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CHARACTERIZATION OF A NEW *Dunalliela salina* STRAIN ISOLATED FROM SAN QUINTIN, BAJA CALIFORNIA (MÉXICO) PRODUCER OF LIPIDS, PIGMENTS AND MICRONUTRIENTS

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ABSTRACT. Some microalgae are recognized for producing pigments and other metabolites with biotechnological importance, particularly *Dunaliella salina*. These kind of compounds are used as food and have an industrial potential. The pigment industry comprises a millionaire market value, being β-carotene one of the most profitable one. In this study, we describe the morphology, molecular identification, growth dynamics, proximal composition, nutrients, and pigment content of a recently isolated *Dunaliella salina* strain from San Quintin, BC, Mexico, under different salinity and light conditions, in order to highlight its remarkable properties for the biotech and biomed industries. *D. salina* SQ reached the highest densities (1.07-1.25 cell mL^{-1} $x10^6$) at low salinities (100 and 500 mM NaCl) under a continuous light regimen. Neoxanthin (Neo) and violaxanthin (Viol) were the most abundant pigments when exposed to 500 mM NaCl (18:6 h Light:Dark cycle). Furthermore, this peculiar strain produces other compounds with high industrial value.

Keywords: Chlorophyte, *Dunaliella salina*, pigments, sea salt fields, salinity challenge.

Caracterización de una nueva cepa de *Dunalliela salina* asilada de San Quintin, Baja California (México), productora de lípidos, pigmentos y micronutrientes

RESUMEN. Algunas microalgas son reconocidas por producir pigmentos y otros metabolitos con importancia biotecnológica, en particular, *Dunaliella salina* es una de las más notables. Este tipo de compuestos se usan como alimento y tienen potencial industrial. La industria del pigmento tiene un valor de mercado millonario, siendo el β-caroteno uno de los más rentables. En este estudio se describen la morfología, la dinámica de crecimiento, composición proximal, composición de nutrientes y contenido de pigmentos de una cepa de *Dunaliella salina* de San Quintín, BC, México, recientemente aislada. La identificación de la especie se corroboró mediante técnicas moleculares. Se cultivó *D. salina* bajo diferentes condiciones de salinidad y luz, con el objetivo de resaltar sus propiedades para las industrias biotecnológica y biomédica. *D. salina* SQ alcanzó las densidades más altas (1.07-1.25 células mL^{-1} $x10^6$) a salinidades bajas (NaCl 100 y 500 mM) en un régimen de luz continua. La neoxantina (Neo) y la violaxantina (Viol) fueron los pigmentos más abundantes en 500 mM NaCl y un ciclo de luz: oscuridad 18: 6 h. Además, esta peculiar cepa produce otros compuestos con alto valor industrial.

Palabras clave: Chlorophyta, *Dunaliella salina*, pigmentos, pozas de sal marina, desafíos de salinidad.

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INTRODUCTION

Pigments and other metabolites produced by microalgae are highly common in nature. These compounds are used as a food, feed or to extract bioproducts with high industrial potential (Dufossé *et al*., 2005). The raw microalgae biomass production in the world has increased in recent years reaching 7500 ton $year^{-1}$ mainly in products for aquaculture, pharmaceutics and as energy feedstock, generating an average annual income of 1.25 billion USD (Pulz & Gross, 2004; Abomohra *et al*., 2016). However, only a small number of species have been successfully placed in the international market. These belong to the genera: *Spirulina*, *Dunaliella*, *Chlorella* and *Synechococcus*. That is why internationally there is interest in developing and exploiting, alternatively, commercially microalgae of the genera *Botryococcus*, *Chlamydomonas*, *Nannochloropsis*, *Nostoc*, *Chlorella*, *Spirulina*, *Haematococcus*, and *Dunaliella* species/strains (Richmond & Hu, 2013). The production costs are higher than in other vectors such as bacteria, higher plants or fungi (Borowitzka, 2013) due to their lower biomass yields (Wen & Chen, 2003), whence, a challenge in this field is to improve production methods or create new processes to increase biomass yields (Cadoret *et al*., 2012). To achieve this, many companies screen particular

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geographic locations searching for specific "high-yielding algae" (Lundquist *et al*., 2010; Ganesan, 2014). Other frequent alternatives are genetically modified strains, but they present risks associated with environmental impact. Thus, another advantage of natural selection is the use of different isolation techniques, which allow to obtain species and strains with certain characteristic features (Rappé *et al*., 2002; Goltekar *et al*., 2006). A biochemical characterization is essential in order to establish the performance of new isolated strains and find the most profitable ones for industrial purposes.

Important bioproducts from microalgae such as phycobilin, astaxanthin and β-carotene are massively produced by a fully established industry. Three genera: *Chlorella*, *Spirulina*, and *Dunaliella* represent 85% of the worldwide production (Pulz & Gross, 2004). More specifically *Dunaliella salina* produces, among others, large amounts of β-carotene and glycerol under high salinity, high irradiance and nutrient limitation conditions (Ben-Amotz & Avron, 1983; Borowitzka, 1988; Borowitzka, 1990). It was the first commercially cultivated microalgae for production of high-value products (Borowitzka, 2013). For example, the β-carotene market value from *D. salina* until 2012 has generated 261 million dollars (Borowitzka, 2013). It has also been subjected to molecular improvements for production of recombinant proteins, and therefore novel compounds and relevant bioproducts (Guo-Zhong *et al.,* 2005; Borowitzka & Silva, 2007; Lamers *et al.,* 2008; Barzegari *et al.,* 2010; Yaakob *et al.,* 2014). Species of the genus *Dunaliella* are very variable in shape (ovoid, spherical, pyriform, fusiform or ellipsoid) and size (between 3 to 13 µm wide and 5 to 25 µm long). These microalgae lack a cell wall and belong to the unicellular eukaryotic group (Eukaryota, Viridiplantae, Chlorophyta, Chlorophyceae, Chlamydomonadales, Dunaliellaceae). *Dunaliella* are motile forms, each cell having two equal size flagella, a central pyrenoid, and a single and large chloroplast (Borowitzka & Siva, 2007). At high densities without seasonal limitations (Kadkhodaei *et al.,* 2011) they undergoe 0.47 to 1.22 divisions per day (Lerche *et al.,* 1937, in Oren, 2005), and are able to grow under a wide range of salt concentrations, from 517 mM to 5,344 mM NaCl, at pH from 1 to 11, and temperatures from 0 to 40 °C (Wen & Chen, 2003; Cadoret *et al.,* 2012; Cai *et al.,* 2013; Borowitzka, 2013). Halophilic strains are widely distributed around the world (Oren, 2010).

Several strains are available and have been described (Table 1). Assunção *et al.* (2013) proposed that some previously identified *D. salina* strains were misnamed and needed taxonomic revision. According to the ITS2 database (Table 1), there are 74 *D. salina* sequences of isolated strains from several countries (Russia, Chile, México, Korea, Australia, Belgium, India, Iran, Israel, New Zealand, Thailand, Spain, Egypt, and France). The correct identification of these strains is difficult since they present a high intra-specific variability (Borowitzka & Siva, 2007; Olmos-Soto *et al.,* 2009; Assunção *et al.,* 2013) and complete information on every strain is not always available. It is also common to find synonyms or old names in alga databases.

In this study we characterize a new *D. salina* strain isolated from San Quintín, BC, México in order to recognize any potential use for productive application. We describe their growth dynamics, morphology, biochemical content, nutrient requirements, pigment profiles, and molecular identification by PCR with specific molecular markers. Its high lipid content, pigments (neoxanthin (Neo), violaxanthin (Viol)) and micronutrients (Ca, Zn and Cu) content reveal its potential for industrial and pharmaceutical applications.

Table 1. Databases for *Dunaliella salina* strains.

Data base	Number of sequences/ strains	Link
National Center for Biotechnology Information-NCBI	495 sequences	https://www.ncbi.nlm.nih.gov/
Internal transcribed spacer 2 sequences - ITS2 database	74 sequences	http://its2.bioapps.biozentrum.uni-wuerzburg.de/
Culture Collection of algae and protozoa-CCAP	6 strains	https://www.ccap.ac.uk/
Culture Collection of Algae at the University of Texas Austin-UTEX	2 strains	https://utex.org/
National Center for Marine Algae and Microbiota-Bigelow	43 strains	https://ncma.bigelow.org
Marine Biological Association-MBA	16 strains	http://www.mba.ac.uk/culture-collection/
Georg-August-Universität Göttingen	2 strains	https://www.uni-goettingen.de

MATERIALS AND METHODS

Cell culture and isolation. Sampling and acclimation processes for the microalgae were previously described in Magdaleno *et al.* (2017). Briefly, single-cell isolates were cultivated in parallel in 500 µL plate in modified Johnson media (MJM) prepared with 3.37 M NaCl, 0.51 mM $NaHCO_3$, 7 mM $MgSO_4$- $7H_2O$, 9.9 mM KNO_3, 0.2 mM $CaCl_2$-$2H_2O$, 0.4 mM KH_2PO_4, 5 µM EDTA, 2 µM $FeCl_3$ $6H_2O$, 185 µM H_3BO_3, 1 µM $(NH_4)_6Mo_7O_{24}$, 1 µM $CuCl_2$-$2H_2O$, 1 µM $CoCl_2$ $6H_2O$, 1 µM $ZnCl_2$ and 7 µM $MnCl_2$ -$4H_2O$ (Johnson *et al.,* 1968). Initially, plates were incubated at room temperature (19-22 °C) under a 18:6 (h) dark:light cycle, irradiated with a led lamp at 21.6 µmol quanta m^2s^{-1} (RGB led 5 mm, Steren). Individual cells were transferred four times to a new plate and incubated under the same conditions. After the isolation process, the cell cultures were upgraded to 5, 50 and 500 mL, and then acclimated to grow in 250 mM of NaCl. Contamination with other microorganisms like bacteria and fungi was avoided using a Penicillin-Streptomycin-Neomycin (PSN) antibiotic mixture: 50 µg mL^{-1} penicillin 50 µg mL^{-1} streptomycin and 100 µg mL^{-1} neomycin (Gibco, 15640055).

Cell morphology and quantification. In order to identify the morphometric characteristics intact cells were harvested with a Pasteur pipette from cell-cultures and processed in two ways: the first one (A- cell series) was fixed with 1% lugol iodine solution (1 g KI in 10 mL H_2O, 0.5 g crystalline I_2 and 1 mL glacial acetic acid) and analyzed by confocal microscopy (MRC 600 BIO-RAD) with a 100× objective using immersion oil. Cell morphology (shape), number of flagella, size (length, diameter) and account of intracellular components (nucleus, pyrenoids, chloroplasts) were recorded (n=30). The second cell aliquots were not fixed (B- cell series); cells were diluted 1:5 and 1:15 to remain into the Scepter cell counter sensor range (Scepter™, Millipore, MD, USA) and were quantified with a 40 µm scale.

PCR identification with specific molecular markers. Genomic DNA was isolated and purified using a commercial system (Axygen: AP-MN-MS-GDNA50) or CTAB method (Sambrook *et al.,* 1989), followed by PCR amplification and 18S rDNA gene sequencing. The PCR reaction was performed with 50 ng of gDNA, 20 pmol of each primer MA1 [5'-CGG GAT CCG TAG TCA TAT GCT TGT CTC-3'] and primer MA2 [5'-CGG AAT TCC TTC TGC AGG TTC ACC-3'] (Olmos-Soto *et al.,* 2000) using a PCR master mix system (Qiagen: 201445). Amplification was conducted in a GenAmp-5700 (Applied BioSystems) thermal cycler with the next thermal profile: 1 cycle for 2 min at 94 °C, 35 cycles; 30 s at 94 °C, 30 s at 52 °C, 2 min 72 °C and then one last cycle of 10 min at 72 °C. PCR products were resolved in 1 % agarose, run at 4 v/cm during 50 min and purified with Qiakit PCR Purification kit (Qiagen: 28104). DNA quality and quantity were determined in a NanoDrop Spectrophotometer (Thermo Scientific: ND1000). Fragment purification and sequencing were done by Eaton BioSciences (San Diego, CA). Provided sequences were analyzed with blast algorithm in the NCBI web site (Zhang *et al.,* 2000).

Growth dynamics. *D. salina* SQ cells were cultured at room temperature (19-22 °C) in 1000 mL crystal flasks with 500 mL of modified Johnson media. Growth kinetics was evaluated at three salinities (100, 500 and 3000 mM NaCl, Table 2) and under two light conditions (18:6 and 24:0 D:L). Culture was irradiated with a led lamp (21.6 µmol quanta m^2s^{-1}), specific conditions are described in Table 2. One mL samples were collected by triplicate with micropipette and fixed with a 1% lugol iodine solution every three days during a 20-day period. Cells were counted in a Neubauer chamber. The cell abundance (δ) and growth rate (µ) were calculated according to Andersen´s (2005) equation 1, which computes the cell divisions average number by day. Data are presented as cell abundance (cells x10^6 mL^{-1}).

$$\mu = (\ln \delta_2 - \ln \delta_1)/t_2 - t_1 \quad (1)$$

where,

δ_n, cell abundance on specific time point.

t_n, specific sampling time (days).

Proximate analysis, macro and micro-nutrients content. Fresh samples (50 mL) were taken from cultures C2 (500 mM NaCl 18:6 D:L, Table 2) and collected by centrifugation (4,300 rpm for 10 min) during early stationary phase (d 15). They were sent to the Research Center and Agricultural Analysis (CINAA, Camalú, B.C., México) for 16 macro-nutrients and 4 micro-nutrients examination. Protein levels were quantified by Lowry method (Lowry *et al.*, 1951). Lipids were quantified following the Bligh and Dyer method (Bligh & Dyer, 1959). Carbohydrates were determined by phenol sulfuric acid method (Dubois *et al.,* 1956). Total phosphorus and P_2O_5 were estimated by ammonium nitro-vanado-molybdate method (Jackson, 1973). Total potassium (KT), K_2O, Mg, MgO, Ca, CaO, Na, Fe, Zn, Mn, and Cu were determined by atomic force spectrophotometry (EAA) (AOAC, 1990); CO_3, HCO_3 by colorimetric acid method; organic carbon (CO) and organic matter (MO) by the Walkley and Black (1934) method, and finally Boron (B) by the Mohr method (Reber & Wallace, 1937). Results were expressed in mg/kg of dried biomass.

Pigments analysis. Fresh samples (5 to 20 mL) were taken during early stationary phase from cultures at 500 mM NaCl 18:6 D:L (Table 2, C2). Culture was filtered through GF/F membrane filters (25

mm diameter, Whatman) applying a <3.3 kPa pressure controlled with a manual pump and a manometer to avoid cell disintegration. The membranes were covered with aluminum foil and frozen at -20 °C to prevent photo-bleaching. Pigments were quantified by high performance liquid chromatography (HPLC) using the standard method (Heukelem & Thomas, 2001) modified by Almazán-Becerril and García-Mendoza (2008) in an Agilent 1260 instrument assembled with a reverse phase-column (150 mm length ×4.6 mm internal diameter) for 3.5 µm size particles (Zorbax Eclipse XDB-C18).

Data analysis. The effect of the experimental conditions on cell cultures was estimated with a one-way ANOVA test using R commander statistical software (Fox, 2017). The assumptions for the variance analysis were verified for homoscedasticity test f-ratio. The significance level was set at $p<0.05$.

Table 2. Summary of culture conditions for growth dynamics analysis of *Dunaliella salina* from San Quintín, BC, México.

Experimental Condition	Light:Dark cycle Period (h)	Salinity NaCl (mM)
C1	18:6	100
C2	18:6	500
C3	18:6	3000
C4	24:0	100
C5	24:0	500
C6	24:0	3000

RESULTS

Isolation and acclimation. Microalgae cultures were successfully transferred and maintained with synthetic media (MJM) at the same salinity than the original water source (3.37 M NaCl), that recovered the green color a week after the incubation started. The typical red color found in wild populations of *Dunaliella* is an indicator of nutrient limitation and usually associated with high light irradiance and salinity. No changes were observed in the cell morphology of *D. salina* SQ as a physiological response the acclimation process.

In order to have a monoculture, a single cell from the sample was obtained by single cell isolation technique. Single cells showed positive growth two weeks after inoculation, as indicated by the development of green color and confirmed by microscopic examination. The subsequent culture upgrade showed positive growth in 5 mL twenty days after culture and in 50 mL thirty-two days after culture.

Morphology. In early stationary phase *D. salina* SQ cells were spherical, ellipsoid or ovoid, with 7±1 µm average length size, with two flagella of similar length 12-16 µm. The flagellated cells were motile. The nucleus and vacuole were visible in the anterior region. A large chloroplast was observed in the posterior region (Fig. 1).

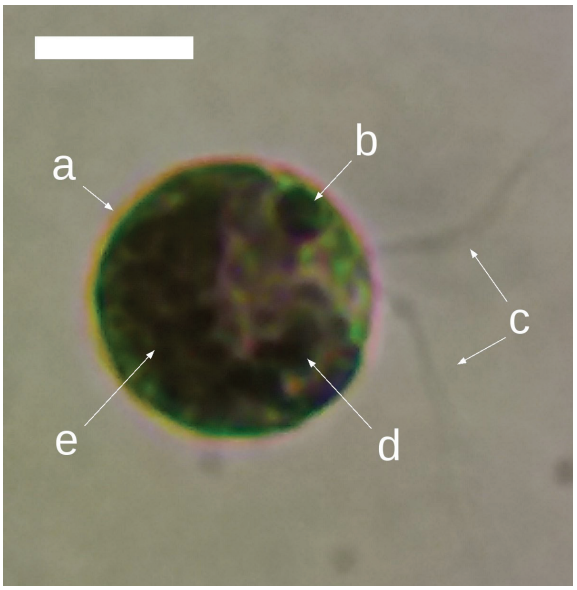

Figure 1. Cell morphology and structure of *D. salina* SQ in early stationary phase cultured in modified Johnson media (250 mM NaCl, 18:6 h light:dark cycle). Image analyzed under an optical microscope with 1000x amplification (scale bar 4 µm). Main cell structures: a, membrane; b, nucleus; c, flagella pair in anterior region; d, vacuole; e, large chloroplast.

Proximate analysis, macro- and micro-nutrients quantification. In early stationary phase (C2), the cellular dry weight biochemical composition was: protein 27.9%, carbohydrates 6.7% and lipids 21.9%. The complete results for macro and micronutrients analyses are listed in Table 3. We included, for comparative purposes, the biochemical composition from published data of other two relevant commercial microalgae species: *Dunaliella salina D.* Gold™ (Plankton Australia Pty Limited) and *Spirulina platensis* (Tokuşoglu & üUnal, 2003).

Pigments analysis. A total of 16 pigments were evaluated in early stationary phase, condition C2. From these, nine photosynthetic and accessory pigment cellular quotas (evaluated from a known volume and microalgae concentration) were reported for *D. salina* SQ (Fig. 2). The contents of Neo (2.33 pg cell^{-1}) and Viol (2.31 pg cell^{-1}) were higher than the content of Chl *a* (2.0 pg cell^{-1}) or Chl *b* (1.16 pg cell^{-1}). The yield of β-carotene was low (0.019 pg cell^{-1}). In addition to these pigments, *D. salina SQ* presented divinyl-PChlde *a* (Mg-DVP), lutein+zeaxanthin, and traces of antheraxanthin.

Growth dynamics. *D. salina* was able to grow under the three experimental salinities and light conditions tested, showing the highest growth rate (μ=0.3) and cell density (δ=10.7-12.5x10^6 cell mL^{-1}) under C4 and C5 conditions (low salinities). No significant differences in growth rate nor cell densities were found between these conditions (p=0.05). Only the highest salinity (3000 mM NaCl) nega-

tively affected the growth of *D. salina* SQ (Fig. 3). The lowest values for μ (0.1) and δ (2.8-0.3x10^6 cell mL^{-1}) were observed at high salinities (C3 and C6 conditions). No significant differences were found (p=0.05).

Molecular identification. The PCR with the set of specific primers MA1 and MA2 for the 18S rDNA amplification in *D. salina* SQ produced the expected fragment of approximate 1770 bp (Fig. 4), which corresponds to that previously published by Olmos-Soto *et al.* (2002) for *D. salina* mexican strain (San Ignacio). The sequence alignment (Blast search) corroborates its identity with 100% similarity to *D. salina* 18S rDNA sequence EU589199.1 (Jayappriyan *et al*., 2010) although the complete mitochondrial and chloroplast genomes analysis of this strain (published as KX530454 and KX641170.1) is phylogenetically closer (Lopez *et al*., 2017; Magdaleno *et al*., 2017) to the GQ250045.1 CCAP_19/18 strain (Smith *et al*., 2010).

DISCUSSION

Nutrients profiles and proximal composition are affected depending on the strain, stage of culture, light, media composition, initial inoculum, and stressors (Cade-Menun *et al.,* 2010; Griffiths *et al.,* 2012; Gonçalves *et al.,* 2014; Chen *et al.,* 2015; Benavente-Valdés *et al.,* 2016). According to our results, intense light conditions and low salinities induce an increase in cell number of *D. salina*. The average proximal composition of the *D. salina* is 40-

Table 3. Proximal composition and nutrient profiles of microalgae species of industrial importance (two chlorophytes and one species of cyanobacteria). All units are expressed in $mg.Kg^{-1}$ of dried biomass. No data available (n.d.).

Sources	***D. salina* SQ** (This study)	***D. salina*** (Datasheet, Plankton Australia Pty Limited)	***Spirulina platensis*** (Tokuşoglu & Ünal, 2003)
Nutrient	Macro-nutrients concentration		
Carbohydrates	6.7%	29.7%	15%
Lipids	21.9%	7%	7%
Protein	27.9%	7.4%	63%
Total nitrogen	61.35±4.73	n.d	n.d
Total phosphorus	18.07±5.40	n.d	7,503.3
Phosphorus pentoxide (P_2O_5)	41,400±12,374	n.d	n.d
Total potassium	2,400±71	20	14,129.7
Magnesium (Mg)	12,025±247	45,910	3,888.7
Magnesium oxide (MgO)	19,932±400	n.d	n.d
Calcium (Ca)	53,725±813	2,130	8,263.3
Calcium oxide (CaO)	75,150±1,131	n.d	n.d
Sodium (Na)	29,625±1,237	n.d	12,620
Chloride	40,500±3,394	n.d	n.d
Bicarbonate (HCO_3)	25,625±1,308	n.d	n.d
Organic carbon	303,200±1,131	n.d	n.d
Organic matter	552,700±1,838	n.d	n.d
Iron (Fe)	604±66	403	653.7
Zinc (Zn)	160±14	30	26.8
Manganese (Mn)	149±11	51.3	42.9
Copper (Cu)	88±7	3	3.1
Boron (B)	78±9	140	n.d.

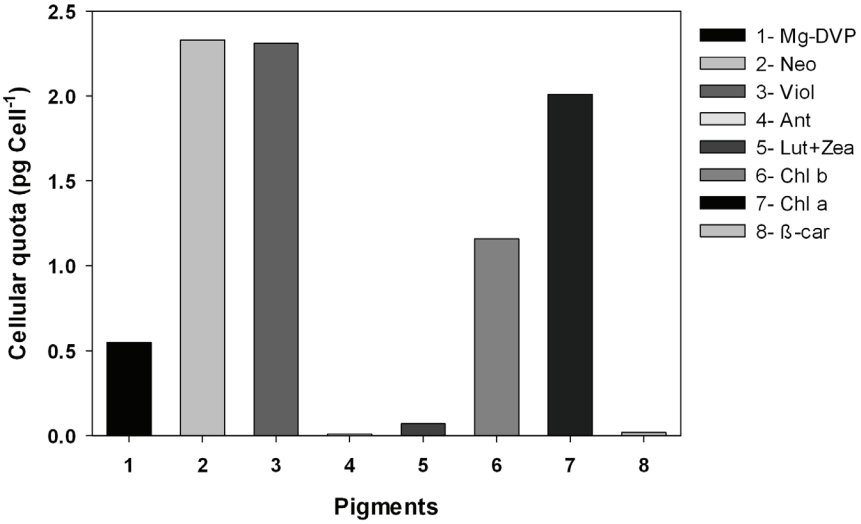

Figure 2. Pigment content as cellular quota from *D. salina* SQ harvested in early stationary phase, incubated with 18:6 (h) light:dark cycle at 500 mM NaCl (C2 condition).

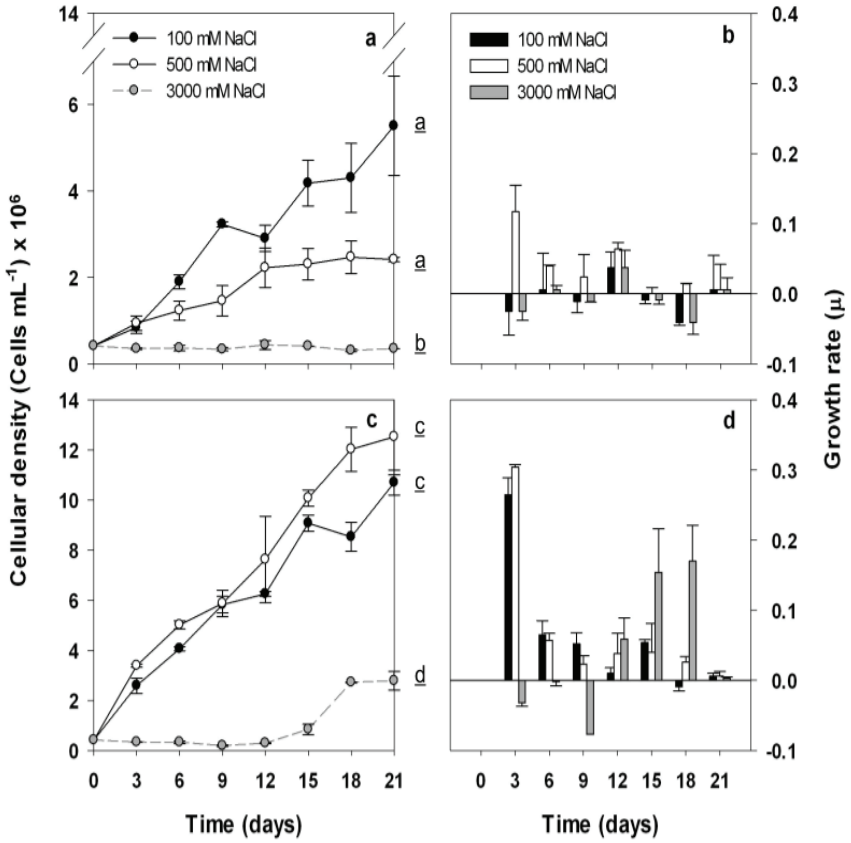

Figure 3. Growth kinetics and grow rates of *D. salina* SQ cultivated in three NaCl salinities and two light:dark cycles. The 100 and 500 mM NaCl salinities were considered as low, and 3000 mM NaCl as high. a and b, at 18:6 (h) light:dark cycle; c and d, 24:0 (h) light:dark cycle. Each sign (squares and others) or bar represents the mean value with the standard deviation (n=3), the same underlined letter was assigned to non- significant differences and different underlined letter for significant differences ($p>0.05$).

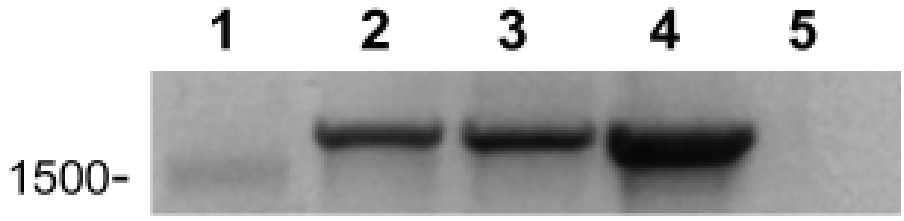

Figure 4. *D. salina* SQ identification by PCR amplification using MA1-MA2 primer set of 1,770 bp size fragment. Line 1, molecular marker; lines 2, PCR product from gDNA sample of *D. salina* SQ; line 3, PCR product from 1 µL of *D. salina* SQ culture; line 4, PCR products from gDNA reference strain *D. salina* LB1644 and line 5, negative control PCR without DNA. Products from lines 2-3 were sequenced.

50% of carbohydrates, 6-18% of lipids and 50-60% of protein (Becker, 2007). However, the *D. salina* SQ cultured in MJM in C2 (500 mM NaCl 18:6 D:L, Table 2) accumulated fewer carbohydrates (6.7%) and proteins (27.9%) in the early stationary phase than the usual average for this species (Becker, 2007), and a higher content of lipids (21.9%). An increase in the lipid content has previously been observed during the stationary and decline phase in *D. salina* (Belghith *et al.,* 2015). In comparison with *D. salina* (*D.* Gold™) lipid production, iron, zinc, manganese and potassium in *D. salina* SQ are higher (Tokuşoglu & üUnal, 2003) (Table 3), but they were lower than in *Spirulina*, which had 29.1% in protein content and 10 times less potassium. These characteristics make this novel isolated strain interesting for lipid obtainment.

The additional results on nutritional compounds such as phosphorus pentoxide (P_2O_5), magnesium oxide (MgO) and bicarbonate (HCO_3) are interesting, since some of them, such as P_2O_5 are used as an industrial desiccator component or in agriculture in fertilizers (Widowati & Asnah, 2014). Magnesium oxide is also used in construction and has environmental applications due to its physical and chemical properties (Speight, 2013). Bicarbonate (in the form of $NaHCO_3$) is used in the food industry and in aquaculture as pH buffer or nutritional supplement (Pancha *et al*., 2015). The presence of these mineral elements and compounds in microalgae is common and their load gives a general approach of nutritional properties, very useful for the food industry (Tokuşoglu & üUnal, 2003). Thus *D. salina* SQ strain is also valuable for obtaining micronutrients.

As for the pigments analysis, Neo and Viol were the most abundant pigments, both reaching higher concentrations than Chl *a* or Chl *b*. The total carotenoids/chlorophylls ratio was 0.68, which is unusual for these pigments, and close (0.84) to those registered in *D. salina* UTEX 1644, but higher when compared to the maximum (0.311) obtained for *D. salina* strain 19.3 (Sammlung von Algenkulturen Gottingen, Germany) (Loeblich, 1982; Pisal & Lele, 2005). Viol is an intermediate for the synthesis of Neo and the activation of the xanthophyll biosynthetic pathway, and it can be accumulated as a β-carotene reservoir (Niyogi *et al*., 1997). Neo and Viol play an important role in photoprotection through the development of non-photochemical quenching (NPQ) in response to short-term exposure to excessive irradiance, de-exiting the Chl protein complex and reducing the O_2 production (Frank *et al.,* 1996; Niyogi *et al.,* 1997; Thaipratum *et al*., 2009). In biomedicine, carotenoids have great potential. Neo has shown to have a strong anti-proliferative effect *in vitro* on human cancer cells (PC-3) by apoptosis (Kotake-Nara *et al.,* 2001). Viol obtained from *Chlorella ellipsoidea* and *Dunaliella tertiolecta* possess an anti-inflammatory activity in mouse macrophage cells (Soontornchaiboon *et al.,* 2012) and an anti-proliferative effect against human mammary cancer cell lines (Pasquet *et al*., 2011). This novel *D. salina* SQ strain produces bio-products that have interesting industrial applications.

Cell densities of *D. salina* SQ increased in five of the six conditions tested excluding C3 (18:6 h L:D, 3000 mM NaCl). When *D. salina* SQ was exposed to high salinity, a significant reduction in growth rate was observed when compared to lower salinities (three times lower). The cell densities and growth rates were similar to those obtained by Guevara *et al*. (2016) for a *D. salina* strain isolated from Arraya Salinas (Venezuela), in which growth was also diminished in similar conditions. Pisal and Lele (2005) reported a similar growth rate with a German *D. salina* strain (Göttingen collection algae cultures). However, Farhat *et al*. (2011) found just the opposite for a *D. salina* strain isolated from Tunisia (Laadhibet); in Farhat´s experiment at low saline conditions (600 mM) density and growth rate were reduced; this was reversed by increasing the salinity from 1500 to 3000 mM. Theoretically, the common variable between these experiments is the salinity. If this is the case, then differential responses must be associated with genetic performance of the strains.

The morphological analysis, physiological responses and taxonomical keys provided in Borowitzka and Silva (2007) for marine and halophilic *Dunaliella* species, effectively classify *D. salina* SQ as a *Dunalliela* taxon. The principal trait is that it is a halophilic species, and the second trait indicates that this species generates a yellow or orange

color under extreme conditions, as we found when we cultivated our strain under a high salinity. The complete genomic identification (chloroplast and mitochondrion) reported by Lopez *et al*. (2017) and Magdaleno *et al*. (2017) for this *D. salina* SQ strain corroborates its identity, although the 18S amplified fragment was informative enough to corroborate its identification. Another interesting feature is its remarkably low β-carotene cellular quota (0.02 pg cell-1) compared with other strains. For example, 1.03±0.03 pg cell-1 (at 1500 mM NaCl, 25 °C, 32.2 µmol quanta m^2s^{-1}) or 1.7 ± 0.01 pg cell-1 (at 1000 mM NaCl, 20 °C, 382 µmol quanta m^2s^{-1}) (Pisal and Lele, 2005). The PCR/sequencing analysis with the molecular markers MA1-MA2 also indicates that this *D. salina* SQ strain has no introns (Olmos *et al*., 2000; Wilcox *et al*., 1992), and it is known that this group does not produce β-carotene, excluding the reference strain *D. salina* LB1644 (Paniagua-Michel *et al*., 2009). More specific analyses are required to prove this feature.

Our study provides the first detailed description of this recently isolated *Dunaliella salina* SQ strain, which is characterized by having a relatively high lipid content (21.9%) and microelements (notably iron, zinc, manganese and potassium). But the most relevant feature are its accessory pigments Violaxanthin (Viol) and Neoxanthin (Neo) contents. It has valuable characteristics for the production of food, cosmetics, and for the biotechnological and biomedical industries. In addition, *D. salina* SQ may be subject to improvements, from growing conditions to genetic modifications to obtain higher yields and other high value substances, and it can be exploited as an alternative to the production of β-carotene from non-producing strains.

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