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Preliminary Assessment of β-carotene Accumulation in Four Strains of *Dunaliella salina* Cultivated under the Different Salinities and Low Light Intensity

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Abstract: Four strains of *Dunaliella salina* (I, G1, A and MUR8) were cultivated under the three salinity regimes (1, 2 and 3 M NaCl) to examine the effect of salinity on the β-carotene synthesis per cell and to compare the β-carotene production per culture volume and the rate of growth. During the 30 days of the experiments the batch cultures maintained in the 300 flasks containing 100 of Johnson's medium under the low irradiance (100 μmol photon m^{-2} s^{-1}) with a 12/8 h light/dark regime and a continuous shaking. The results showed that the inductive effect of salinity on β-carotene accumulation per cell was strain dependent and when the values for the strains IR-1 and Gh-U were slightly higher at 3 M NaCl compare to lower salinities, there were no direct relationships between the concentrations of salt and the β-carotene contents per cell in other two strains (MUR-8 and Au-W). On the other hand, increase the salinity result in serious decreasing in the cell number and the β-carotene content per culture volume of all strains during the experiment. The highest β-carotene content per (14.2 mg L^{-1}) was obtained at 1 M NaCl in the suspension culture of the strain Au-W.

Key words: β-carotene, *Dunaliella salina*, salinity, strain selection

INTRODUCTION

Dunaliella genus are unicellular, motile, green algae which can grow in wide range of salt concentration ranging from 0.5% to saturation around 35% and in contrast to other algae have no a rigid cell wall. Among them, *D. salina* is the best commercial source of natural β-carotene in the world (Borowitzka, 1995). This alga accumulates large amounts of β-carotene as droplets in the chloroplast in conditions such as high light intensity, increased temperature, high salinity and nutrient deficiency to prevent chlorophyll photo-damage (Ben-Amotz and Avron, 1983; Ben-Amotz and Shaish, 1992; Salguero *et al*., 2003). The β-carotene content of up to 14% of dry weight has been reported for *D. salina* (Borowitzka and Borowitzka, 1990). Natural β-carotene is used in the food, cosmetic and pharmaceutical industries as a colorant, antioxidant, anti-tumor agent and heart diseases preventive in addition to its characteristic as precursor of Vitamin A (Davison *et al*., 1993; Laurent *et al*., 2005; Tornwall *et al*., 2004). Among the factors, which influence the productivity of biomass and β-carotene content in *D. salina*, the salinity is very important. Some studies have shown a direct relationship between β-carotene production and growth-limiting conditions such as increasing salinity (Ben-Amotz and Avron, 1983; Ben-Amotz and Avron, 1990; Borowitzka *et al*., 1984). Therefore, several strategies have been used for the induction of β-carotene in *Dunaliella* mass cultures by changing the concentration of salt (Borowitzka and Borowitzka, 1990; Hosseini Tafreshi and Shariati, 2006). Other authors commented that the salinity does not have a clear effect on β-carotene accumulation per cell (Gomez *et al*., 2003; Orset and Young, 1999). It seems that the effect of salinity like other inducing factors on β-carotene production in *D. salina* is strain dependent and that only a few strains of *Dunaliella* have the potential to produce the β-carotene to up to 10% of the algal dry weight under appropriate condition (Ben-Amotz and Avron, 1983; Lers *et al*., 1990). The present study, considered the effect of increasing the salinity on growth rates and pigment contents of four existing strains of *D. salina* during a period of 30 days and examined their possible potency for use in mass β-carotene production.

MATERIALS AND METHODS

The strains of *Dunaliella* utilized in this experiment were: strain IR-1, isolated from the salt marsh of Gavkhoni, Iran; strain Gh-U, a kind gift from the Biotechnology Center of Gheshm, Iran; strain Au-W, a kind gift from the

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Prof. Lilley s laboratory at the University of Wollongong, Australia and strain MUR8, a kind gift from Prof. Borowitzka at the Murdoch University, Australia. The experiment was accomplished during year 2005 in the Biology Department of the University of Isfahan, Iran. Cells were cultivated separately in 300 mL cotton-plugged Erlenmeyer flasks containing 100 modified Johnson's medium (Johnson *et al*., 1968), at three different salinities (1, 2 and 3 M NaCl). The batch cultures were maintained in a growth cabinet at a temperature of 27/24°C (light/dark) and an irradiance of 100 μmol photon m^{-2} s^{-1} with a 12/8 h light/dark regime and continuously were shake on an orbital shaker at around 80 rpm for a time period of 30 days. The sampling performed at the days of 0, 2, 5, 8, 12, 16, 19, 23, 27 and 30 to study the trends of the cell concentration and the quantitative changes of β-carotene per cell and per culture volume during the experiment.

The cell concentration was calculated by cell counting using a light microscope and haemocytometer. Pigments were determined spectrophotometrically in 85% acetone extracts using equations previously derived (Eijckelhoff and Dekker, 1997). The mean and standard deviation values of the parameters were calculated. All statistical analyses of variance (one-way ANOVA) were performed using SPSS Software.

RESULTS AND DISCUSSION

It has been claimed that severe conditions such as high salinity, low nutrient levels, high irradiance and high temperature induce β-carotene production in the cell but, at the same time decrease the cell number per culture volume by affecting cell division (Ben-Amotz and Avron, 1983, 1990; Ben-Amotz and Shaish, 1992; Borowitzka *et al*., 1990; Marin *et al*., 1998). Indeed, the higher the salinity and as a result the lower the growth rate of the alga, the more is the amount of the light absorbed by the cell during one division cycle. This situation can led to the higher accumulation of β-carotene per cell. In contrast, other studies did not find such a clear pattern on the carotenoids accumulation per cell in different *D. salina* strains (Orset and Young, 1999; Gomez *et al*., 2003). The time dependences of β-carotene accumulation per cell by the four strains in response to different salt concentrations were different (Fig. 1). The general decreasing in the β-carotene contents of all strains observed at the beginning of the growth period is due to the rapid cell division during the exponential phase of growth and consequently dilution of carotenoids per cell. According to Fig. 1 the β-carotene contents of the strains IR-1 and Gh-U on the basis of per cell were

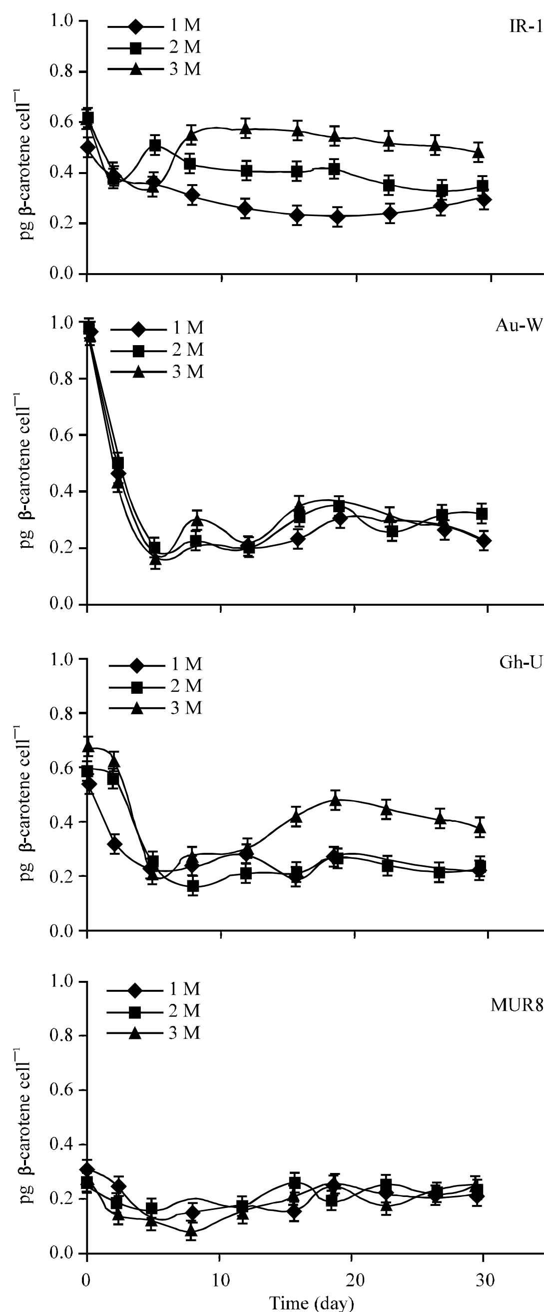

Fig. 1: Quantitative changes in β-carotene as a function of time per cell of four strains of *Dunaliella salina* (IR-1, Au-W, Gh-U and MUR8) cultured in three different NaCl concentrations (1, 2 and 3 M) in the medium. The values are means of three replicates±SD

slightly higher at higher salinities, compare to lower concentrations of salt during the experiment whereas, there were no significant differences among the trends of

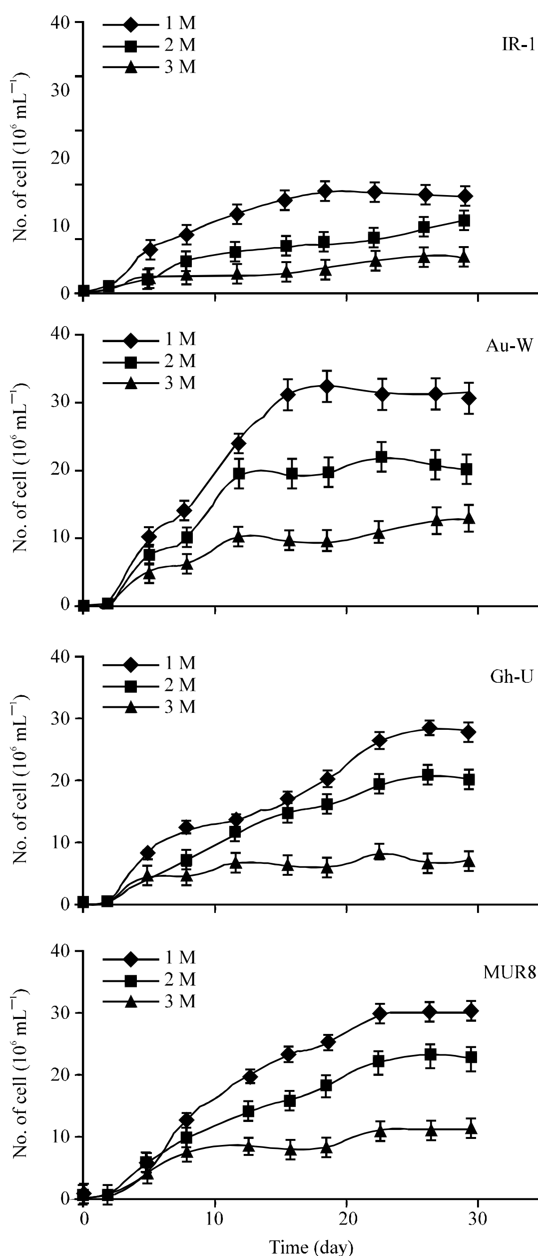

Fig. 2: Cell concentrations of four strains of *Dunaliella salina* (IR-1, Au-W, Gh-U and MUR8) cultured in three different NaCl concentrations (1, 2 and 3M) in the medium. The values are means of three replicates±SD.

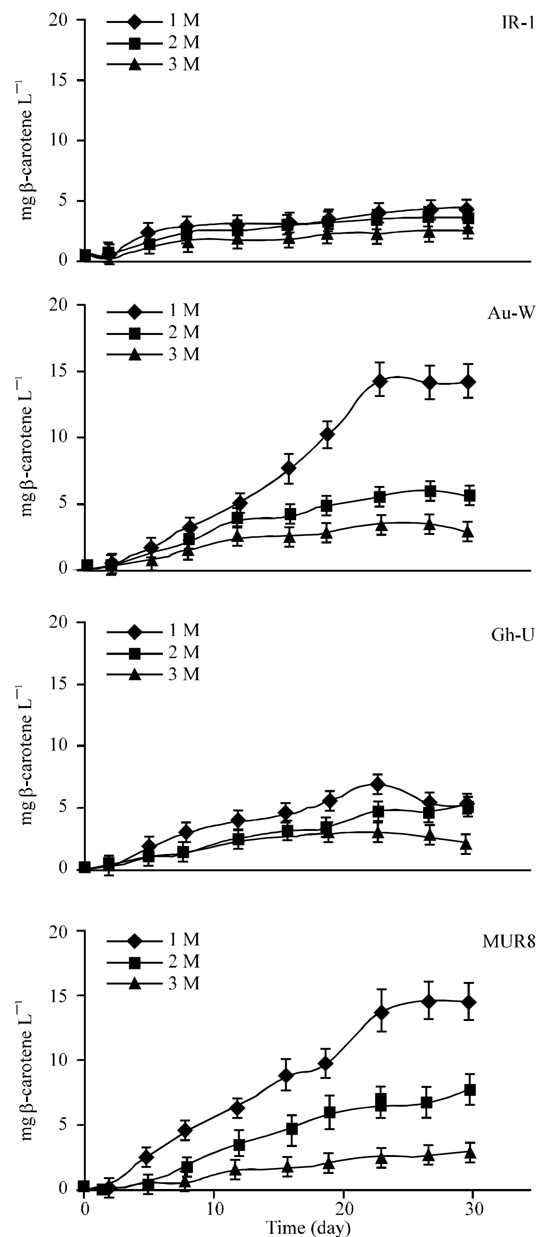

Fig. 3: Quantitative changes in β-carotene as a function of time per unit volume of four strains of *Dunaliella salina* (IR-1, Au-W, Gh-U and MUR8) cultured in three different NaCl concentrations (1, 2 and 3 M) in the medium. The values are means of three replicates±SD

β-carotene production per cell of other two strains (Au-W and MUR8) in these conditions. This shows that the presence of a direct relationship between the β-carotene accumulation in *Dunaliella* and the salt concentration in the medium is strain dependent. The highest β-carotene content per cell was observed in strain IR-1 at 3 M NaCl, which was not significant, compare to that of other studies. For example, total carotenoids contents of eight strains of *D. salina* isolated from salt ponds in Chile, ranged from 4-to 42 pg cell^{-1} (Cifuentes *et al*., 1992) whereas, the maximum β-carotene concentration in present study was only 0.6 pg cell^{-1}.

The time dependence of cell growth (growth curves) of four strains at different salinities shown in Fig. 2. Increase in the salt concentration of the suspension cultures resulted in a general decreasing in cell number per culture volume of all strains. This is accordance with the results of Marin *et al.* (1998) who showed that *D. salina* isolated from the Araya salt ponds in Chile can grow faster during the exponential phase at the salinity of 9% than the higher salinities. In this experiment, the levels of the salt effect on cell division of four strains were different. It seems that the growth rate of the strain IR-1 at all salinities is lower than that of the other strains whereas, the growth rate of the strains Au-W and MUR8 are highest in these conditions. Thus, it can be conclude that these two strains can genetically grow better in different salinities than the other two strains (IR-1 and Gh-U). In comparison, the cell density maxima recorded in our experiment (> 30×10^6 cell mL^{-1}) were relatively higher than those reported by Ben-Amotz and Avron (1990) who found that the cell density maxima for large and small-celled species of *D. salina* were 2×10^6 and 20×10^6 cells mL^{-1}, respectively.

The most important part of the results is formed by the time dependences of β-carotene production on the basis of per unit volume (mL) by the four strains shown in Fig. 3. According to this data, in spite of the highest β-carotene content per cell of strain IR-1 in present experiment, the β-carotene content of this strain per mL was lower compare to that of other strains seemingly due to the genetically lowest growth rate of this strain at all salinities during the growth period. Figure 3 shows well that the β-carotene contents per mL of four strains were decreased at higher salinities which can easily attributed to the number of cell severely decreased by the salinity stress. The highest β-carotene content per unit volume was obtained at 1 M NaCl being for strain Au-W (14.2 mg L^{-1}). From a commercial point of view, it is desirable to obtain not only high β-carotene content per culture volume and per unit time but also high β-carotene to chlorophyll ratio. The β-carotene to chlorophyll ratio up to 10.0±2.0 has been reported for *D. bardawil*, a related strain of *D. salina* (Ben-Amotz, 1995). Although in this experiment, the β-carotene concentrations per mL of the suspension cultures were comparable to those reported by Cifuentes *et al.* (1992) (7.2 to 38.2 mg L^{-1}) for eight Chilean strains, but the ratios of β-carotene to chlorophyll were much lower in this study (data was not shown). This is because the β-carotene content per cell is significantly much low in present study (Fig. 1).

Ben-Amotz and Avron (1983) showed that the salinity and irradiance have an additive or synergistic effect on carotenogensis in *D. salina*. However, prolonged nutrient starvation can led to high mortality of algae. Therefore, it is recommended that adjusting light and salinity likely is one of the best strategies to achieve optimal β-carotene production in mass cultures of *D. salina* (Marin *et al.*, 1998). In addition, increase the salinity can cause the growth inhibition of *Dunaliella* predators and non-carotenogenic *Dunaliella* species naturally occur in open ponds (Borowitzka and Borowitzka, 1990). The conditions of our experiment (low light intensity = 100 μmol photon m^{-2} s^{-1}) were not very effective for massive accumulation of carotenoids compare to other experiment. One comment is that in spite of reduction in the division cycles of the strains in higher salinities, the overall β-carotene productivities were low because the cells would not be subjected to enough light per division cycle during the experiment. So, if it is the case, the relatively low values of β-carotene (based on per cell) recorded in this experiment will likely be improved by the use of concomitant inductive factors such as the higher light intensities and high salinities. It is also possible that these strains are genetically non-carotenogenic strains of *Dunaliella*.

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