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Biochemical Effects of Different Salinities and Luminance on Green Microalgae *Tetraselmis chuii*

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Abstract: In this study the effects of (20, 30, 40, 50 ppt) salinities and (2500, 4500, 6500 Lux) luminances on the changes of chlorophylls, carbohydrates, proline and proteins of unicellular microalga *Tetraselmis chuii* have been studied in the controlled conditions. The results indicated that the amounts of all of these parameters have been influenced by the salinity and light intensities. The amount of proteins have not been changed significantly in all cases. Carbohydrats and proline contents as osmoregulators have been increased in stress conditions. But the amount of chlorophylls have been reduced while carotenoids contents have been increased. So we conclude that as like as higher plants high and low (in halophytes) salinities and light intensities cause some biochemical changes in unicellular green microalgea. In many cases adaptation mechanisms to stress conditions are the same in them, such as increased accumulation of proline and carbohydrates and some changes in pigments and protein contents of the cells of organism. Due to these results and other similarities, perhaps higher plants have been originated from these green unicellular algae.

Key words: *Tetraselmis* (*chuii*), salinity, luminance, protein, proline, carbohydrate, pigment

INTRODUCTION

Green algae and land plants (Viridiplantae') are sister groups which are phylogenetically subdivided into the Chlorophyta and the Streptophyta. The common ancestor of this lineage is believed to have been a member of the Prasinophyceae (Bhattacharya and An, 1998). Microalgae are rich sources of proteins, carbohydrates and especially essential fatty acids. *Tetraselmis* ssp (prasinophyte) is a green, motile and about 10×14 μm microalgea. This organism as a phytoplankton is used greatly by the artemia, mussels, oysters, clams, scallops and corals (Eirik *et al*., 1998). It is also one of the preferred foods for rotifer cultures (Makridis *et al*., 2006). *Tetraselmis chuii* is very important due to its higher proteins, lipids, essential fatty acids and sterols. From many years ago man was interested in to study aquatic plants. He makes experimental lakes (220 years ago) to research about algae. Unicellular ukaryotic microalgae have especial importance about these organisms due to the simpelicity of their structures, showing all of the methabolic activities and having similarities to the higher plants (having cellulose in their cell walls, starch storage, a and b chlorophylls as pigments). microalgae differ in their adaptability to salinity and another stress condition. In either case, the algae produce some metabolites to protect from salt injury and also to balance as per the surroundings osmotica (Richmond, 1986). *Dunaliella*, the unicellular green alga is an example for its ability to survive extreme salt stress and serve as a useful model to comprehend the strategies of cell response to high salt concentration. The present study focused on the adaptation of *Tetraselmis chuii* to varied range of salinity and different lumuinance conditions and their effect on the protein, proline, pigments (a, b), carotenoid and carbohydrate production.

MATERIALS AND METHODS

The green unicellular *Tetraselmis chuii* (Prasinophyte) was supplied by the department of university of Tarbiat Moddares of Noor. Microalgae was grown photoautotrophycally in filtered and sterilised natural seawater medium (20 min at 121°C) enriched with Walne medium (modified from Laing and Verdugo, 1991). Cells were grown in 500 mL erlenmeyer flasks containing 450 mL of medium at 20°C with different irradiance 2500, 4500 and 6500 lux and different salinity of seawater 20, 30, 40 and 50 ppt for 9 days and the initial pH of 8. At the end of treatment the biomass of the algal cells is harvesred to use for some biochemical analysis.

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Measurement of carbohydrate content: The amount of soluble sugars was determined by the the method of Dubois *et al.* (1956). Two mililiter of the supernatant of aliquot was mixed with 1 mL of 5% phenol and 5 mL of sulfuric acid. The mixtures incubated for 30 min, after cooling, the changes in absorbance were estimated at 485 nm on a spectrophotometer.

Measurement of proline content: To show the changes of proline 0.04 g of every sample was grinded in glass powder. The proline content was determined by the method of Bates *et al.* (1973) using 3% aqueous sulphosalicylic acid for preparing the microalgae homogenate. The homogenate was centrifuged at 1000 rpm for 15 min. Two mililiter of the supernatant was mixed with an equal volume of glacial acetic acid and acid ninhydrin and incubated for 1 h at 100°C. Then 4 mL of toluene was added to mixture tube were put in ice and the chromatophore containing fraction was then aspirated from the aqueous phase and its absorbance was determined at 520 nm on a spectrophotometer.

Measurement total proteins: Total proteins content were measured by Folen-Lowry method (Lowery *et al.*, 1951).

Chlorophyll and carotenoid: Intracellular content of chlorophyll a, chl b and carotenoids were extracted from the cells in the filterby 12 mL of aseton (90 %) and were determined spectophotometrically by reading absorbance at 470, 645 and 662 nm as described by Lichtenthaler and Wellburn (1985).

RESULTS AND DISCUSSION

Carbohydrates: The results have indicated that the amounts of carbohydrates have been increased by the low and high salinities and light intensities The amount of this component in 50 ppt and 4500 and 6500 lux was the highest and in 40 ppt, 6500 lux was lowest (Fig. 1). Vazquez and Arredodondo (1991) has reported that at higher salinity in alga *B. braunii* the amount of carbohydrates has been increased. Stress-induced increase in carbohydrate levels in plants is one of the known daptation mechanisms to salt and luminance stress (Ashraf and Harris, 2004; Naeini *et al.*, 2004). Many of the previous studies have indicated that soluble sugars play an important role in osmotic regulation of the cells during reproduction and stress conditions (Gill *et al.*, 2002). There is a relationship with the physiological processes such as photosynthesis, translocation and respiration and carbohydrates metabolism, in the other words when stress condition

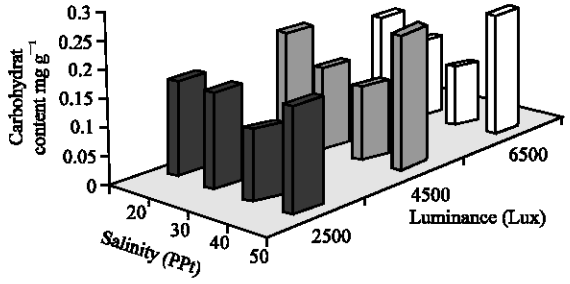

Fig. 1: Effect of differen salinity (20, 30, 40, 50 ppt) and different luminance (2500, 4500, 6500 Lux) carbohydrate contents in *tetraselmis chuii.* Data represents an average of three replicates

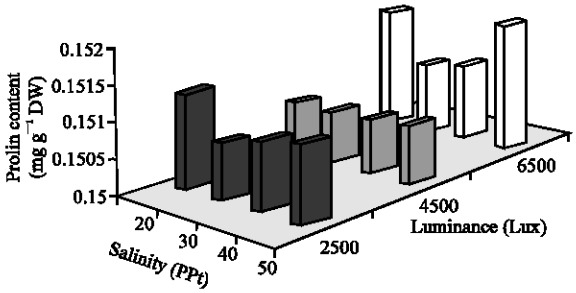

Fig. 2: Effect of differen salinity (20, 30, 40, 50 PPt) and different luminance (2500,4500,6500 Lux) on total proline contents in *tetraselmis chuii.* Data represents an average of three replicates

influences the growth of plants about mentioned physiological processes, carbohydrates metabolism changes too, as, the soluble sugars accumulates in the cells. The reasion of this result is probably because of induced degradation of starch (Chang *et al.*, 2001) and inhibition of starch and other polysacharids synthesis that cause to accumulation of soluble sugars (Kerepesi and Galiba, 2000).

Proline: Proline content in microalgae cells has been increased and maximum proline reported in 20, 50 ppt salinity and in 6500 lux luminance and in 30 ppt, 4500 lux was lowest (Fig. 2). Proline plays an important role in osmoregulation of plant cells (Ahmad and Hellebust, 1988; Laliberte and Hellebust, 1989), protection of enzymes (Nikolopoulos and Manetas, 1991; Laliberte and Hellebust, 1989; Paleg *et al.*, 1984), stabilization of the protein synthesing machinery (Kadpal and Rao, 1985), regulation of cytosolic acidity (Venekemp, 1989) and scavenging of free radicals (Smirnoff and Cumbes, 1989). It also acts as an effective singlet oxygen quencher (Alia and Matysik, 2001). Proline is reported as osmoprotectant in plants subjected to hyperosmotic

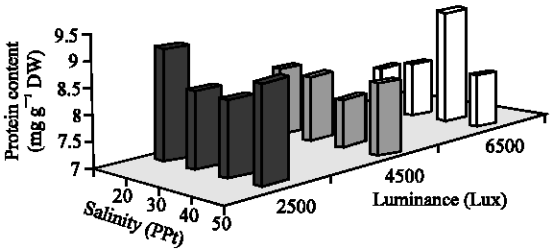

Fig. 3: Effect of differen salinity (20, 30, 40, 50 ppt) and different luminance (2500, 4500, 6500 Lux) on total protein contents in *tetraselmis chuii*. Data represents an average of three replicates

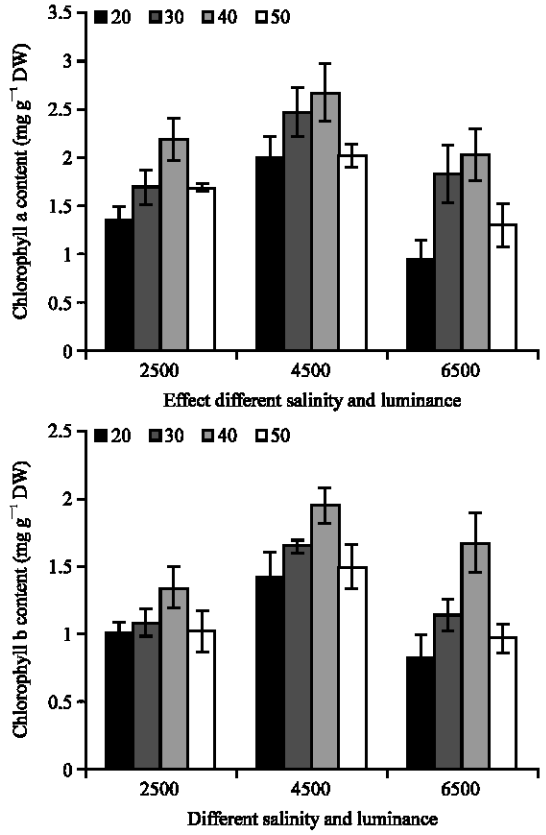

Fig. 4: Effect of differen salinity (20, 30, 40, 50 ppt) and different luminance (2500, 4500, 6500 Lux) on Chlorophyll a and b contents in *tetraselmis chuii*. Data represent the mean±SE. of three replicates

stresses such as drought and soil salinity (Hong *et al*., 2000; Arshi *et al*., 2005; Stewart and Lee, 2004) suggested that the capacity to accumulate proline is correlated with salt tolerance. Hong *et al*. (2000) suggested that increased resistance to oxidative stress is due to some indirect metabolic or physiological consequences of the accumulation of proline and other metabolites. Therefore,

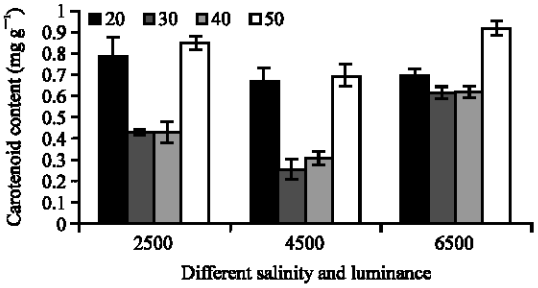

Fig. 5: Effect of differen salinity (20, 30, 40, 50 ppt) and different luminance (2500, 4500, 6500 Lux) Carotenoid contents in *tetraselmis chuii*. Data represents an average of three replicates. Bars indicate mean±SE. of 3 replicates

in microalgae and other plants proline acts as a free radical scavengering and increases salt tolerance of these microorganisms.

Proteins: The results indicated that the amount of proteins have been increased by the salt stress. Its amount in 20 ppt salinity and 2500 lux was the highest (Fig. 3). It shows that the lowest salinity and light intensity have the highest effect in protein contents. The protein contents have also increased in 6500Lux irradiance too. This reasult is probably because of the synthesis of new stress proteins (Sadka *et al*., 1991).

Chlorophylls contents: In this research chlorophylls contents have been decreased by the salinity and light intensity and highest chlorophyll content was observed in 40 ppt salinity, 4500 lux and lowest content was in 20, 50 ppt salinity in 6500 lux (Fig. 4). When microalgal cells are exposed to high light intensity, photosynthesis is inhibited and therefore the growth rate and chlorophylls contents of the algal culture is reduced (Barnes and Mann 1999; Toro, 1989). This process is known as photo-inhibition. This effect is caused by photo-oxidation reactions inside the cell due to excess light that cannot be absorbed by the photosynthetic apparatus; the increase in ultraviolet light also has detrimental effect on the cell (Barnes and Mann, 1999; Hart *et al*., 1991). Damage to photosynthetic organisms caused by excess light is believed to result in part from damage to Photosystem II (PSII) caused by the oxidation of lipids, proteins and pigments by reactive oxygen species, such as single Oxygen (O_2), Hydrogen peroxide (H_2O_2) and the Hydroxyl radical (OHN) (Niyogi, 1999; Oceanogr, 2006). Reduced chlorophyll (a,b) contents (Fig. 4) with reduced growth rate at higher salinities are also due to decrease in photosynthetic rate because of salt osmotic and toxic ionic effects (Moradi and Ismail, 2007).

Carotenoids: Caroteniods contents in *Tetraselmis chuii* cells have been increased, highest. Caroteniod content was showed in 50 ppt salinity, 6500 lux and lowest content was in 40 ppt salinity in 4500 lux (Fig. 5). These results are in agree with the results that Fazeli *et al.* (2005) have reported recently on increased total carotenoid production in *Dunaliella tertiolecta* exposed to salinity. The results of Vazquez *et al.* (1991) has also indicated that the *B. braunii* has adapted to lower levels of salinity with an increased production of biomass, carbohydrate and carotenoids.

CONCLUSION

Finnaly we can conclude that, to have a suitable medium culture to grow a unicellular organism especially commercially important spieces such as *Tetraselmis* sp.(*chuii*), it needs to do some experiments that describe the best condition for its growth. This knowledge allows algal culture staff to be able to avoid photo-inhibition and culture crashes, predict rates and manipulate algal culture harvests to coincide with feed demand in a hatchery situation.

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