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Note

Studies on the growth of the marine microalga *Dunaliella salina* (Teodoresco)

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ABSTRACT

The present paper reports on the growth pattern of *Dunaliella salina* cells cultured in different salinities and also in stressed conditions on exposure to mutagens (UV and PEG). The cultures were maintained in different salinities *viz.*, 20, 25, 30, 35, 40, 45 and 50 ppt for a period of two weeks in triplicates and the growth rate was monitored. The peak growth (14.29 lakhs) was observed in 35 ppt on eleventh day indicating the ideal salinity for the culture of this species. The cultures in mid-exponential growth phase were exposed to UV light for 30 and 60 minutes and PEG at four doses *viz.* 0.125, 0.25, 0.5 and 1 gm/ml. Poorest cell growth was observed for half an hour UV treated cultures (3.51 lakhs/ml). A proportionate decrease in cell count was noticed with increase in the concentration of PEG.

Dunaliella salina is a unicellular, eukaryotic green alga. (Chlorophyta, Chlorophyceae). The rapid changes in salt concentrations along with mutagens resulted in the synthesis and accumulation of b-carotene and the compatible solute glycerol in this algae (Borowitzka and Borowitzka, 1988).

Several studies have been made in the past to determine the range of salt concentrations at which various *Dunaliella* spp. grows (Borowitzka Brown, 1974 and Ben Amotz & Avron, 1983). Heriant and Pralampita (1987) studied the influence of salinity on population growth of *Dunaliella* species. Borowitzka & Brown (1974) investigated the ability of *Dunaliella* spp. to tolerate high solute concentrations in the environment. The adaptation of the unicellular alga *Dunaliella parva* to a saline environment was illustrated by Ben Amotz (1975). b-carotene production can be increased substantially by subjecting the cells to either physical or chemical mutagens (Shaish *et al.*, 1991). The present study was undertaken to find out the behaviour of halotolerant *Dunaliella salina* at different salinities, UV treatment and polyethylene glycol treatment.

The effect of salinity on cell growth was studied in different concentrations. Culture at 35 ppt was taken as control. Growth rates were determined by counting the cell number with a haemocytometer. Samples in triplicate were counted and the average counts were taken for evaluation of results. To study the effect of UV treatment on cell growth, a set of cultures with different salinities ranging

from 20 to 50 ppt were pipetted into 5 cm petri dish and exposed to UV light in the laminar flow hood (254 nm). One set of culture was exposed to UV light for half an hour and another set for one hour. UV treated cultures were kept in dark for 48 hours to prevent photo activation repair and their survival rates were determined by cell counting method.

Chemical mutation was done with polyethylene glycol (PEG). PEG was added to cultures at lag phase and the cultures were maintained with the compound for 25 hours. Four different concentrations were fixed namely 0.125, 0.25, 0.5 and 1 gm/ml and 50 ml of the culture sample was used for treatment. Cell count was taken for a period of twelve days and the results were interpreted.

The purpose of this experiment was to study the behaviour of *D. salina* in different salinities. In the first set of experiment the cell concentration on day 0 was 1.38 lakhs/ ml. The cell growth in different salinities *viz*. 20, 25, 30, 35, 40, 45 and 50 ppt was estimated from day 0 onwards upto day 13. (Fig.1).

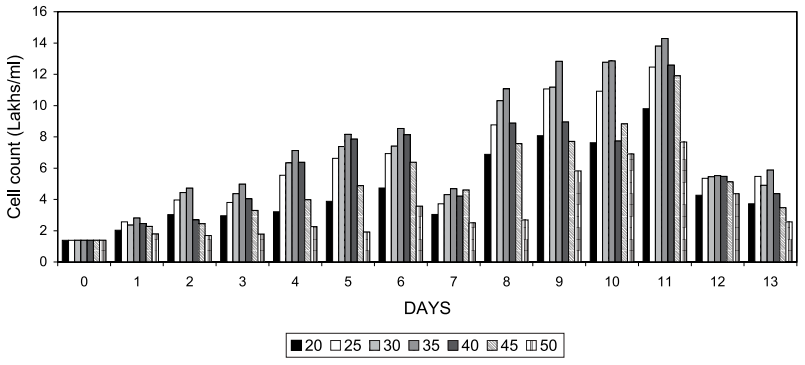

Fig. 1. Growth of *Dunaliella salina* in different salinities (experiment I)

An increase in salinity resulted in a rapid increase in the total carotenoid content and decrease in cell growth (Borowitzka and Brown, 1974). The cell concentration of *D. salina* in different salinities was found to be optimum at 35 ppt. At lower and higher salinities the cell count was found poor (25, 30, 40, 45 and 50 ppt). Cells grown in low salinities suffered severe osmotic shock because of the lower equivalent osmolalities. The inhibition of growth could be attributed to a slow penetration by Cl ions, which may interfere with the activity of enzymes. In the first experiment the cell density showed an increasing trend and the peak growth was obtained on day 11 in all salinities. The highest cell concentration was observed in 35 ppt (14.29 lakhs/ ml) and the lowest in 50 ppt (7.67 lakhs/ ml). On succeeding days a sharp decline in cell count was observed in all salinities. Growth inhibition was observed in all salinities above 35 ppt. Cells grown in a medium of higher concentration suffered osmotic shock and lysis occurred eventually, whereas cells grown in hypoosmotic medium became swollen.

One-way ANOVA programmed in SYSTAT was performed for the culture experiment. No significant difference was noted between culture days and salinities in the experiment ($P > 0.05$).

The cells of *D. salina* were exposed to UV irradiation for half an hour and one hour (Fig. 2). Cell growth was arrested in UV treated cultures. Among the control, maximum growth of 7.6 lakhs/ml at 35 ppt and minimum of 3.4 lakhs/ml at 50 ppt were observed with an average cell count of 6.14 lakhs/ml, whereas the average value in half an

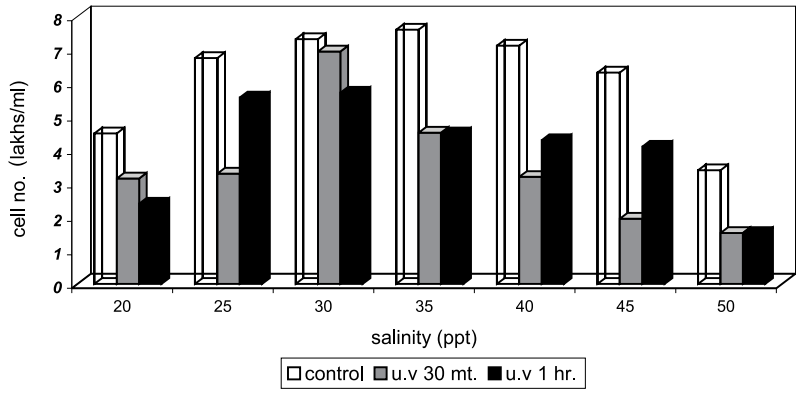

Fig. 2. Effect of U.V irradiation on cell culture of *Dunaliella salina*

hour and one hour UV treated samples were 3.51 and 4.01 lakhs/ml respectively.

UV irradiation resulted in poor cell growth but the mutations induced by UV treatment could enhance the carotenoid content of the cells. The results of one-way ANOVA on cell growth of UV irradiated cells showed no significant difference between two exposures ($P > 0.05$).

The cell concentrations of *D. salina* after PEG treatment are given in Fig. 3. Among the control, maximum cell growth occurred at 30 ppt with 8.85 lakhs/ml. Maximum survival was observed in 0.125 mg/ml concentration of PEG with 4.89 lakhs/ml. At higher doses poor cell growth was noticed. i.e., 4.71 lakhs/ml for 0.25 mg/ml, 4.70 lakhs/ml for 0.5 mg/ml and 3.89 lakhs/ml for 1 mg/ml of PEG. The results showed that higher concentration of PEG induced mutation in the cells and resulted in poor cell growth. The data of the two experiments indicated that the exposure to UV light for half an hour could bring about cell mutations more effectively than both one hour UV treated and chemical treated samples. One-way ANOVA performed on the cell growth of PEG treated cultures showed no significant difference between the doses of PEG ($P > 0.05$).

A sudden increase in salinity resulted in a lag phase in growth and the length of this lag phase was dependent on the final salinity and the magnitude of salinity change (Ben Amotz & Avron, 1983). The results shows that 35 ppt is the ideal salinity for the growth of the species. A lag before an increase in the total carotenoid content was observed in *Dunaliella salina* (Ben Amotz, 1975 Borowitzka, *et al*., 1990). The response to changes in NaCl concentration indicated

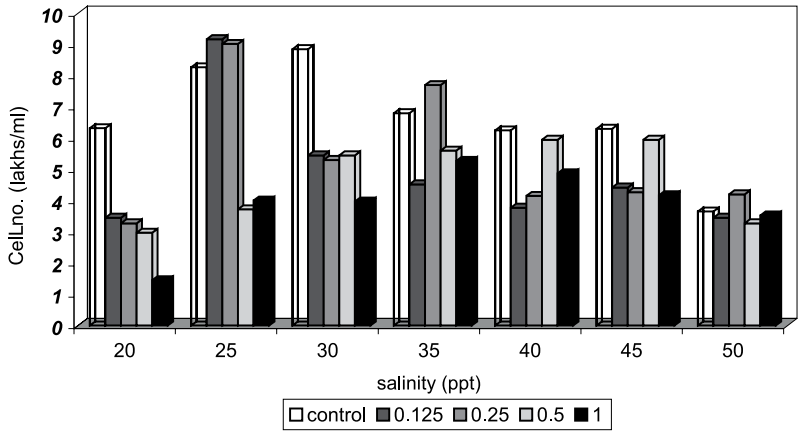

Fig. 3. Effect of polyethylene glycol on cell culture of *Dunaliella salina*

a complicated relationship between NaCl concentration and carotenoid biosynthesis. The onset of carotenoid biosynthesis within 12 hour of NaCl increase indicated activation of enzymes with continued biosynthesis at the rate of 60 mg carotenoid g^{-1} cell protein d^{-1} for 5-6 days. In cultures grown in 25 % NaCl, 400 mg carotenoid g^{-1} cell protein was obtained (Borowitzka, *et al*., 1984). The results showed that increase in total carotenoid content was mainly due to b-carotene.

Acknowledgments

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