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

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Abstract: In this study, Dunaliella viridis was isolated from Čamaltı salt works 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/m²/sec). In this experiment, maximum growth rate was at 2 M salinity with 28°C temperature and 50 µmol photon/m²/sec light intensity. Maximum cell density for D. viridis was obtained at 25°C, 50 µmol photon/m²/sec, 2M cultures. The highest chlorophyll-a and total carotenoids were calculated as 2.84±0.50 and 11.4±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 Čamaltı 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, 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 Čamaltı 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 Čamaltı 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/m²/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: MgCl₂, 1.5 g L⁻¹; KCl, 0.2 g L⁻¹; CaCl₂ 1.5 g L⁻¹; NaNO₃, 1.5 g L⁻¹; NaHCO₃ 0.043 g L⁻¹; KH₂PO₄ 0.035 g L⁻¹; Fe solution (Na₂EDTA, 189 mg L⁻¹; FeCl₃·6H₂O, 244 mg L⁻¹), 10 mL and trace metal solution (H₃BO₃, 61.0 mg L⁻¹; (NH₄)₆Mo₇O₂⁴, 4H₂O, 38.0 mg L⁻¹; CuSO₄·5H₂O, 6.0 mg L⁻¹; CoCl₂·6H₂O, 51.0 mg L⁻¹; ZnCl₂, 4.1 mg L⁻¹; MnCl₂·4H₂O, 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 1 L flasks at two different temperatures (25, 28°C) and two light intensities (50 and 75 μmol/m²/sec). These experimental conditions were selected to monitor 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 Scour-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 Haemocytometer). Specific growth rate (μ) and doubling time (d.t.) were calculated as in following equation:

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

\[
d.t. = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}
\]

where, \(X_0\) and \(X_t\) represent the cell density at the times \(t_0\) 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* Çamlıca 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/m²/sec, 2 M cultures (8.56±0.12 x10⁶ cell mL⁻¹) and the lowest concentrations were at 25°C, 50 μmol photon/m²/sec, 3 M cultures (4.92±0.25 x10⁶ 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/m²/sec and 3 M cultures. Between the all experimental groups, the lowest chlorophyll-a content was obtained from 28°C, 75 μmol photon/m²/sec.

![Fig. 1: Increase in cell density under the conditions of (a) 50 and (b) 75 μmol photon/m²/sec light intensities at 1, 2 and 3 M salinity and 25 and 28°C temperatures](image-url)
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

<table>
<thead>
<tr>
<th>Light intensity (μmol photon/m²/sec)</th>
<th>Temperature (°C)</th>
<th>Salinity (M)</th>
<th>Specific growth rate (day⁻¹)</th>
<th>Doubling time (day)</th>
<th>Maximum cell density (10⁶ cell mL⁻¹)</th>
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<tbody>
<tr>
<td>50</td>
<td>25</td>
<td>1</td>
<td>0.77</td>
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<td>0.72</td>
<td>0.94</td>
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<td>3</td>
<td>0.98</td>
<td>1.18</td>
<td>4.92</td>
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<td></td>
<td>28</td>
<td>1</td>
<td>0.31</td>
<td>2.25</td>
<td>7.76</td>
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<td>2</td>
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<tr>
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<td>1</td>
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<td>0.97</td>
<td>0.72</td>
<td>5.12</td>
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</table>

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 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* Çamalı strain was determined. The maximum specific growth rate of 1.08 day⁻¹ reported here for *D. viridis* Çamalı 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 1 M 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 (Garcia 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 Çamalı salt works was found at 25°C (Table 1). This result agrees with the findings of Jiménez and Niell (1991), who studied *D. viridis* Yucaatan 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 photocaccumulation 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 Çamalı salt works will help better understanding the production system. This study shows that cell divisions and pigment yields of D. viridis Çamalı strain are strongly dependent on salinity, temperature and light intensity.

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