In vitro evaluation of β-carotene production in two different strains of Dunaliella salina Teodoresco (Chlorophyta)

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Abstract

The description of the genus Dunaliella, the unicellular green alga which lacks a cell wall. These organisms have high concentrations of beta carotene to protect against the intense light and high concentrations of glycerol to provide protection against osmotic pressure, which accumulates massive amounts of carotenoids under appropriate growth conditions. Effect of four different physical-chemical parameters such as NaCl, NaNO\textsubscript{3}, pH and light intensity made on the organism revealed that the algal strains required a slightly alkaline pH (pH 7.5), high light intensity (40 \(\mu\)Em\textsuperscript{-2} \textsuperscript{s}\textsuperscript{-1}), low NaCl concentration (0.5M), and moderate NaNO\textsubscript{3} concentration (1.18M) for the maximum production of the valuable pigment, β-carotene. At such conditions, the level of chlorophyll concentrations was also more.

Key words: Dunaliella, β-carotene, Chlorophyll and carotenoids

Introduction

Dunaliella, the unicellular green alga is most responsible for the major production of β-carotene in hyper saline environments worldwide. It was first discovered by a French Scientist Michel Felix Dunal in saltern evaporation ponds (Dunal, 1838). Later it was discovered by Teodoresco in 1905 and is now named as Dunaliella Teod. (Teodoresco, 1905). Twenty eight species of Dunaliella are presently recognized. Few organisms can survive in such highly saline conditions as salt evaporation ponds. The unique morphological feature of Dunaliella is that it lacks a cell wall. The cell is enclosed by a thin plasma membrane or periplast, which permits rapid changes in cell shape and volume in response to osmotic changes (Gimmler, 1992). To survive, these organisms have high concentration of beta carotene to protect against the intense light and high concentration of glycerol to provide protection against osmotic pressure, which accumulates massive amounts of carotenoids under appropriate growth conditions.

Different technologies are used for commercial cultivation of Dunaliella for the production of beta carotene throughout the world which is now one of the success stories of halophile biotechnology (Ben-Amotz, 1993; Ben-Amotz et al., 1992; Fazeli, 2005). The beta carotene occurs as a number of isomers, two of which, 9-cis and...
all-trans, make up approximately 80% of the total beta carotene in Dunaliella (Ben-Amotz et al., 1988; Borowitzka and Borowitzka, 1988). When maintained under growth limiting conditions high amounts of beta carotene was synthesizing and accumulated (Ben-Amotz et al., 1982; Ben-Amotz et al., 1988; Borowitzka et al., 1984).

The present paper provides knowledge about the optimal production of β-carotene in two different strains of Dunaliella salina obtained from the Centre for Advanced Studies in Botany, University of Madras, Chennai, India. Salinity is an important parameter, easy to control in outdoor cultures of Dunaliella for commercial purposes. In addition to that, some of the other parameters like pH, light intensity are also involved in promoting the β-carotene production. The aim of this work was to study the effect of these parameters evaluated by concentration of chlorophyll a, chlorophyll b and total carotenoids in two different strains of Dunaliella salina cultured in a media with different dosage of nutritional composition. These studies are immensely useful to find out the exact dosage (optimization) of the nutrition which were used in a basal medium called De walne’s medium (Orset and Young, 1999).

Materials and method

Two different strains of Dunaliella viz., Dunaliella sp.V-101 and Dunaliella sp. V-102 obtained from the Centre for Advanced studies in Botany, University of Madras, were investigated in the present study. The strains were identified based on their morphological and cultural characteristics. They were grown in DeWalne’s medium (Orset and Young, 1999) and their growth characteristics were studied. The effect of different concentrations of NaCl, NaNO₃ and levels of light intensity and pH on the growth characteristics of Dunaliella V-101 and Dunaliella V-102 was studied.

Extraction and estimation of pigments

The experiments were conducted in 250ml conical flask containing 90ml medium inoculated with 10ml of optimally grown culture and kept at 30 µEm⁻² s⁻¹, 12/12 light dark cycle and 24±1°C for a period of 30 days. At every 3 days intervals 5 ml of culture was taken and centrifuged at 5000 rpm for 10 minutes and the supernatant was discarded. The algal pellet was then added with 5ml of 100% acetone and covered with black paper and kept overnight at 4°C. The sample was then centrifuged at 5000rpm for 10 minutes. The supernatant was collected and the chlorophyll a, chlorophyll b and total carotenoid contents of the samples and its absorbance was measured (Lichtenthaler, 1987) using Milton Roy UV/Visible spectrophotometer.

Results and Discussion

The two different strains of Dunaliella viz., Dunaliella sp.V-101 and 102 were identified based on their morphological and cultural characteristics. The size of the Dunaliella sp.V-102 was more than the Dunaliella sp.V-101.

Fig. 1a. Dunaliella sp.V-101
Effect of different pH

*Dunaliella* is not a true halophile but it is an acidophilic alga that grows optimally at pH values between 0.5 and 2.0. In recent years, it has become a popular research object for the study of adaptation of life to low pH environment that was reported by Gimmler *et al.*, (1992). In the present investigation, similar observations were obtained when strains were grown under acidophilic conditions at pH values 5.0, 5.5, 6.0 and 6.5, and neutral. But more amount of for chlorophyll a, chlorophyll b and total carotenoid was observed at halophilic conditions. Generally the two different strains of *Dunaliella* sp. survived in all the different pH.

**Fig.1b. Dunaliella sp.V-102**

The results showed that, the *Dunaliella salina* V-101 was having maximum concentration of chlorophyll a (6.302 μg/ml) and chlorophyll b (5.002 μg/ml) at pH 7.5 on 21st day. Maximum amount of β-carotene is around 72.322 μg/ml recorded at pH 7.5 on 30th day. This result was more than the control pH of 8.0. Maximum concentration of chlorophyll a and chlorophyll b of 6.395 μg/ml and 4.821 μg/ml of *Dunaliella salina* V-102 were recorded at pH 7.5 on 21st day. Maximum amount of β-carotene was around 71.304 μg/ml recorded at pH 7.5 on 30th day. At this condition the β-carotene production was slightly more than the control pH of 8.0.

**Effect of different Light Intensities**

The two strains of *Dunaliella* sp. kept under different light intensities revealed that, maximum concentration of chlorophyll a and chlorophyll b (7.585 μg/ml and 9.561 μg/ml) in *Dunaliella salina* V-101 were recorded at 2 μEm² s⁻¹ and 4 μEm² s⁻¹ light intensities on 21st day. However, the maximum amount of β-carotene (80.879 μg/ml) was recorded on 30th day at 40 μEm² s⁻¹. This result was more than the control (30 μEm² s⁻¹). *Dunaliella salina* V-102 contained maximum amounts of chlorophyll a and chlorophyll b (9.952 μg/ml and 9.564 μg/ml) at 2 μEm² s⁻¹ and 4 μEm² s⁻¹ light intensities on 21st day. The maximum amount of β-carotene of 82.684 μg/ml recorded at the above condition was 28.8% more than that of control (30 μEm² s⁻¹). The results are in line with observations made by Borowitzka, (1999) on the production of carotenoids when micro algal cells grown in full nutrient culture media and incubated under specific conditions of high intensity light.

**Effect of different concentrations of NaCl**

The results of the medium amended with different concentrations of NaCl showed that the maximum concentration of chlorophyll a and chlorophyll b of *D. salina* V-101 of 6.302 μg/ml and 5.002 μg/ml was recorded at 1M NaCl concentration on 21st day. However, the maximum amount (67.232 μg/mL) of β-carotene was recorded at the concentration of 0.5M NaCl on 30th day. In this state β-carotene production was higher than the control (2.14M NaCl). *Dunaliella salina* is able to survive between 0.5M and 5.0M NaCl concentration and it was reported by Fisher *et al.*, (1994). Maximum concentration of chlorophyll a and chlorophyll b of 6.395 μg/ml and 4.821 μg/ml of *D. salina* V-102 were recorded at 1.5M NaCl concentration on 30th day. Maximum amount of β-carotene was
around 86.370 µg/mL at 1M NaCl concentration on 30th day. The amount of β-carotene recorded at this condition was more than 85.1% when compared to control (2.14M NaCl).

**Effect of different concentrations of NaNO₃**

The results obtained from medium amended with different concentrations of NaNO₃ showed that, the maximum concentration of chlorophyll a and chlorophyll b of 18.311 µg/ml and 10.411 µg/ml of *D. salina* V-101 was recorded at 4M NaNO₃ concentration on 24th day. However, the amount of β-carotene of 69.370 µg/ml recorded on 30th day at 0.5M NaNO₃ concentration was more than that of control (1.18M NaNO₃). The parallel investigation made on *D. salina*, *D. tertiolecta* and *D. viridis* recorded that NaNO₃ could be the best nitrogen source (Goldman and Brewer, 1980). It has also been reported by Geider *et al.*, (1998) that the ratio of accessory photo protective pigments like Chlorophyll a increased under nitrogen limited conditions. Recording the nitrogen concentrations, *Dunaliella* strains showed growth in the medium amended with different concentration of NaNO₃. The highest concentration of chlorophyll a and chlorophyll b of 14.45 µg/ml and 9.31 µg/ml of *Dunaliella salina* V-102 was recorded at 5M NaNO₃ concentration on 24th day. Maximum amount (76.482 µg/ml) of β-carotene recorded on 30th day at 1M NaNO₃ concentration and its increments was more than 16% when compared to control (1.18M NaNO₃).

**Conclusion**

*Dunaliella salina* V-101 and *Dunaliella salina* V-102 showed two phases during their growth period. In the first phase, there was an increase in cell number, accumulation of β-carotene and the synthesis of chlorophyll a, chlorophyll b while in the second phase, there was a steady level in cell number, gradual decline in the concentrations of chlorophyll a, chlorophyll b but there was accumulation of β-carotene. Effect of four different physical-chemical parameter such as NaCl, NaNO₃, pH and light intensity made on the organism revealed that the algal strains required a slightly alkaline pH (pH 7.5), high light intensity (40 µEm⁻² s⁻¹), low NaCl concentration (0.5M), and moderate NaNO₃ concentration (1.18M) for the maximum production of the valuable pigment, β-carotene. At these conditions, the level of β-carotene increased up to 51.2%, when compared to control (DeWalne’s medium).

**Fig. 2. The cultures were kept at cold room conditions**

**References**


