of this species (Ben-Amotz and Avron, 1983; Borowitzka *et al*., 1984; Borowitzka, 1986). Different from *D. salina* and *D. viridis* was considered a pest because it appeared to compete with *D. salina* and reduced β-carotene yield (Borowitzka *et al*., 1984; Moulton *et al*., 1987). However, *D. viridis* could be a potential candidate for mass culture on commercial scale; it produces predominantly oxygenated carotenoids (Moulton and Burford, 1990).

Dunaliella growth responses are complicated interactions of many variables such as temperature, salinity and light intensity. Optimum values of these variables depend on the species. *Dunaliella viridis* grows optimally in 5.8-8.9% (w/v) NaCl and tolerates up to 23.2% (Borowitzka *et al*., 1977; Borowitzka and Borowitzka, 1992). The optimum temperature for *D. viridis* lies in the range 14 to 30°C, with an upper limit for survival of about 35°C (Gibor, 1956). The marine *D. bioculata* and *D. primolecta* have temperature optima between 25 and 29°C and *D. tertiolecta* grew optimally at 30°C (Goldman, 1977).

The objective of this study was to determine growth rates, cell density, chlorophyll-a and carotenoid content of *Dunaliella viridis* Çamaltı strain under different combinations of temperature, salinity and light intensity.

MATERIALS AND METHODS

Isolation of *Dunaliella viridis* and cultivation: *Dunaliella viridis* cells were isolated from the Çamaltı solar salt works (İzmir, Turkey). The water temperature and salinity of the salt works were measured from 10 to 27°C and from 0.32 to 23.8%, respectively in 6-7 months period between the years 2004-2006. Total nitrogen concentration ranged from 0.08 to 3.08 μmol L^{-1}. Identification of isolates were established based on morphological characters following Preising (1992). After isolation; stock cultures was established under laboratory conditions (25± 1°C, 50 μmol photon/m^2/sec) in a modified Johnson Medium (Johnson *et al*., 1968) at 2 M NaCl.

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The composition of the modified Johnson Medium was as follows: $MgCl_2$, 1.5 g L^{-1}; KCl, 0.2 g L^{-1}; $CaCl_2$ 1.5 g L^{-1}; $NaNO_3$ 1.5 g L^{-1}; $NaHCO_3$ 0.043 g L^{-1}; KH_2PO_4 0.035 g L^{-1}; Fe solution (Na_2EDTA, 189 mg L^{-1}; $FeCl_3.6H_2O$, 244 mg L^{-1}), 10 mL and trace metal solution (H_3BO_3, 61.0 mg L^{-1}; $(NH_4)MO_7O_{24}.4H_2O$, 38.0 mg L^{-1}; $CuSO_4.5H_2O$, 6.0 mg L^{-1}; $CoCl_2.6H_2O$, 5.1 mg L^{-1}; $ZnCl_2$, 4.1 mg L^{-1}; $MnCl_2.4H_2O$, 4.1 mg L^{-1}), 10 mL.

Experimental culture conditions: *Dunaliella viridis* strain was cultivated at three NaCl concentrations (1, 2 and 3 M) in 1L flasks at two different temperatures (25, 28°C) and two light intensities (50 and 75 μmol/m^2/sec). These experimental conditions were selected to monitored the in cell density and pigment composition (chlorophyll a and total carotenoids) over time. Cultures of *D. viridis* at the mid-exponential phase were used for inoculation. Cells were grown using modified Johnson Medium and NaCl added as needed to obtain target salinity. Experiments were conducted over a 30 day period. These experiments were conducted between December 2005 to July 2006.

Analytical methods: For the extraction of chlorophyll-a and total carotenoids, 5 mL of algal culture was taken daily from each flask. The cells were pelleted by centrifugation (Sigma, 1-6) at 5000 rpm for 10 min at room temperature and then resuspended in 5 mL of 90% acetone. Cellular debris was removed by centrifugation at 5000 rpm for 10 min into a screw cap tube. The concentration of chlorophyll a and total carotenoids in the supernatant was spectrophotometrically at 450, 630, 645 and 663 nm wavelengths. Chlorophyll-a and total carotenoids were calculated using the equations of Scor-Unesco (1966). Absorbance measurements were made by using a Jasco UV/Visible Spectrophotometer. Algal growth was monitored by counting cells numbers in a counting chamber (Neubauer Heamocytometer). Specific growth rate (μ) and doubling time (d.t.) were calculated as in following equation:

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

$$\text{d.t.} = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$$

where, X_2 and X_1 represent the cell density at the times t_2 and t_1, respectively.

Statistical analysis: Data were tested for homogeneity (Levene). Analysis of variance (ANOVA) and t-test were used to determine the significance of the differences between treatments.

RESULTS

Growth of *D. viridis* Çamaltı strain at different salinities, temperatures and light intensities is shown in Fig. 1. Maximum cell density for *D. viridis* was obtained at 25°C, 50 μmol photon/m^2/sec, 2 M cultures (8.56±0.12 $\times 10^6$ cell mL^{-1}) and the lowest concentrations were at 25°C, 50 μmol photon/m^2/sec, 3 M cultures (4.92±0.25 $\times 10^6$ cell mL^{-1}).

Temperature clearly affected the cell density in *D. viridis*. The optimum temperature for growth of *D. viridis* strain was around 25°C. There was a significant decrease ($p<0.002$) of the maximum cell number with increasing in temperature. At low salinities cells grew much faster than at high salinities and the length of the growth phase decreased with decreasing salinity. Significant differences in cell density were found at the end of cultivation period for all tested salinity degrees ($p<0.05$). No significant differences in cell density were found for two light intensities values ($p>0.05$). Increasing the light intensities resulted in decreasing in maximum cell numbers (Table 1).

The highest chlorophyll-a content per cell was calculated as 2.84±0.50 pg $cell^{-1}$ at 25°C, 50 μmol photon/m^2/sec and 3 M cultures. Between the all experimental groups, the lowest chlorophyll-a content was obtained from 28°C, 75 μmol photon/m^2/sec,

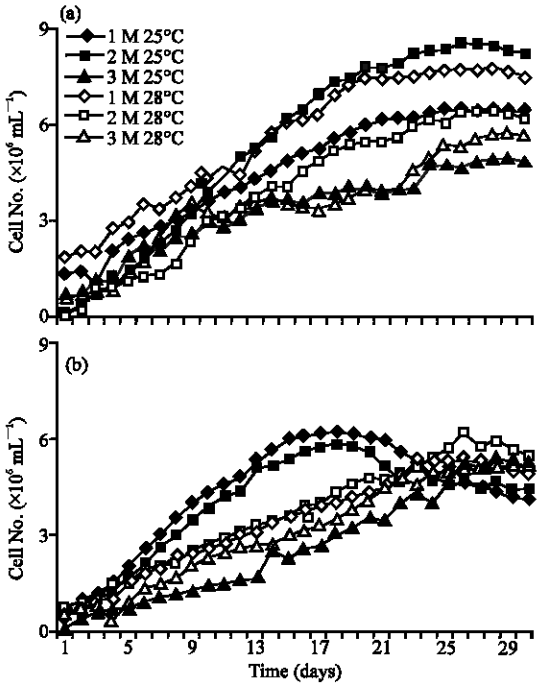

Fig. 1: Increase in cell density under the conditions of (a) 50 and (b) 75 μmol photon/m^2/sec light intensities at 1, 2 and 3M salinity and 25 and 28°C temperatures

Table 1: Specific growth rates, doubling times and maximum cell densities at 1, 2 and 3 M salinities, 25 and 28°C temperatures and 50 and 75 μmol photon/m²/sec light intensities

Light intensity (μmol photon/m²/sec)	Temperature (°C)	Salinity (M)	Specific growth rate (day⁻¹)	Doubling time (day)	Maximum cell density (×10⁶ cell ml L⁻¹)
50	25	1	0.77	0.90	6.46
		2	0.72	0.94	8.56
		3	0.58	1.18	4.92
	28	1	0.31	2.25	7.76
		2	1.08	0.64	6.50
		3	0.56	1.23	5.74
75	25	1	0.44	1.56	6.07
		2	0.35	2.00	7.14
		3	0.72	0.96	5.93
	28	1	0.40	1.74	5.31
		2	0.37	1.88	6.21
		3	0.97	0.72	5.12

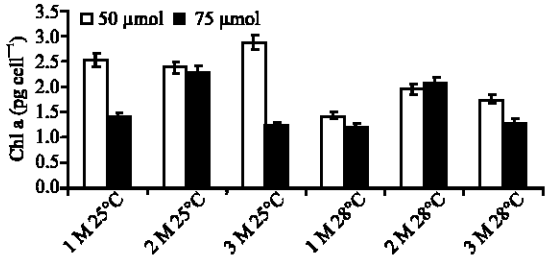

Fig. 2: Maximum chlorophyll a concentrations per cell in *D. viridis* grown at different light intensities, salinities and temperatures

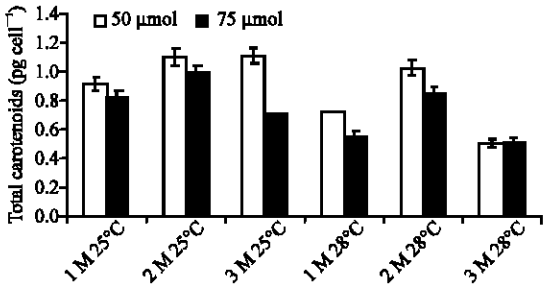

Fig. 3: Maximum total carotenoids concentrations per cell in *D. viridis* grown at different light intensities, salinities and temperatures

1 M cultures (1.19±0.20 pg cell^{-1}) (Fig. 2). Chlorophyll-a concentration was affected by temperature and light intensity. Increasing temperature and light caused chlorophyll content to decrease. Salinity of the culture affected the chlorophyll production as well and maximum content was achieved with 3 M in 25°C and 2 M in 28°C. Significant differences in chlorophyll a content per cell were found for all tested temperature ($p<0.05$), salinity ($p<0.05$) and light intensity ($p<0.05$) values.

Between experimental groups, carotenoid content ranged from a minimum of 0.50±0.09 pg cell^{-1} to a maximum of 1.11±0.05 pg cell^{-1} (Fig. 3). In *D. viridis* cultures, carotenoid content is obviously affected by salinity. The maximum carotenoid accumulation on per cell was achieved with 3 M in 25°C and 2 M in 28°C. And also, the highest carotenoid production (pg cell^{-1}) decreases with increasing light intensity and temperature (Fig. 3). Significant differences in carotenoid content per cell were found for all tested temperature ($p<0.05$), salinity ($p<0.05$) and light intensity ($p<0.05$) values.

DISCUSSION

In the present study, the effect of light intensity, salinity and temperature on growth of *D. viridis* Çamaltı strain was determined. The maximum specific growth rate of 1.08 day^{-1} reported here for *D. viridis* Çamaltı strain with a doubling time of 0.64 d at salinity around 2 M NaCl; at lower or higher salt concentration this strain did not grow well. This specific growth rate is in agreement with that of Ginzburg and Ginzburg (1981), who reported doubling times for members of members of the *D. viridis* type at 29°C, 2 M NaCl. It has been observed to grow optimally at salinity around 1M NaCl (5.8%) (Jiménez and Niell, 1991), however from salt works in Mexico, *D. viridis* has been reported to grow well at 15-20% NaCl concentrations (García *et al*., 2007). The Fig. 3 is comparable to the results of the study.

The optimum temperature for the growth of *D. viridis* was around 30°C, as has been earlier reported by Gibor, (1956) and Jiménez and Niell, (1990). The highest growth of *D. viridis* of Çamaltı salt works was found at 25°C (Table 1). This result agrees with the findings of Jiménez and Niell (1991), who studied *D. viridis* Yucatan strain.

There was also a clear decrease of both chlorophyll a and carotenoids content with increasing light intensity. On a per cell basis, chlorophyll a and carotenoids concentrations were the highest at 50 μmol photon m²/sec light intensity. This was probably due to the fact that growth at higher light intensity was faster, so pigment accumulation could not be promoted. By the adaptation of microalgae to high light, the dimensions of light harvesting antenna lessen, and thylakoid membranes become more efficient, which is a natural process. During so called photoacclimation phenomenon, cellular chlorophyll components come to minimum and thylakoid membranes start to work more efficiently (Falkowski, 1980; Prezelin and Matlick, 1980; Ramus, 1990). Salinity also has a strong influence in pigment production (Borowitzka and Borowitzka, 1992). Maximum pigment yield increased with

the increasing of salinity to up to 2 M; however at higher concentration there was a decrease in pigment yield. This result agrees with the findings of Jiménez and Niell, (1991). Data on growth characteristics of *D. viridis* from Çamaltı salt work will help better understanding the production system. This study shows that cell divisions and pigment yields of *D. viridis* Çamaltı strain are strongly dependant on salinity, temperature and light intensity.

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