First Successful Isolation and Cultivation of *Dunaliella salina* (Dunal) Teodoresco from a Hypersaline Lagoon, Kingdom of Bahrain

Layla Hazeem
Dept. of Biology, College of Science
University of Bahrain

ID # (2743)
Received: 05/ 05/ 2013
In-revised: 24/11/2014
Correspondent Researcher:
Layla Hazeem
E-mail: hazeemlayla@gmail.com

KEYWORDS

Dunaliella salina, halotolerant species, growth rate, chlorophyll

ABSTRACT

The halotolerant species *Dunaliella salina* is known to contain valuable products such as glycerol, *β*- carotene and unsaturated fatty acids. The commercial cultivation of *Dunaliella* for the production of *β*-carotene is now very successful in many areas of the world. A successful attempt to isolate and cultivate this species from a hypersaline lagoon located in the Southern part of Bahrain has been made in 2009. Growth rate and chlorophyll *a* and *b* concentrations were measured under controlled conditions. Mass production of *D. salina* for commercial reasons can be greatly implemented in Bahrain.

أول عملية عزل وزراعة ناجحة لطحلب *Dunaliella salina* (Dunal) Teodoresco من بحيرة شديدة الملوحة، مملكة البحرين

ليلى جاسم هزيم
قسم الأحياء، كلية العلوم، جامعة البحرين

رقم المسودة: (2743)
تاريخ استلام المسودة: 2013/05/05
تاريخ المسودة المُعَدَلة: 2014/11/24
الباحث المُرَاسِل: ليلى جاسم هزيم
بريد الكتروني:
hazeemlayla@gmail.com

الكلمات الدالة

Dunaliella salina، الأنواع التي تتحمل الملوحة العالية، معدل النمو، الكلوروفيل

المُستلخص

من المعروف أن الطحلب *Dunaliella salina* الذي يتكاثر في المياه شديدة الملوحة ذو فائدة اقتصاديه كبيرة لاحتوائه على المنتجات الثمينة مثل الجلسرين، بيتا كاروتين والأحماض الدهنية غير المشبعة. إن زراعة الطحلب الآن لأغراض تجارية واقتصاديه لإنتاج البيتا كاروتين ناجحة جدا في مناطق كثيرة من العالم. وفي هذه الدراسة تمت محاولة ناجحة لعزل وزراعة هذا النوع من بحيرة شديدة الملوحة وتقع في الجزء الجنوبي من البحرين في عام 2009. وقد تم قياس معدل النمو و تركيز كلوروفيل أ و ب تحت ظروف مختبريه محددة. إن إنتاج كميات كبيرة من طحلب *Dunaliella salina* لأسباب تجارية يمكن تنفيذها بشكل كبير في مملكة البحرين.

Introduction

Dunaliella salina (*D. salina*) species is a motile, unicellular green algae (Chlorophyta). It has two long flagella but lack rigid cell wall, the cells are rod to ovoid shaped (9 - 11 µm), and are common in marine waters. This type of organisms are relatively simple to cultivate and do not clump or form chains. *Dunaliella salina* is one of the most salt- tolerant eukaryotic alga and can grow in saturated media with Sodium Chloride (Ben-Amotz and Avron, 1990). It is also known to tolerate high temperatures, high photon flux densities and low nutrient concentrations (Borowitzka and Borowitzka, 1990); (Phadwala and Singhb, 2003).

This species is able to yield three major valuable products: glycerol, *β*- carotene and proteins (Tsai *et al*., 2012). It is capable of accumulating large amount of glycerol in response to extracellular osmotic pressure. Additionally, *Dunaliella salina* is a unique source of *β*- carotene that is produced in response to high light intensities, nitrogen starvation, high salinity and high temperature

(Pisal and lele, 2005). This species has the highest concentrations of *β*- carotene of any organism (Borowitzka and Borowitzka, 1990). It has been found that the concentration of *β*- carotene increased with the increase in salt concentrations (Al- Hasan *et al*., 1987) . In 1986, commercial production of *Dunaliella salina* has been commenced in Australia, the USA and Isreal (Borowitzka and Borowitzka, 1988) cited in (Borowitzka and Borowitzka, 1990). The anti-oxidants and anticancer properties of *Dunaliella salina* were found to be associated with the more production of *β*- carotene under stress conditions (Emtyazjoo *et al*., 2012).

Dunaliella salina has been isolated and cultivated in many areas of the world. A successful attempt has been made in Kuwait (Al-Hasan *et al*., 1987). It is known to occur in some salt ponds in Bahrain but no attempts were made previously to isolate this species. Consequently, the main purpose of this present study was to isolate *Dunaliella salina* and maintain it as unialgal culture to be used in future for more biotechnological applications.

Material and Methods

(1) Sampling Location

Water samples were collected from a hypersaline lagoon located in the southern part of Bahrain near Al-Jazear beach. Figure 1 shows the location of the sampling site. This area is characterized by its high salinity values and the spread of halotolerant plants.

(2) Isolation and Identification

Single cells of *Dunaliella salina* isolated from the collected water samples were spread on Petri plates containing agar dissolved in sterile seawater obtained from the salt pond (original salinity in the pond is approximately 300 PSU). After several weeks, cells from microcolonies on these plates were transferred aseptically to sterile test tubes containing Walne's liquid media. The unialgal culture was incubated at 18°C under 12:12 hours dark: light cycles. Isolation and cultivation protocol was carried out according to (Anderson and Kawachi, 2005); (Al-Ansari, 2007). The isolated and purified algal culture was identified according to morphological properties (Lee, 2008); (Graham *et al*., 2009). The culture was deposited at Scandinavian Culture Collection for Algae & Protozoa in October, 2012 and have been accepted in December, 2012 (SCCAP K-1830).

(3) Biomass Determination

Algal growth was determined by cell count and chlorophyll content. Cell count was performed using three replicates by a counting chamber (Neubauer Hemocytometer, 0.1mm deep). Chlorophyll *a* and *b* concentrations were determined by analyzing thirty milliliters of the culture extracted in 90% acetone following UNESCO protocol (Vohra, 1966) and the absorption spectra were recorded using (Perkin Elmer Lambda XLS) spectrophotometer . Cell count, Chlorophyll *a* and *b* measurements were carried out for 26 days after transferring the culture into a fresh medium in 250 mL Erlenmeyer flasks. These flasks were incubated at 18°C under 12/12 hours dark/ light cycles with occasional shaking in an incubator equipped with cool white fluorescent lamps.

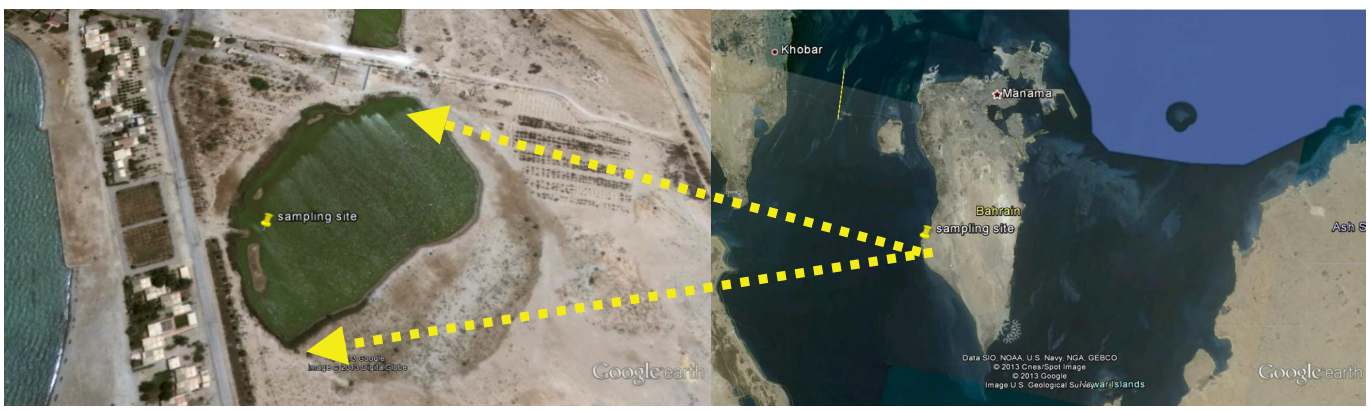

Figure 1: Location of Sampling Site

Results and Discussion

The isolated and purified strain was identified as *D. salina* by morphological examinations under Zeiss microscope (figure 2) based on cell shapes, the presence of two flagella and the changes in cells shape and color in different temperatures and nutrient concentrations (data not shown). The isolated species occurred as large red and small green cells according to their growth stage and media conditions.

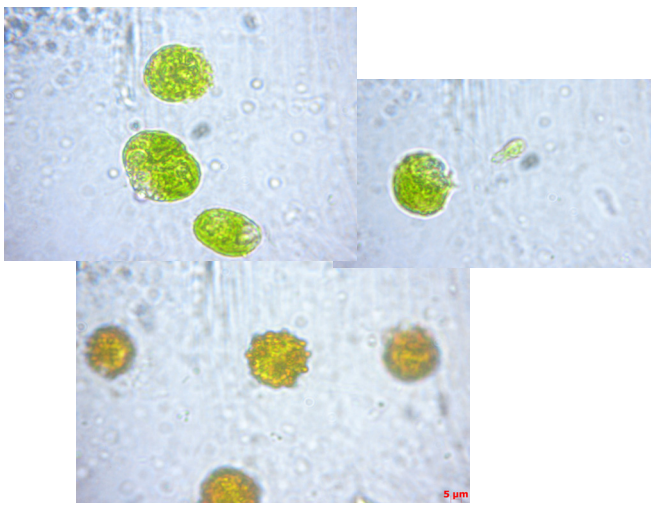

Figure 2: Different Images of *Dunaliella salina* Showing Different Growth Stages and Color

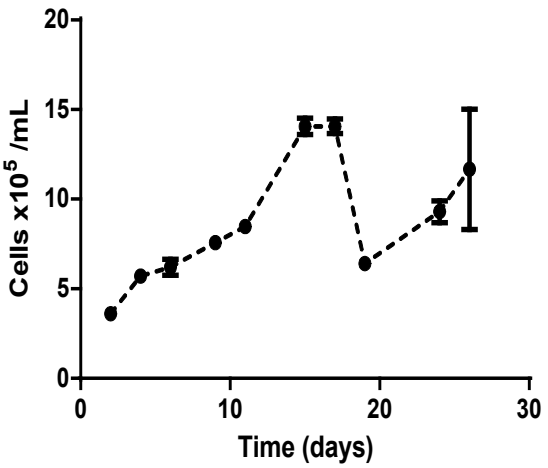

Figure3: Changes in Cell Concentration of *Dunaliella salina* Over 26 Days

Similar results have been found in (Al-Hasan *et al*., 2007). Figures 3 and 4 show the changes in cell densities and chlorophyll *a* and *b* concentrations during the 26 days from new sub-culturing. The maximum cell densities achieved after 15 days ($14x10^5$ cells. mL^{-1} [±0.45]) which coincided with the highest concentration of both chlorophyll *a* and *b* ($1mgL^{-1}$ [±0.3], $0.24mgL^{-1}$ [±0.04] respectively).

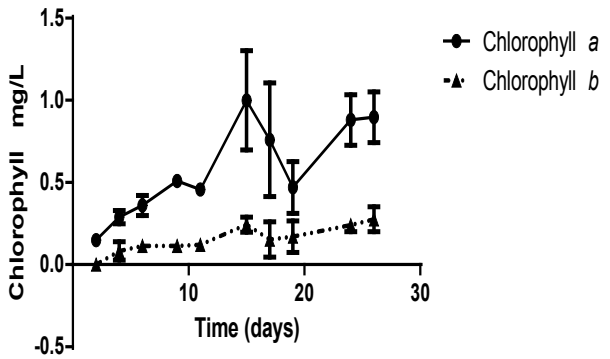

Figure 4: Changes in Chlorophyll *a* and *b* Concentration During 26 Days.

There was a decline in cell density and chlorophyll concentration on day 19 but there is a quick recovery on day 24. This is due to the fact that this species is capable of growing at low nutrient concentration. There was a significant correlation between cell count and chlorophyll *a* concentration (R^2= 0.8066) (Figure. 5).

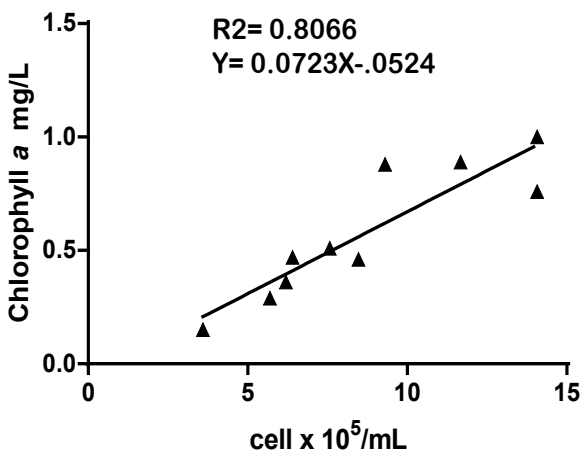

Figure 5: Correlation between Cell Concentration and Chlorophyll *a* Concentration.

The specific growth rate (μ) of *Dunaliella salina* in this study was 0.1. Nevertheless, *Dunaliella salina* have been found to achieve a maximum growth rate (μ) of 0.66 when macroalgae extract added to the media (Raja *et al*., 2007). Halostress may decrease the growth rate and cause harmful effects to the chloroplast, but the thylakoids that remain intact is capable of photosynthesis (Al-Hasan *et al*., 2007). It was found that maximum cell number obtained at pH 7, 5 mM NaNO3 and 20% NaCl concentrations (Çelekli and Dönmez ,2006) . Additionally, Sodium Chloride (NaCl) concentration more than 1.5M can inhibit cell growth but an increase in NaCl concentration from 0.5 to 1.0 M produced more intracellular lipid content (Takagi *et al*., 2006). (Abd El-Baky *et al*., 2004) has demonstrated that increasing salt concentration with decreasing nitrogen levels in the growth medium resulted in higher concentration of polyunsaturated fatty acids.

The results of this study shows that cultivation of *Dunaliella salina* species for commercial use is a promising project in the Kingdom of Bahrain either in open salt ponds or in closed bioreactors. It is quite important to find the best areas where environmental conditions are optimal for outdoor cultivation. The southern part of Bahrain is one of these possible areas that can be used for production of *Dunaliella salina*. This is due to the fact that this part of the island is characterized in addition to the hot climate that is dominant along Bahrain Island, it has very high salinity. This part of the island is distant from potential contamination and pollution, which are direct products of industrial or agricultural activities.

Acknowledgements

The author is grateful to Dr. Hashim Al-Sayed, Dean of College of Science, University of Bahrain and Mr. Adly Al-Ansari from National Mariculture Centre, Ministry of Municipalities and Urban Planning. Special thanks to Mr. Makki Daffalla and Mr. Ali Ibrahim from Department of Chemistry, University of Bahrain for technical assistance.

References

Abd El-Baky HH; El Baz FK; and **El-Baroty GS** (2004) Production of Lipids Rich Omega 3 Fatty Acids from the Halotolerant Alga *Dunaliella salina*. *Biotechnology,* **3** (1): 102- 108.
Available at: http://www.darkage.org/darkage/letolt/Pdf%20iratok/biotech31102-108.pdf

Al-Ansari A (2007) *Technical Manual for Live Food Production. Republic Commission for the Protection of Marine Resources, Environment and Wildlife.* Directorate of Marine Resources, Kingdom of Bahrain. pp31.

Al-Hasan RH; Ghannoum MA; Sallal AK; Abu-Elteen KH; and **Radwan SS** (2007) Correlative Changes of Growth, Pigmentation an Lipid Composition of *Dunaliella salina* in Response to Halostress. *Journal of General Microbiology,* **133** (9): 2607- 2616.
Available at: http://www.mic.sgmjournals.org/content/133/9/2607.full.pdf

Anderson R; and **Kawachi M** (2005) Traditional Microalgae Isolation Techniques. *In*: **Anderson R** (ed.), *Algal Culturing Techniques*. Elsevier Academic Press, San Diego, USA, pp83-101.
Available at: http://www.store.elsevier.com/Algal-Culturing-Techniques/isbn-9780120884261/

Ben-Amotz; and **Avron M** (1990) The Biotechnology of Cultivating the Halotolerant Alga *Dunaliella*. *Trends Biotechnology,* **8** (5): 121- 126.
Available at: http://www.cell.com/trends/biotechnology/abstract/0167-7799(90)90152-N

Borowitzka MA; and **Borowitzka LJ** (1988) *Dunaliella*, *In*: **Borowitzka MA; and Borowitzka LJ** (eds), *Micro-algal Biotechnology*. Cambridge University Press, Cambridge, UK, pp27-58.
Available at: http://www.bsb.murdoch.edu.au/groups/asrg/MABpublications.html

Borowitzka LJ; and **Borowitzka MA** (1990) Commercial Production of *β*- Carotene by *Dunaliella salina* in Open Ponds. *Bulletin of Marine Science,* **47** (1): 244- 252.
Available at: http://www.researchgate.net/publication/233633747_Commercial_

Çelekli, A; and **Dönmez G** (2006) Effect of pH, Light Intensity, Salt and Nitrogen Concentrations on Growth And B- Carotene Accumulation by a New Isolate of *Dunaliella sp*. *World Journal of Microbiology & Biotechnology,* **22** (2): 183-189.
Available at: http://www.link.springer.com/article/10.1007%2Fs11274-005-9017-0#page-1

Emtyazjoo M; Moghadasi Z; Rabbani M; Emtyazjoo M; Samadi S; and **Mossaffa N** (2012) Anticancer Effect of *Dunaliella salina* under Stress and Normal Conditions against Skin Carcinoma Cell Line A431 in Vitro. *Iranian Journal of Fisheries Sciences*, **11** (2): 283- 293.
Available at: http://www.jifro.ir/browse.php?a_code=A-

Graham LE; Graham JM; Wilcox LW (2009) *Algae*. Benjamin Cummings Pub Co. San Francisco, USA.
Available at: http://www.abebooks.com/Algae-Linda-Graham-Lee-

Lee ER (2008) *Phycology*. Cambridge University Press, United Kingdom.
Available at: http://www.cup.cam.ac.uk/9780521682770

Phadwala K; and **Singhb PK** (2003) Effect of Nutrient Depletion on β- carotene and Glycerol Accumulation in Two Strains of *Dunaliella sp*. *Bioresource Technology* **90** (1): 55- 58.
Available at: http://www.eurekamag.com/research/010/530/influence-nutrient-depletion-beta-carotene-glycerol-accumulation-strains-dunaliella-sp.php

Pisal DS; and **Lele SS** (2005) Carotenoid Production from Microalga, *Dunaliella salina*. *Indian Journal of Biotechnology,* **4** (4): 476- 483.
Available at: http://www.nopr.niscair.res.in/bitstream/pdf

Raja R; Iswarya SH; Balasubramanyam D; and **Rengasamay R** (2007) PCR- Identification of *Dunaliella salina* (Volocales, Chlorophyta) and its Growth Characteristics. *Mircobiological Research,* **162** (2): 168- 176.
Available at: http://www.sciencedirect.com/science/article/pii/S0944501306000309

Takagi M; Karseno T; Yoshida (2006) Effect of Salt Concentration on Intracellular Accumulation of Lipids and Triacylglyceride in Marine Microalgae *Dunaliella* Cells. *Journal of Bioscience and Bioengineering,* **101** (3): 223-226.
Available at: http://www.researchgate.net/publication/7064657_Effect_of_salt_

Tsai CF; Lu FJ; Hsu YW (2012) Protective Effects of *Dunaliella salina*- a Carotenoids Rich Alga against Ultraviolet B-induced Corneal Oxidative Damage in Mice. *Molecular Vision,* 18: 1540- 1547.
Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3380915/
Available at: http://www.ncbi.nlm.nih.gov/pubmed/22736944

Vohra FC (1966) Determination of Photosynthetic Pigment in Seawater. Monograph on Oceanographic Methodology I. UNESCO, Paris, France, pp1-66.
Available at: http://www.unesdoc.unesco.org/images/0007/000716/071612eo.pdf