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Characterization of an Indigenous Isolate, *Dunaliella tertiolecta* ABRIINW-G3, from Gavkhooni Salt Marsh in Iran Based on Molecular and Some Morpho-physiological Attributes

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ABSTRACT

***Dunaliella* is a green halotolerant microalga, which has several industrial applications e.g. β-carotene production. Identification of different *Dunaliella* species has been carried out by morpho-physiological and recently molecular studies. To achieve an improved understanding of taxonomy, these studies are required to be in linkage. The present study describes molecular and specific morpho-physiological properties of a *Dunaliella* isolate obtained from Gavkhooni salt marsh in Iran. Phylogenetic analysis of Internal Transcribed Spacer region demonstrated that the isolate was associated with different species except *D. salina* (CCAP 19/18 and 19/30) and *D.viridis*. 18S rDNA size of the isolate was identical to that of *D. tertiolecta* and intron-lacking strains of *D. salina*. 18S rDNA fingerprint profile and phylogenetic analysis revealed *D. tertiolecta* as the closest taxon to the isolate. Features of optimum growth salinity (1.5-3% w/v) and maximum carotenoid per cell (0.7 pg cell^{-1}) were comparable with reported data for *D. terrtiolecta*. Morphological characteristics including the size and color of the cells, presence and location of stigma and refractile granules were similar to those of *D. tertiolecta*. Totally, considering molecular and morpho-physiological properties, the isolate was attributed to the species *D. tertiolecta* and was named as *Dunaliella tertiolecta* ABRIINW-G3.**

Keywords: Carotenoid, *Dunaliella*, 18S rDNA, Internal Transcribed Spacer, Optimum growth salinity.

INTRODUCTION

The green alga *Dunaliella* is a unicellular, eukaryotic and photosynthetic microorganism that lacks a rigid cell wall (Ben-Amotz and Avron, 1987). As one of the main sources of natural ß-carotene, *Dunaliella* has attracted growing interest in nutraceutical, cosmetic and pharmaceutical industries (Hejazi *et al*., 2002). Further, because *Dunaliella* represents unique biological and technical features, it has been the subject of study in molecular farming (Barzegari *et al*., 2010). *Dunaliella* can adapt to a wide range of salt concentrations varying from low (0.05M) to saturation (5.5M) level (Ben-Amotz and Avron, 1973; Borowitzka and Brown, 1974). In addition, it can survive high light intensities (Ben-Amotz and Avron, 1982; Borowitzka *et al*., 1984) due to carotenoid accumulation ability, which protects *Dunaliella* against intense irradiation (Ben-Amotz *et al*., 1989; Gómez *et al*., 1992). Under stress conditions such as elevated light densities, high salinity and nutrient deficiency,

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ß-carotene accumulation may reach a level more than 10% dry weight of *Dunaliella* (Ben-Amotz and Avron, 1983; Ben-Amotz, 1995). Commercial considerations and achieving effective classification require increasing attention to isolation and delineation of various strains of *Dunaliella*. Different species of *Dunaliella* have been identified based on morphological characteristics and physiological behaviour. However, lack of a rigid cell wall and presence of compensatory adaptation processes in the members of *Dunaliella* (Sciandra *et al*., 1997) lead to variable morpho-physiological features. Consequently, misidentifications and confusions are possible in the taxonomy of *Dunaliella*. Molecular techniques may provide a powerful tool in distinguishing different species (Gómez and Gonzaléz, 2004; Olmos *et al*., 2000, 2009). In order to better understand the taxonomy of the genus, molecular investigations are recommended to accompany morphological/ physiological studies (Borowitzka and Siva, 2007) as was observed in the recent research by Hejazi *et al.* (2010) and Azua-Bustos *et al*. (2010).

Analysis of ribosomal spacer sequences, including internal transcribed spacer region (ITS1 and ITS2 plus the 5.8S rDNA gene) has been carried out for phylogenetic studies in *Dunaliella* genus (González *et al*., 1999; González *et al*., 2001; Gómez and González, 2001, 2004). Further, molecular markers based on 18S rDNA gene have been used for the identification of *Dunaliella* species (Olmos *et al*., 2000, 2002; Fazeli *et al*., 2006; Raja *et al*., 2007; Hejazi *et al*., 2010). Using 18S rDNA region, four different structures within the genus of *Dunaliella* have already been described (Olmos *et al*., 2000, 2002; Hejazi *et al*., 2010): the two structures which contain one intron but in two different positions, the structure that has two introns, and the structure that lacks any intron in 18S rDNA gene. The latter is reported in the species of *D. tertiolecta* and some strains of *D. salina* (e.g. UTEX LB1644).

Having several salt lakes and marshes, Iran can be considered as an appropriate habitat for microalga *Dunaliella*. In this study, an indigenous isolate was obtained from Gavkhooni salt marsh in Iran. The isolate was described based on the genomic regions of 18S rDNA and ITS. Cell morphology as well as the physiological features of optimum growth salinity and maximum cellular carotenoid were determined.

MATERIALS AND METHODS

Isolation and Cultivation

Water sample was collected from Gavkhooni salt marsh in Iran and was then inoculated with a liquid medium described by Hejazi and Wijffles (2003). After several recultivations, the cultured specimen was spread on the same medium prepared by 1.8% agar. An individual isolate was selected from the culture for the experiment. The cultured isolate was maintained at 26ºC and a light intensity of 80 µmol photon m^{-2} s^{-1} under the light to dark photoperiod of 16: 8 hours.

DNA Extraction and PCR Amplification of ITS Region and 18S rDNA Gene

Isolation of genomic DNA was carried out by the method suggested by Hejazi *et al.* (2010). ITS region was amplified using two primers of AB1 (5'-AATCTATCAATAACCACACCG-3') and AB2 (5'-TTTCATTCGCCATTACTAAGG-3'). Using Oligo5, these primers were designed from the nucleotides 1-21 of ITS1 and 80-100 of 28S rDNA gene, respectively. PCR reactions were performed in 50 µl volumes containing 20 ng of template DNA in TE (Tris/EDTA) buffer and 50 ng of each primer using 1X PCR Master Kit (CinnaGen PCR Master Kit, Cat. No. PR8250C). The amplification was performed in 35 cycles using a TECHNE Thermal Cycler (Model: FTGRAD2D) according to the method described by Hejazi *et al*. (2010).

18S rDNA amplification was performed using two conserved primers called MA1 and MA2 (Olmos *et al*., 2000). To delineate at species level, three species-specific primers of DSs, DBs and DPs were utilized; DSs, DPs and DBs were designed (Olmos *et al*., 2000, 2002) according to the single intron of *D. salina*, the first intron of *D. parva* and *D. bardawil*, respectively. They were used as forward primers along with the reverse primer of MA2. Thermocycling consisted of 5 minutes initial denaturing time at 95ºC, followed by 35 cycles at 95ºC for 1 minute, 52 ºC for 1 minute and 72ºC for 2 minutes with final extension of 72 ºC for 10 min.

Purification and Sequencing of PCR Products

Purification was performed according to the manufacturer's instructions described in the PCR purification kit (Roche, Product No. 1732668). The purified amplicon was sent to Macrogen Company (Seoul, Korea) for sequencing.

Alignment and Phylogenetic Analysis of Sequences

ITS and 18S rDNA sequence of several strains (Table 1) including *Chlamydomonas reinhardtii* as outgroup was collected from National Center for Biotechnology Information (NCBI). The sequence alignment was performed using MEGA version 4 (Tamura *et al*., 2007). The alignment statistics were estimated by 'DNA Sequence Polymorphism' (DnaSP), version 5 (Librado and Rozas, 2009). Phylogenetic analyses were conducted using Neighbour-Joining method. The evolutionary distances were computed by the Maximum Composite Likelihood model. Reliability of the branches was assessed by bootstrapping the data with 1,000 replicates.

Determination of Cellular growth and Carotenoid Production

In order to determine the optimum growth salinity and carotenoid content of the isolate, the media were prepared with 7 salt concentrations of 0.25, 0.5, 1, 1.5, 2,

Table 1. List of *Dunaliella* strains investigated in this study.

No.	*Dunaliella* strains	Accession NO. (18S rDNA)	Accession NO. (ITS)
1	*Dunaliella sp*. ABRIINW-M1/1	EU522092	EU927374
2	*Dunaliella sp*. ABRIINW-U1/1	FJ164062	FJ164063
3	*Dunaliella salina* CCAP 19/30	EF473749	EU932917
4	*Dunaliella salina* UTEX LB 1644	DQ009765	
5	*Dunaliella salina* SAG 42.88	EF473740	EF473741
6	*Dunaliella tertiolecta* CCAP 19/27	EF473747	EF473748
7	*Dunaliella tertiolecta* CCMP 1320	EF537907	
8	*Dunaliella tertiolecta* CCMP 1302	DQ009771	DQ377096
9	*Dunaliella tertiolecta* SAG 13.86	EF473737	EF473738
10	*Dunaliella tertiolecta* UTEX LB 999	DQ009773	
11	*Dunaliella primolecta* UTEX LB 1000	DQ009764	DQ377092
12	*Dunaliella bioculata* UTEX LB 199	DQ009761	DQ377086
13	*Dunaliella salina* CCAP 19/18	EF473745	EF473746
14	*Dunaliella salina*	M84320	
15	*Dunaliella viridis* CONC002	DQ009776	DQ377098
16	*Dunaliella bardawil* LB2538	AF150905	
17	*Dunaliella parva*	M62998	DQ116746
18	*Dunaliella sp*. ABRIINW-M1/2	EU678868	EU927373
19	*Dunaliella salina* MSI-1		GQ337903
20	*Dunaliella atacamensis*	FJ917192	
21	*Chlamydomonas reinhardtii*	AB511836	AJ749638

2.5 and 3 M NaCl each in 3 replications. The cultures were maintained at an irradiance of 80 µmol photon m^{-2} s^{-1}. Cell counting was performed every other day using Neubauer counting chamber (HBG, Germany, Tiefe depth profondeur 0.10 mm and 0.0025 mm^2 area). Carotenoid content of the cells was determined once every four days. For carotenoid extraction, the following procedure was used; 2 ml of the culture was centrifuged (Centrifuge Beckman, Allegra X-22R, USA) at 948 xg for 10 minutes. Supernatant was thrown away and 5 ml of KOH (5%) dissolved in methanol (30%) was added to the biomass. The sample was maintained at 60°C for 20 minutes to remove chlorophyll. Then the container was centrifuged at 2132 xg for 10 minutes. The upper fluid was discarded. To the remaining biomass of yellow/orange-color, 2 ml of Hexane-Acetone (1:9) was added and the tubes were well spun. Finally, after 5 min centrifugation at 658 xg, carotenoid concentrations were determined using a spectrophotometer (Genesys 5, Model 336001, USA). Total carotenoid content was calculated according to the equation obtained by calibration curve (Hejazi and Wijffels, 2003) using the following formula:

$C= A_{446}\times3.26\times V_C/V_S$

Where, C= Total carotenoid (µg ml^{-1}), V_C= Volume of culture sample and V_S= Volume of extract (ml).

The sample absorbance was scanned over the wavelengths of 350-800 nm and λ_{max} was recorded at 446 nm.

Statistical Analysis

The data comparison for different salinity levels was performed based on Completely Randomized Design (CRD) method using SAS software (Release 6.12, SAS Institute Inc., Cary, NC, USA). Means were separated by the Duncan's multiple range test (Duncan, 1995).

Light Microscopy

Morphological characteristics including the shape and size of the cells, absence/presence and location of stigma and refractile granules were examined using a light microscope (Zeiss, Axiostar Plus, Germany). To observe pyrenoids, the cells were treated with 0.05% bromophenol blue (BPB) dissolved in 0.1% $HgC1_2$ (Brown and Arnott, 1970).

RESULTS

Amplification and Sequence Analysis of ITS Region

A fragment of ~700 bp was amplified as ITS region with primer pair (AB1-AB2) specific for ITS region of *Dunaliella*. The amplification confirmed the isolate as a member of *Dunaliella* genus. To achieve ITS sequence composition, the amplified region was sequenced. Using the software "Basic Local Alignment Search Tool" (BLAST) at NCBI, the sequence of the isolate was compared with other recorded data. It exhibited various similarities of 99% to *D. tertiolecta* CCAP 19/27, *D. salina* SAG 42.88, *D. tertiolecta* SAG 13.86, *D. primolecta*, *D. bardawil*, *D. parva*, 94% to *D. salina* CCAP 19/18 and 92% to *D. salina* CCAP 19/30. During the next analyses, the isolate of our interest was coded as *Dunaliella sp*. ABRIINW-G3. To analyze the ITS region and establish a phylogenetic tree, the sequences of different strains given in Table 1 were utilized. Because analysis of the entire ITS region (including 5.8S rDNA) was aimed, the sequences of the six following strains were excluded: *D. salina* M84320, *D. bardawil* LB2538 and *D. atacamensis* for which there was no recorded ITS sequence; *D. salina* UTEX LB 1644, *D. tertiolecta* CCMP 1320 and *D. tertiolecta* UTEX LB 999 for which ITS regions were registered as separate ITS1 and ITS2 sequences, lacking 5.8S rDNA gene, at NCBI. After alignment, using DnaSP software, the sequence conservation was determined as C: 0.619 and polymorphic sites were calculated as 228 out of 598 nucleotides. The tree (Figure 1) illustrated that *Ch. reinhardtii* was the most divergent from all other analyzed *Dunaliella* taxa. The 14 sequences belonging to *Dunaliella* members were divided into three lineages of a, b and c. *Dunaliella sp.* ABRIINW-G3 appeared as an individual entity which was grouped (Bootstrap value: 96%) with the clade containing *D. tertiolecta* strains 'CCMP 1302', SAG 13.86 and CCAP 19/27, *D. primolecta* UTEX LB 1000, *D. bioculata* UTEX LB 199, *D. salina* SAG 42.88 and *D. parva*. The members of this clade showed the least evolutionary distance (0.009) with the isolate. Evolutionary distance, as a measure of evolutionary divergence, is the number of substitutions per site that have occurred between a pair of homologous sequences. The second lineage (b) contained *Dunaliella sp.* ABRIINW-M1/1, *D. salina* CCAP 19/18, *D. salina* CCAP 19/30, *Dunaliella sp.* ABRIINW-U1/1 and *D. salina* MSI-1. Finally *Dunaliella sp.* ABRIINW-M1/2 and *D. viridis* were included within the third lineage (c). The ITS sequence of *Dunaliella sp.* ABRIINW-G3 was registered at NCBI database with accesion number of HQ590542.

Amplification and Sequence Analysis of 18S rDNA Gene

Amplification of 18S rDNA region with MA1-MA2 resulted in the production of a ~1770 bp DNA fragment. Using three species-specific primers of DSs, DPs and DBs (Olmos *et al*., 2000, 2002), no fragment was produced.

To obtain the sequence fingerprint profile of the 18S rDNA, sequencing was performed. Then the obtained sequence was aligned with 18S rDNA sequences related to other known species (Table 1). Out of 1,702

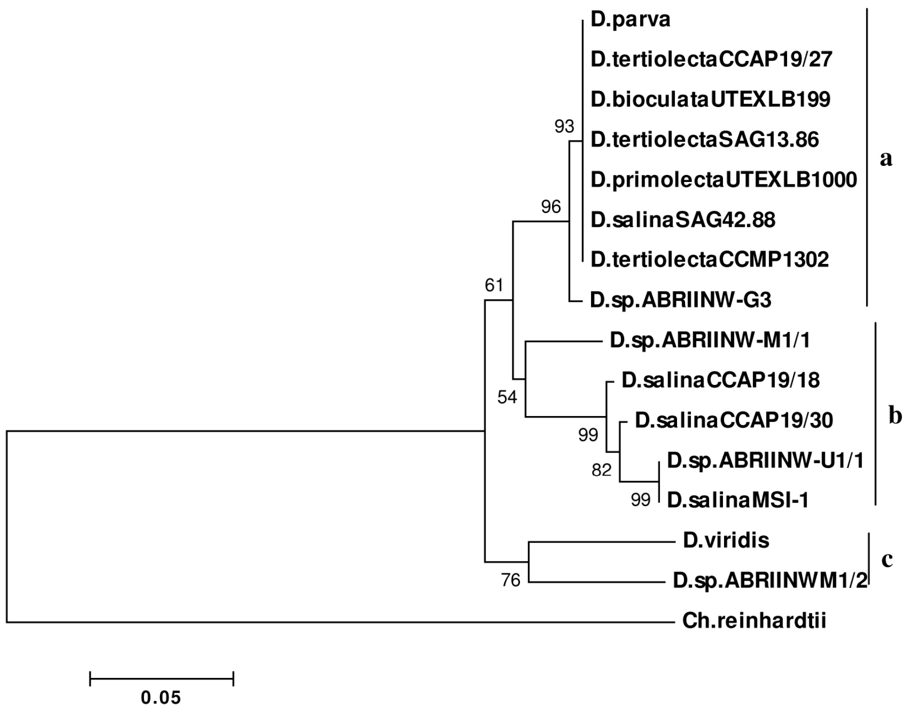

Figure 1. Phylogenetic tree based on ITS region of various strains of *Dunaliella* using neighbor-joining method. Bootstrap values, as measures of supporting a given grouping, were calculated from 1,000 replicates. Bar represents 0.05 nucleotide substitutions per position. Three lineages of a, b and c were produced. Within lineage a, *Dunaliella sp.* ABRIINW-G3 was grouped with different members of *Dunaliella*. *D. salina* MSI-1 isolated from Maharlou Lake was included in Lineage b.

net number of analyzed sequence sites, 14 polymorphic sites and a high conserved region extending from 33 to 1,645 nucleotides were determined. Sequence conservation was estimated to be 0.992. According to the phylogenetic tree (Figure 2), five lineages of A, B, C, D and E were supported with high bootstrap value (more than 80%). As an outgroup, *Ch. reinhardtii* appeared in a distinctive branch. The isolate of our interest, was located in lineage B which contained *D. tertiolecta* CCMP 1302, *D. tertiolecta* UTEX LB 999, *D. viridis* and *Dunaliella sp.* ABRIINW-M1/2. Based on the evolutionary analysis (data not shown), *D. tertiolecta* CCMP 1302, with evolutionary distance of 0.002, was identified as the closest taxon to *Dunaliella sp.* ABRIINW-G3. The sequence of our isolate was registered at NCBI database with accesion number of GU984571.

Optimum Growth Salinity and Maximum Carotenoid Content

To measure the optimum growth salinity, the starting Optical Density of inoculums in

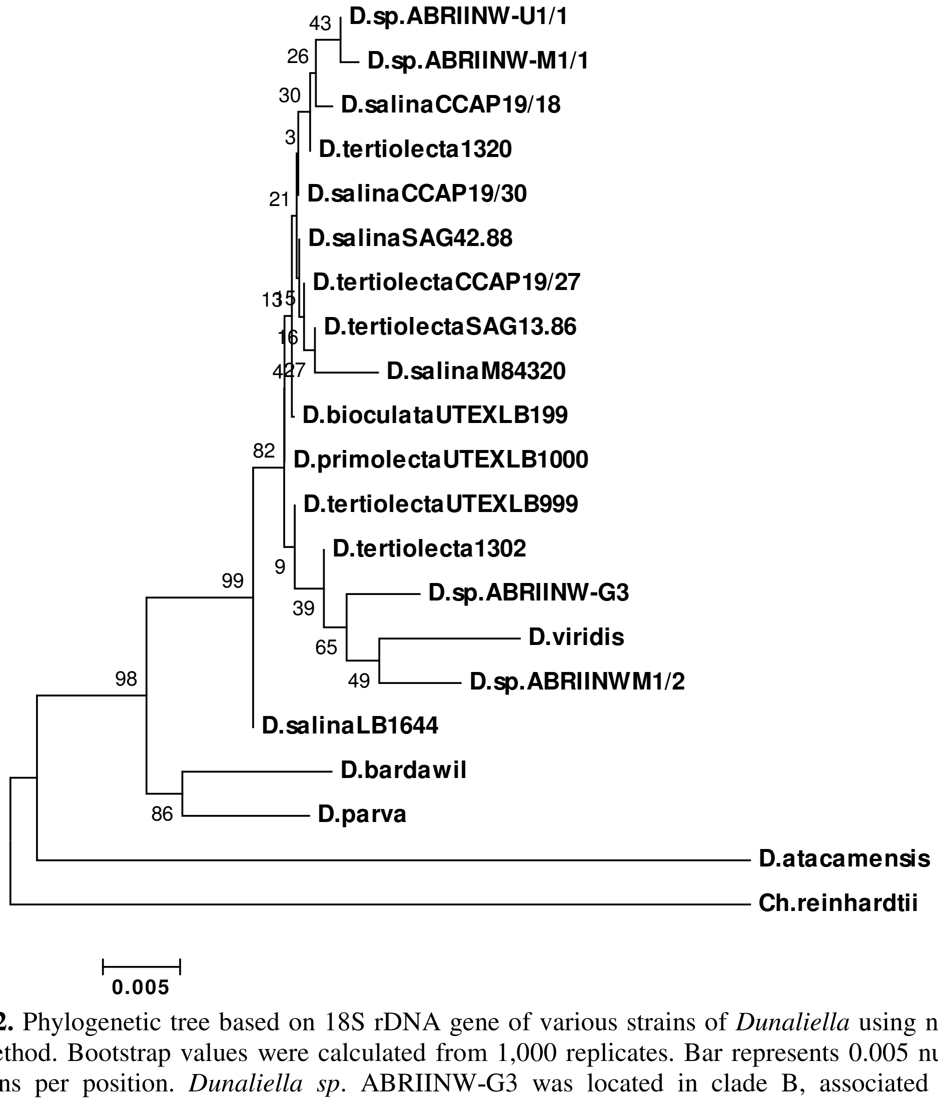

Figure 2. Phylogenetic tree based on 18S rDNA gene of various strains of *Dunaliella* using neighbor-joining method. Bootstrap values were calculated from 1,000 replicates. Bar represents 0.005 nucleotide substitutions per position. *Dunaliella sp.* ABRIINW-G3 was located in clade B, associated with *D. tertiolecta* CCMP 1302 and *D. tertiolecta* UTEX LB 999.

all 7 salinity levels was adjusted to the same quantity of ~0.08 at 550 nm. Because there was not any significant growth at 2.5 and 3M NaCl, these measurements were excluded from subsequent investigations. The data revealed that cell growth was significantly affected by NaCl concentration (P< 0.01). 26 days after inoculation, the isolate attained maximum cell density of 16.4×10^6 and 15.83×10^6 cell ml^{-1} at 0.25 and 0.5M NaCl, respectively (Figure 3). In contrast, the lowest cell density was determined at 2M NaCl. The maximum carotenoid content was measured as 0.7 pg $cell^{-1}$ at 2M NaCl, which was significantly higher than the other salinities (P< 0.01). During the absorbance scanning procedure (Figure 4), the main carotenoid type was assessed as ß-carotene.

Cell Morphology

Morphological schema of the cells in 10 day old cultures of different salinities is depicted in Figure 5 (a-f). At different salt concentrations, the cells color was constantly green. However, cell shape changed varying from ellipsoidal at low NaCl levels of 0.25-1.5M to spherical at the salinities of 2-3M. The cells lack any cell wall and have one stigma (Figure 5-g) near the location where two equal flagella are inserted. Cells contain a cup-shaped chloroplast with a basal pyrenoid surrounded by starch granules (Figure 5-h). The lateral lobes of the chloroplast are not extended to the anterior end of the cell, which contained small refractile granules (Figure 5-h). The cell size was calculated by averaging the sizes measured at optimum growth salinity. The average cell length was about 11.6 µm with the range of 9 to 15 µm. The average width of the cells was 7.2 µm ranging from 5 to 9.5 µm. The average size for two equal flagella, inserted apically, was about 12.5 µm. Proportion of flagella length to cell length was ~ 1.1 (based on average sizes).

DISCUSSION

To date, identification of *Dunaliella* species/strains has already been the subject of morpho-physiological and recently molecular studies. However, these studies were mostly performed separately and led to ambiguity in achieving a thorough understanding of *Dunaliella* taxonomy. In 2007, Borowitzka and Siva illustrated the

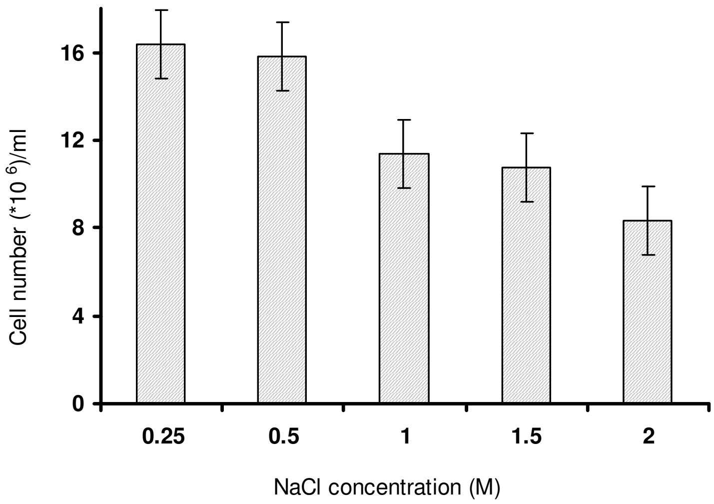

Figure 3. Diagram showing cell density at five salt concentrations (26 day after cultivation) under light intensity of 80 µmol photon m^{-2} s^{-1} in *Dunaliella sp.* ABRIINW-G3.

deficiencies of this separation, emphasizing that these studies need to be paired. Iran has different salt lakes and marshes in which *Dunaliella* can grow. However, very few studies have been performed on native isolates of Dunaliella (Fazeli *et al.*, 2006; Hadi *et al.*, 2008; Zamani *et al.*, 2011) and there is still demanding need to isolate and characterize the Iranian isolates of this microalga. In this regard, this research was performed to identify and describe an indigenous isolate from Gavkhooni. Gavkhooni is a salt marsh located in the center of Iran where *Dunaliella* exists.

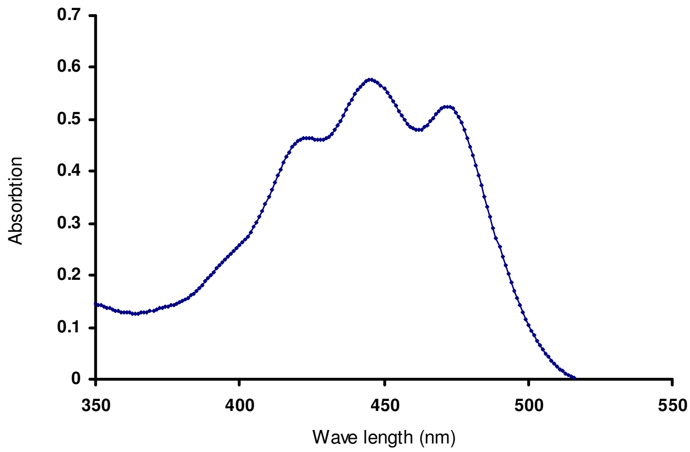

Figure 4. Absorption spectrum of the carotenoid extracted from 12-day old culture of *Dunaliella sp.* ABRIINW-G3.

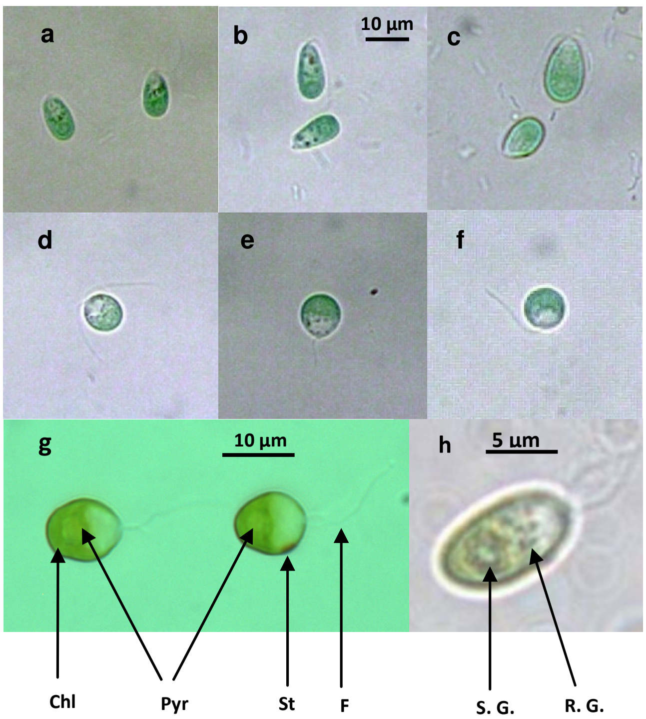

Figure 5. Light micrographs of *Dunaliella sp.* ABRIINW-G3 in 10 day old cultures: **a-f** (Scale= 10 µm) show cell morphology at different salinity levels (a: 0.5; b:1; c:1.5; d: 2; e: 2.5, and f: 3 M NaCl), **g** and **h** are of the sections: Chl: Chloroplast; Pyr: Pyrenoid; St: Stigma; F: Flagella; S.G.: Starch grains, and R.G.: refractile granules.

Previously, two species of *D. salina* and *D. viridis* had been identified from Gavkhooni (Hadi *et al*., 2008). The salinity and pH of the collected water sample were respectively in the range of 0.5-2M NaCl and 7-8. The studied isolate was obtained from a water sample with salinity close to 0.5M and pH equal to 7.5. The ITS region size (~ 700 bp) of the isolate was identical to the sizes reported for different *Dunaliella* species. It was demonstrated that there was no ITS length variation at intra- or interspecific level of *Dunaliella* (González *et al*., 1999). Based on phylogenetic analysis of ITS region, *Dunaliella sp*. ABRIINW-G3 was grouped with the clade containing *D. tertiolecta*, *D. primolecta*, *D. bioculata*, *D. bardawil*, *D. parva* and intron-lacking strains of *D. salina*. The mentioned different species within this clade were not differentiated using phylogenetic analysis of ITS region. The previously reported isolate of *D. salina* MSI-1 from Maharlou lake in Iran (Zamani *et al.*, 2011) was placed on a separate lineage and was grouped with *D. salina* strains 19/18 and 19/30. This Iranian isolate showed 92% homology with *Dunaliella sp*. ABRIINW-G3. From Iran, another strain, *D. tertiolecta* (DCCBC26) had been identified originating from Urmia Lake (Fazeli *et al.,* 2006). Because only ITS2 region of this strain was available, it was compared with the corresponding region in our isolate. The comparison showed that the two isolates shared 98% nucleotide similarity with each other.

The amplification by MA1-MA2 primers, which allows amplification of nearly full length of 18S rDNA in *Dunaliella*, resulted in the production of ~1770 bp fragment. According to Olmos *et al*. (2000) an amplicon of the same size is amplified in *D. tertiolecta* as well as some strains of *D. salina* (e.g. LB1644). 18S rDNA of the mentioned size is reported to have no intron within the gene and hence no fragment is amplified using the species-specific primers. It should be noted that in other strains of *D. salina* (e.g. 19/3, 19/18, M84320), which contain one intron, a band of ~2170 bp is created. Thus, in the studied isolate 18S rDNA size and amplification of no band by species-specific primers confirmed the lack of intron(s) in 18S rDNA. According to the analysis of evolutionary distances, the studied isolate showed the closest distance with *D. tertiolecta* 1302.

Optimum growth of *Dunaliella sp*. ABRIINW-G3 occurred at 0.25-0.5 M (1.5-3% (w/v)) NaCl. For *D. terrtiolecta* DCCBC26 (from Urmia Lake, Iran) growth preference was reported as 0.3-0.7 M NaCl (Fazeli *et al.*, 2006). It has been specified that *D. terrtiolecta* and *D. salina* can grow well at the salinities of no more than 6% and 20-25%, respectively (Borowitzka and Siva, 2007). Therefore, optimum growth salinity of the studied isolate was comparable with that of *D. terrtiolecta.* The maximum carotenoid per cell (0.7 pg cell^{-1}) was close to the quantity reported for *D. tertiolecta* CCMP 19/22. Maximum carotenoid per cell occurred at 2 M NaCl (12%) in which cell growth was limited. This was in congruence with the studies that claim although elevated salinity (here 2M NaCl) favors carotenoid biosynthesis, it represses the growth (Gómez *et al*., 2003; Jahnke and White, 2003).

Morphologically, the green color of the cells in all media containing 0.25- 3 M NaCl was retained. However, cell shape changed with salinity of the cultures; when cultured at elevated salinities the ellipsoidal shape turned to rounded. This was in agreement with previous studies which had demonstrated that cell shape of a certain species often became spherical under unfavorable conditions (Borowitzka *et al.,* 1984; Ben-Amotz *et al*., 2009). *Dunaliella sp*. ABRIINW-G3, matched in cell size with *D. tertiolecta* except for the proportion of flagella length/cell length (1.1) which was less than *D. tertiolecta* (1.5-2).

In conclusion, based on molecular investigations of 18S rDNA gene and ITS regions, low carotenoids content, growing well at low salinities and morphological properties, the studied isolate was attributed to the species *D. tertiolecta* and named as *Dunaliella tertiolecta* ABRIINW-G3.

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شناسایی ایزوله بومی *Dunaliella tertiolecta* ABRIINW-G3 از باتلاق گاوخونی در ایران براساس خواص مولکولی و برخی صفات مورفو-فیزیولوژیکی

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چکیده

دونالیلا یک ریزجلبک سبز مقاوم به شوری است که کاربردهای تجاری متعددی نظیر تولید بتاکاروتن دارد. شناسایی گونه‌های مختلف دونالیلا براساس مطالعات مورفو-فیزیولوژیکی و اخیرا مطالعات مولکولی صورت گرفته است. برای دست‌یابی به درک صحیح از تاکسونومی دونالیلا، توصیه می‌گردد این مطالعات به طور همزمان در شناسایی به کار گرفته شوند. تحقیق حاضر مطالعات مولکولی و برخی خصوصیات مورفولو-فیزیولوژی ایزوله دونالیلای جدا شده از دریاچه گاوخونی در ایران را توصیف می‌کند. مقایسه توالی ناحیه حد فاصل بین نواحی رونویسی شونده (ITS) رابطه نزدیک این جدایه را با یک سری از توالی‌های متعلق به گونه‌های مختلف دونالیلا به استثنای دونالیلا سالینا نژادهای CCAP 19/18و CCAP 19/30 و دونالیلا ویریدیس نشان داد. اندازه ۱۸S rDNA ایزوله مورد مطالعه با اندازه ژن مربوطه در دونالیلا ترتیولکتا و نژادهای فاقد اینترون گونه دونالیلا سالینا یکسان بود. تجزیه و تحلیل پروفیل انگشت نگاری ۱۸S rDNA و آنالیز فیلوژنتیکی آشکار ساخت که دونالیلا ترتیولکتا نزدیک‌ترین تاکسون به جدایه مورد مطالعه است. دو مشخصه شوری مطلوب رشد (۱/۵-۳٪ وزنی-حجمی) و حداکثر کاروتنویید سلولی (۰/۷ پیکوگرم بر سلول) با داده‌های گزارش شده برای دونالیلا ترتیولکتا قابل مقایسه بود. تشابه این ایزوله با گونه دونالیلا ترتیولکتا از نظر صفات ظاهری نظیر اندازه سلول، وجود و محل لکه‌چشمی و دانه‌های انکساری تایید شد. در مجموع با درنظرگیری بررسی‌های مولکولی و مورفو-فیزیولوژیکی، ایزوله مورد مطالعه به گونه ترتیولکتا نسبت داده شد و به عنوان *Dunaliella tertiolecta* ABRIINW-G3 نامگذاری گردید.