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Research Article

Culturing Marine Green Microalgae *Dunaliella salina* Teod. and *Dunaliella tertiolecta* Masjuk in Dewalne's Medium for Valuable Feeds Stock

Subramaniyan Venkatesan[1], Munuswamy Senthil Swamy[2], Chinnasamy Senthil[3], Sailendra Bhaskar[3] and Ramasamy Rengasamy[1]*

[1]*Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India.*

[2]*EID Parry India Ltd, Parry Nutraceuticals Division, Research and Development Centre, Chennai 600 019, Tamil Nadu, India.*

[3]*Aban Infrastructure Pvt. Ltd., Biotechnology Division, Thandalam, Chennai 602 102, Tamil Nadu, India.*

*Correspondence Author e-mail: profrrengasamy@yahoo.com

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Abstracts

The single cell, marine, halo tolerant carotenogenic species of *Dunaliella salina* have the ability to accumulate secondary carotenoids, β-carotene as they grow in high salinity and high irradiance. Commercially β-carotene has many applications such as nutritional supplements to chemotherapeutic agent in cancer therapy and antioxidant. Pro vitamin-A is a natural colorant; aqua feed and prevents night blindness, enhances immunity and reduces the risk of heart diseases. Whereas, non-carotenogenic species have the ability of fast growth and lipid production and can be used for biofuel production. In the present study, the carotenogenic *Dunaliella salina* and non-carotenogenic *Dunaliella tertiolecta* obtained from the Centre for Advanced Studies in Botany, University of Madras and were cultured using Dewalne's medium for a period of 30 days under laboratory conditions. Maximum cell number in *Dunaliella salina* of 6.83 x10^6 cells/ml was recorded on 16th day and the maximum amount of total carotenoid was produced as 55 mg/L on 26th day, while the maximum cell number recorded in *Dunaliella tertiolecta* was 8.48 x10^6 cells/ml on 20th day and maximum lipid content of 295 mg/L on 30th day.

Key words: *Dunaliella salina*, *Dunaliella tertiolecta*, growth rate, total carotenoids, Dewalne's medium.

INTRODUCTION

Microalgae culture is one of the commercially important aspects in modern biotechnology. The first unialgal culture was achieved by Beijerinck (1890), on the green alga *Chlorella vulgaris* and the use of such culture for studying plant physiology was developed by Warburg in the early 1900 (Warburg, 1919). Certain microalgae, namely *Chlorella*, *Spirulina* and *Dunaliella* are grown commercially for nutraceuticals and other algal products such as hydrocarbons, fatty acids, phycocolloids, bioactive molecules and fine chemicals.

The genus *Dunaliella*, includes about 30 species of which 25 are found in brackish water and 5 in freshwater (Melkonian and Preisig, 1984; Leonard and Cascers, 1993). The optimal conditions for carotenogenesis are those that limit growth and include exposure to high light intensities and other stress factors, especially nutrient deprivation.

The biflagellate microalga, *Dunaliella* growing profusely in salt marshes was first discovered by a French Scientist Dunal in 1837. This naked allay of *Chlamydomonas*, responsible for the red coloration characteristic of salt

marshes was later described by Teodoresco in 1905 and is now named as *Dunaliella* Teod. The unique morphological feature of *Dunaliella* is 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. The genus *Dunaliella* of the order Volvocales includes a variety of well-defined species of unicellular, ovoid and biflagellate green algae. *Dunaliella* demonstrates a remarkable degree of environmental adaptation to salt and is widely distributed in natural habitats. The cells are motile and have two equal, long, smooth, whiplash flagella. The cells have one large cup shaped chloroplast, which occupies about half the volume and it is oriented around the nucleus (Ben-Amotz et al., 1982; Loeblich, 1982; Garcia Gonzalez et al., 2003; Gomez et al., 2003; Gomez and Gonzalez et al., 2005).

Species of *Dunaliella* are very well adopted to propagate in media ranging from less than seawater concentration (0.1M sodium chloride or 0.5844 g/1000 ml) to saturated salt solution (5M sodium chloride or 292.2 g/1000 ml). This extremely halotolerant organism is one of the eukaryotic organisms found under such extreme conditions. *Dunaliella salina* collected from the lakes without any addition of chemicals is a potent source of natural mixed carotenoids.

Dunaliella salina has 90% of β-carotene and 10% of other carotenoids. Carotenoids are made up of α-carotene and xanthophylls like lutein, zeaxanthin, and cryptoxanthis similar to the ones found in food and vegetables (Gouveia and Emphis, 2003). These xanthophylls have a wide spread application in the pharmaceutical and cosmetics as well as in animal feed. Approximately 500 different carotenoids have been identified so far. *Dunaliella salina* is a mixture of two stereoisomer all-trans carotene and 9-cis carotene (synthetic β-carotene has only the all trans isomer). 9-cis β-carotene is a powerful antioxidant and it is well known that it contributes significantly in maintaining the health. In the present study carotenogenic strains of *Dunaliella salina* and non carotenogenic strains of *Dunaliella tertiolecta* obtained from Centre for Advanced Studies in Botany, University of Madras were cultured in Dewalne's medium under laboratory condition. The growth, pigment composition, total protein, carbohydrate and lipid content were estimated at every three days interval up to 30 days.

MATERIAL AND METHODS

Growth study of *Dunaliella salina* and *D. tertiolecta*

Experiments were carried out in 1000 ml Erlenmeyer's flasks containing 500 ml sterilized Dewalne's medium inoculated with 50 ml of optimally grown cultures of *Dunaliella salina* and *Dunaliella tertiolecta* and kept under 25±1^{o}C at 30μE m^{-2} s^{-1} light intensity, 12/12 light/dark photoperiod for a period of 30 days. At every alternate day interval, 5 ml of samples were drawn from the culture and recorded for the following parameters. (i) cell count using Haemocytometer (ii) concentration of photosynthetic pigments viz., Chlorophyll a, Chlorophyll b and total carotenoid (Lichtenthaler, 1987) (iii) Total protein (Bradford *et al.*,1976) (iv) Total carbohydrate (Dubois *et al*., 1956) (v) Total lipid (Folch *et al*., 1956). Growth curve was plotted against time and $\log_{10}$ of cell number/ml was calculated by the formula given by Guillard (1973). The concentration of pigments i.e., Chl *a*, Chl *b* and total carotenoids were expressed as μg/10^{6} cells and total protein, carbohydrate and total lipid were expressed as μg/ml. All the experiments were carried out in triplicates and means are presented.

RESULTS AND DISCUSSION

An industrially important unicellular green alga, *Dunaliella* which is recognized for the production of β-Carotene was screened for its growth and carotenogenesis. Besides, the concentrations of total protein, carbohydrate and lipid contents were also estimated in the present study. Up to date, around 30 different species of *Dunaliella* were reported (Melkonian and Preisig, 1984; Leonard and Cascers, 1993). Among the two different strains chosen in the present study, one of them was identified as carotenogenic strain *Dunaliella salina* (Teodoresco, 1905) and the other as non-carotenogenic *Dunaliella tertiolecta* (Masjuk, 1973) based on their morphological characteristics. Dewalne's medium was used to grow the organism since *Dunaliella* is reported to perform best for the growth, biomass and β-carotene production in *Dunaliella*.

Five different types of media were tested for their effects on the growth of *Dunaliella* sp.*:* M1 (BG-11 medium, Stanier *et al*., 1971), M2 (f2 medium, Jeffrey and LeRoi 1997), M3 (f/2 medium, Guillard 1962), M4 medium of Ben-Amotz et *al.* (1989), and M5 of Sallal *et al.* (1987).

Under constant illumination at room temperature 25 ± 2 °C after 14 days, the maximum growth of Chlorophyll-*a* was 7.5 mg/l while β-carotene was 5.2 mg/l and the maximum number of *Dunaliella* cells was found to be $6.5x10^6$ cells/ml in M1 medium (Nader Fareid Abu Sara, *et al*., 2011). A comparative study was made on *D. salina* with respect to its growth characteristics in Dewalne's medium (control) and modified Dewalne's medium. The algae grown in the modified Dewalne's medium showed a maximum number of 6.71 $\log_{10}$ cells/ml whereas in Dewalne's medium (Control) was 6.63 log10 cells/ml. Similarly, the concentration of Chl-*a* and Chl-*b* were found to be maximum in the modified Dewalne's medium and their increment were 8.7% and 7.0%, respectively, on 12^{th} day. A maximum amount of 6.64 mg/L β-carotene was recorded in the modified Dewalne's medium on 18^{th} day, which was more than 7.5% to that of control. The division rate (μ) of *Dunaliella salina* in the Modified Dewalne's medium was 0.43 when compared to 0.40 in the control (Raja *et al*., 2004).

In the present study, *Dunaliella salina* showed a maximum accumulation of total carotenoids of 55 μg/10^6 cells in Dewalne's medium on 26^{th} day. The amount of Chlorophyll-*a* and Chlorophyll-*b* were 8.64 and 6.51 μg/10^6 cells on 16^{th} and 14^{th} day respectively. The maximum of cell number *Dunaliella salina* was 6.83 x 10^6 cells/ml on 16^{th} day. The maximum amount of total protein, carbohydrate and lipid for *Dunaliella salina* were 68.6, 72 and 126 μg/ml on 18^{th}, 20^{th} and 30^{th} day respectively (Figure 1, 2 and 3). While, the maximum accumulation of total catotenoids in *Dunaliella tertiolecta* was 3.22 μg/10^6 cells on 26^{th} day. The amounts of Chlorophyll-*a* and Chlorophyll-*b* recorded were 4.48 μg/10^6 cells and 4.03 μg/10^6 cells on 18^{th} and 16^{th} day respectively. The maximum of cell number recorded in *Dunaliella tertiolecta* was 8.48 x 10^6 cells/ml 20^{th} day in Dewalne's medium. The maximum concentration of total protein, carbohydrate and lipid recorded in *Dunaliella tertiolecta* were 79, 101 and 295 μg/ml on 20^{th}, 18^{th} and 30^{th} day, respectively (Figure 1, 4 and 5).

CONCLUSION

The growth and lipid content were found to be maximum in non-carotenogenic strain of *Dunaliella tertiolecta* when compared to carotenogenic strain *Dunaliella salina*. Whereas, Chlorophyll-*a*, Chlorophyll-*b* and β-carotene were maximum in *Dunaliella salina* when compared to the pigment of the non carotenogenic strain. Therefore it is concluded that the carotenogenic *D. salina* can be mass cultured for β-carotene production, while the non-carotenogenic strain *D. tertiolecta* can be used for achieving the maximum biomass and for biofuel production.

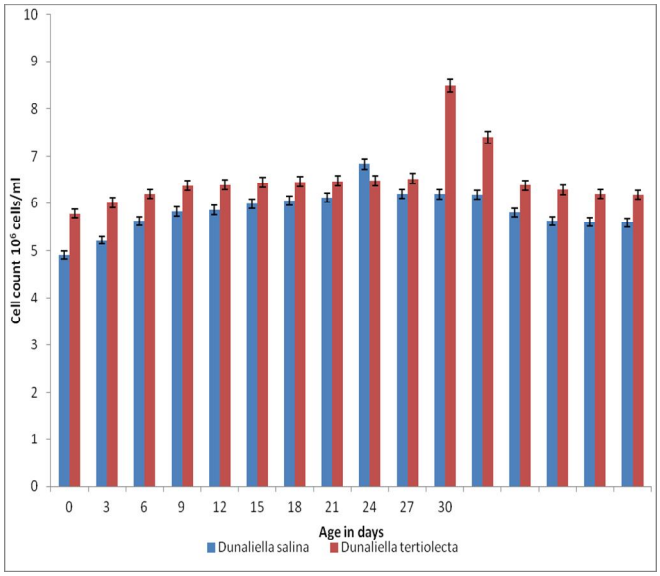

Figure 1: Growth curve of *Dunaliella salina* and *Dunaliella tertiolecta* in De Walne's medium

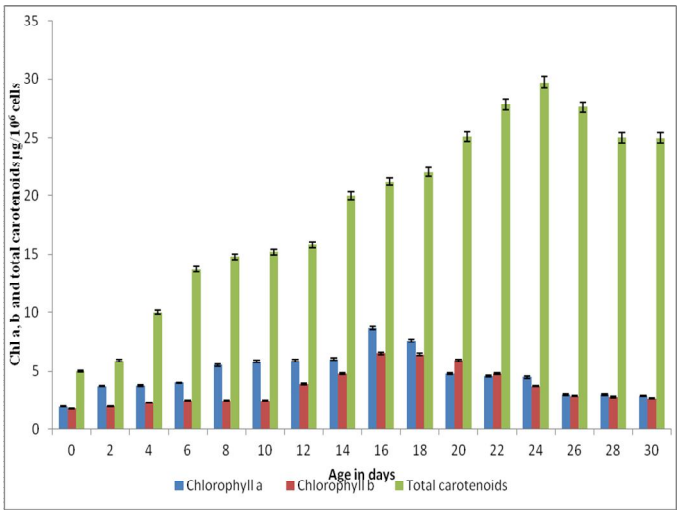

Figure 2: Estimation of chlorophyll-a, chlorophyll-b concentration of *Dunaliella salina* in De Walne's medium

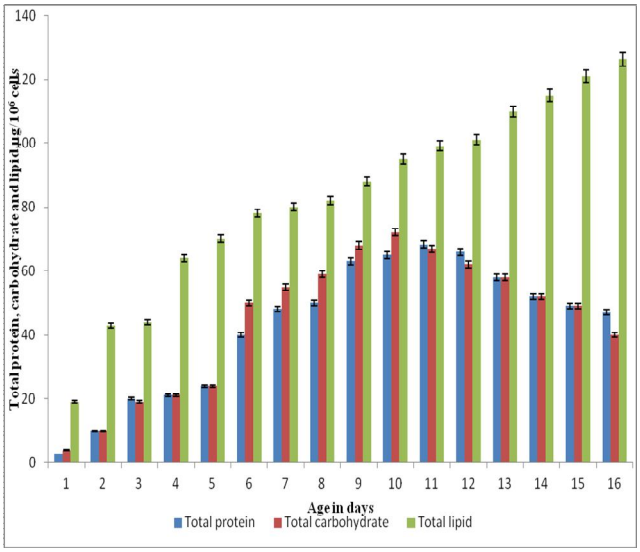

Figure 3: Estimation of total protein, carbohydrate and lipid content of *Dunaliella salina* in De Walne's medium

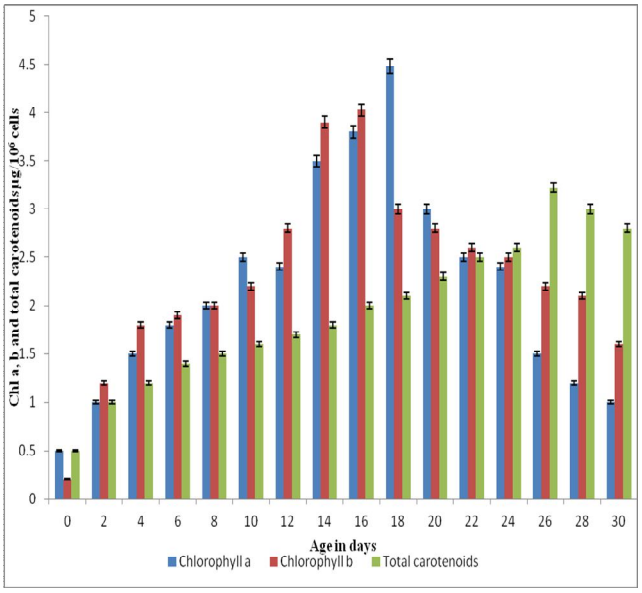

Figure 4: Estimation of chlorophyll a, chlorophyll b and total carotenoids concentration of *Dunaliella tertiolecta* in De Walne's medium

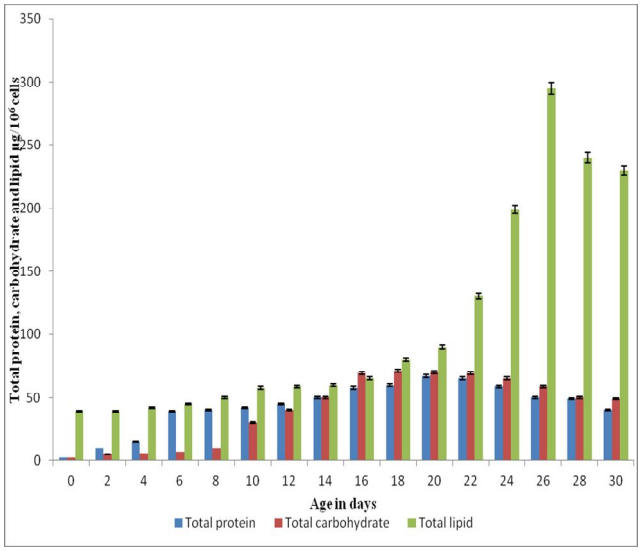

Figure 5: Estimation of total protein, carbohydrate and lipid content of *Dunaliella tertiolecta* in De Walne's medium

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