Microbiological Communication

Identification and Growth Characterization of Native Microalgae Isolated from Different Environments of Saudi Arabia

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ABSTRACT

Selection of appropriate strains of microalgae that work well in local conditions is important for a establish algae-based production system. The general aim of this study was to isolate and identify native microalgae species for exploring its further potential applications. To achieve this aim, 25 samples were collected from different locations of western region of Saudi Arabia. Standard isolation and purification techniques were applied *In vitro* to obtain axenic cultures. Among the best performing isolates, seven predominant strains were chosen for characterization based on morphological and molecular features. Morphological observations and molecular markers analysis using internal transcribed spacer sequence (ITS) were performed. Moreover, phylogenetic relationship of these strains was constructed. According to the DNA sequence analysis of the seven isolates, they were belonged to six genera of *Chlamydomonas, Dunaliella, Chlorococcum, Graesiella, Coelastrella* and *Chlorella*. Screening the growth rates of all strains showed that *Chlorella sorokiniana* had the highest growth rate (0.180 day^{-1}) and biomass productivity (150 mg. $L^{-1}.day^{-1}$). Whereas other strains showed comparable growth rates under same growth conditions. This study found that *Chlorella sorokiniana* UJ as robust species which holds a great potential to be used in different commercial and environmental applications. In conclusion the identification of microalgae is considered key step in microalgae-based industry. This work screened microalgae strains isolated from the local environment of Saudi Arabia. The best performing algal strains were selected to be identified and characterized to discover strains that can be utilized for mass cultivation. This study successfully isolated and identified seven local strains, most of them are already known with high biomass productivity (fast growers) and are considered as good candidate to serve as platform for many further applications.

KEY WORDS: CHARACTERIZATION, GROWTH RATE, IDENTIFICATION, MICROALGAE SAUDI ARABIA.

INTRODUCTION

Algae constitute a diverse group of photosynthetic organisms, which ranging from single cellular bodies to multicellular seaweeds, with a broad diversity in morphological, physiological and biochemical characteristics, distributed in almost all environments (Buijks, 2012). Using microalgae have attracted much attention in various industrial and environmental sectors such as human food, animal and aquaculture feed, pharmaceuticals, cosmetics, wastewater treatment, and bio-fertilizers (Olaizola, 2003; Draaisma et al., 2013). Moreover, microalgae are considered as promising alternative biofuel feedstocks due to their rapid growth, high biomass productivity and their capability to grow under divers conditions (Wijffels and Barbosa, 2010; Elliott et al., 2012; Ratha and Prasanna, 2012; Ratha et al., 2012; Markou and Nerantzis, 2013). Over the last decade, a number of microalgae have been cultivated on large scale to be used in industry because of their ability to produce valuable products (Olaizola, 2003; Markou and Nerantzis, 2013; Wijffels et al., 2013; Kaspar et al., 2014; Mulders et al., 2014; Ciurli et al., 2021). The common genera used are *Spirulina* and *Chlorella* as nutritional supplement, *Haematococcus*, to produce the antioxidant astaxanthin (Guerin et al., 2003; Yuan et al., 2011), and *Dunaliella salina* to produce carotenoids (Borowitzka, 1999; Hosseini

Article Information:*Corresponding Author: waalshehri@uj.edu.sa
Received 28/06/2021 Accepted after revision 09/10/2021
Published: 30th September 2021 Pp- 1065-1076

Available at: https://bbrc.in/
Article DOI: http://dx.doi.org/10.21786/bbrc/14.3.25

Tafreshi and Shariati, 2009; Knothe, 2010; Camacho et al., 2019; Ciurli et al., 2021).

Due to these successful achievements of the using algae, the unexploited and undiscovered microalgae offer great possibilities for future. Until now, the actual number of microalgae species are still unknown, it is estimated that there are between 200,000 and 800,000 species (De Clerck et al., 2013; Lizzul et al., 2018; Camacho et al., 2019; Yin et al., 2020; Ciurli et al., 2021). Only 50,000 species have been defined and characterized (Yin et al., 2020). New genera and species are being discovered very consistently indicating the presences of large portion of undescribed species that exist (De Clerck et al., 2013). Saudi Arabia could be a good source of algal biodiversity due to variation of geographical and environmental nature. The capability of microalgae to grow in the local environmental conditions is an important prerequisite toward successful cultivation of microalgae-based for industrial production. Therefore, there is a need for a proper identification and characterization of local species. Microalgae are usually identified based on their morphological features.

However, environmental factors may cause some of the phenotypic plasticity, therefore morphological identification could be insufficient and misleading (Hoshina et al., 2010; Gour et al., 2016). A new classification based on the ultra-structure of the basal body in the flagellation cells and cytokinesis during mitosis has been suggested. Nevertheless, these principles are difficult to practice especially by non-taxonomists (Gour et al., 2016). The presence of molecular-based techniques such as polymerase chain reaction (PCR) and sequencing have improved such studies and helped in demonstrating evolutional relationships between different organisms and species. Molecular based techniques are usually recommended to confirm morphological classifications (Chung et al., 2018). Nowadays, internal transcribed spacer sequence (ITS) is considered a very powerful and helpful tool to discriminate between microalgae at the genus and species level (An et al., 1999; Van Hannen et al., 2002; Coleman, 2003, 2009; Hegewald et al., 2005, 2010; Jeon and Hegewald, 2006; Schultz et al., 2006; Schultz and Wolf, 2009; Hegewald et al., 2013; Lizzul et al., 2018; Wang et al., 2019; Goecke et al., 2020; Karm and Dwaish, 2021).

Thus, in current study, amplification and sequencing of internal transcribed spacer sequence (ITS 1 and ITS 4) was chosen as molecular markers to confirm the primary morphological identification of isolated strains, compared with other known sequences of species from public databases. The results of these comparisons were represented in a phylogenetic tree. This sort of studies is limited in Saudi Arabia and therefore this work will contribute in the field of discovering and exploiting microalgae from the local environment.

MATERIAL AND METHODS

Sampling, isolation and purification of microalgae: Twenty-five samples were collected through duration (Jan-April, 2018) from different environments of western region of Saudi Arabia (Table 1). Once the samples were collected and transferred to the laboratory, they were exposed to light, enriched with BG-11 medium and incubated for few days (Rippka et al., 1979). In order to obtain an axenic culture, the basic microbiological techniques for isolation and purification were used (serial dilution in liquid media and streak plate method).

In the serial dilution method, a series of test tubes with 9 ml of sterilized distilled water were prepared. One ml of the mixed enriched sample was taken, diluted in the first test tube (10^{-1}) and mixed. Next, 1 ml was taken from the first dilution and transferred to the second test tube (10^{-2}), this process was repeated until reaching to dilution of (10^{-6}). Serial dilution increases the chance of getting individual colonies. Then, each diluted tube was cultured in both liquid and semi-solid agar plates media, incubated in controlled conditions at 22 ± 1°C, exposed to continues light using LED fluorescent tube of intensity 2000 LUX 28 $\mu mol.m^{-2}. s^{-1}$ (Figure 1a). Further purification was achieved by consecutive streaking on semi-solid BG-11 agar (Figure 1b). This process was repeated several times until axenic culture was obtained. To ensure purity, the cultures were regularly monitored using light microscope. Subculturing was performed every 2 weeks. Axenic culture was preserved in cell culture flasks (Figure 1c).

Figure 1: Isolation and purification of algae. a) Algae stock cultures, b) Streaking purification of the microalgae on semi-solid BG-11 agar plates, c) Axenic culture preserved in cell culture flasks.

Growth of microalgae isolates in different media: Algae isolates were cultivated on different growth media, Kuhl (SAG Göttingen, 2013), BG-11 (Rippka et al., 1979), F2 (Guillard and Ryther, 1962) and Johnson's (Johnson et al., 1968). The cultures were aerated and incubated under controlled condition mentioned previously. The

growth of algae was estimated and determined as shown in (Table1).

Morphological identification: To identify morphological characterization of isolated strain, cell shape and arrangement were documented using light microscope (BX51; Olympus, Tokyo, Japan) equipped with a built-in digital camera, and microphotographs were processed with cell Sens Standard program. Morphological identification was determined according to Sime (2004) and Serediak and Huynh (2011).

Genomic DNA Isolation and PCR amplification of ITS Regions: For DNA isolation, 50 ml of each culture were harvested using centrifuge at 5000x g for 15 minutes, the genomic DNA (gDNA) then extracted using Qiagen kit following the manufacturer's instructions. The extracted gDNA was confirmed by agarose gel electrophoresis (1%) stained with ethidium bromide and visualized under ultraviolet light (UV). The extracted DNA was kept at –20 °C till using as PCR template. To amplify the ITS gene, the universal oligonucleotide primer set described in Van Hannen et al., (2002) was used. The sequences of these primers are: FW primer ITS1: (5′- TCCGTAGGTGAACCTGCGG -3′) RE primer ITS4: (5′- TCCTCCGCTTATTGATATGC -3′).

The PCR reaction was performed in a total volume of 25 ml by mixing up the following reagents: 12.5 µL master mix (2x), 8.5 µL dH_2O, 1 ml from each forward primer (FW) and reverse primer (RE) (10 pmoles) and 2 ml of genomic DNA. The PCR reaction was carried on at the thermocycler (Bibby Scientific, UK) using the following conditions: 5 minutes at 94 °C for the initial denaturation followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute, extension at 72 °C for 2 minutes, followed by a final extension step at 72 °C of 10 minutes. Then the PCR products (860 bp) were resolved in 1.5 % agarose gel stained with ethidium bromide and visualized using UV light detector system. To perform DNA sequencing, the PCR products were shipped to (Macrogen, Seoul, Korea) to perform DNA sequencing reaction using Sanger sequencing methods.

Sequence analyses of ITS region: In order to identify the isolated strains, the obtained sequences were analyzed by searching for homology in the National Center for Biotechnology Information (NCBI) database using BLAST tool. All sequences were submitted to GenBank under the number SUB9546968. The phylogenetic tree of identified microalgae was constructed using the neighbor-joining (NJ) method (Ratha et al., 2012) of Mega 5 software (Draaisma et al., 2013). A bootstrap analysis of 1000 replication was used to test the degree of support of the branches produced by NJ analysis (Figure 2).

Growth characteristics: To study algal growth, four liters of BG-11 medium (except for S2 and S6 Jonson medium) was inoculated with active 7 days old inocula and incubated under controlled conditions as described above. Culture's densities were adjusted to be (OD_{680} = 0.25) in zero day for all strains. Optical densities were measured on regular basis using spectrophotometer (T60 UV–visible spectrophotometer, PG instruments, UK). To determine the dry weight of the biomass, 60 ml of each culture were centrifuged at 4000×g for 10 minutes and the washed several times with distilled water to remove residual salts. Then, the pellets dried at 60 °C oven for 48 h, weighed. The specific growth rate (µ) of each strain was calculated using equation below (Gill et al., 2016):

$$\mu = ln\frac{x_2 / x_1}{t_2 - t_1}$$

where x_1 is weight of dry biomass at the beginning of the selected time interval, x_2 is weight of dry biomass at the end of the selected time interval, (t_2-t_1) is the selected time for the determination of dry biomass. Biomass productivity (Pdwt) was expressed as dry biomass produced per litter per day (mg. L^{-1}.day^{-1}) during the exponential growth phase (Song et al., 2013) according to the following equation:

$$Pdwt = dwt \cdot \mu$$

RESULTS AND DISCUSSION

The pre-enrichment step allowed strains with high growth performance to compete with other weak strains. Therefore, this step is important to screen the best candidate strain for further scale up cultivation. After two weeks of initial incubation, most isolates were showed growth in liquid media. The growth performance was noticed and reported as ++++, +++, ++, + or – (Table 1). It was clear that the maximum growth was mostly observed in BG-11 medium except for (S2 and S6) were they had the highest growth (++++) in Johnson's medium. Samples with good growth were examined under light microscope to determine the type of microalgae present in them. Even though sub-culturing was done with extreme care, in some samples, especially when they were grown in BG-11 medium, protozoa and rotifers prey on microalgae rapidly and could not be processed or rescued, because BG-11 medium was improved microscopic grazer growth along with microalgae. In case of marine-origin samples, the growth was very weak, thus, those cultures were discarded.

Microscopic investigation of several samples during the screening process revealed the presence of flagellated, coccoid and filamentous algae. In some cases of mixed culture with filamentous algae, the growth of filamentous algae were very dense and no single cell strains could be purified. In addition, filamentous algae have sticky nature and very hard to handled, hence, they were excluded from selected collection. Also, it was noticed that S11 (obtained from 70 °C hot spring) requires at least 40°C incubation temperature to grow, therefore it is excluded too. Based on the growth performance and microscopic examination, seven isolates (S1, S2, S3, S4, S5, S6, S7) were chosen for morphological and molecular identification. For subsequent sub-culturing rounds, most microalgae were showed growth after seven days in the agar surface, probably due to acclimatization in the new conditions. Whereas in case of S2 and S6 (which later defined as *Dunaliella salina*), they

did not show growth on agar plates, however, they grew well in liquid media.

Morphological and molecular identification and characterization: In this study, seven strains were chosen to be identified and characterized. The selection criteria were focused on strains that could be easily cultivated. Based on morphological and reproductive features, all chosen strains were belonging to the phylum of Chlorophyta under the genera of *Chlamydomonas, Dunaliella, Chlorococcum, Graesiella, Coelastrella*, and *Chlorella*. To verify their taxonomical positions, the sequences of internal transcribed spacer (ITS) obtained from our samples were compared with the sequences available in the NCBI database, the results are summarized in (Table 2). Similar sequences were used to construct independent molecular phylogenetic trees. The reliability of the phylogenetic tree was evaluated using neighbor-joining analysis.

Table 1. Growth of the isolated microalgae on different media after 14 days. Excellent growth (++++), moderate growth (+++), poor growth (++), very poor growth (+), no growth (-).

Isolate ID	Source	Habitat	Isolation location	Kuhl	Medium		
					BG-11	F2	Jonson
S1	Fresh	Agriculture soil	Al-Madina	+	++++	-	-
S2	Fresh	Anthropogenic silt soil	Al-Baqi cemetery	-	++	-	++++
S3	Fresh	Agriculture soil	Al-Madina	++	++++	-	-
S4	Fresh	Stagnant water (effluent from air conditioner).	Jeddah	+	++++	-	-
S5	Fresh	Agriculture soil	Al-Madina	+	++++	-	++++
S6	Marine	Al Khumra salt marshes	Jeddah	+	+	++	++++
S7	Fresh	Agriculture soil	Jeddah	+	++++	-	-
S8	Marine	Sharm beach	Yanbu	-	-	+	-
S9	Fresh	Asphalt surface	Jeddah	++	++++	+	-
S10	Fresh	Agriculture soil from public walkway	Jeddah	-	++++	-	-
S11	Fresh	Gomygah hot spring	Al-Lith	+	++	-	-
S12	Fresh	Damp walls	Jeddah	-	++++	-	-
S13	Fresh	Garden irrigation dripper	Jeddah	+	++++	-	-
S14	Fresh	Birdbath	Jeddah	+	++++	-	-
S15	Fresh	Water barrel	Jeddah	+	++++	-	-
S16	Fresh	Planter	Jeddah	+	++++	-	-
S17	Fresh	Fountain	Jeddah	+	+++	-	-
S18	Fresh	Roadside mud	Al-Lith	+	++++	-	-
S19	Marine	Waterfront Corniche	Jeddah	-	-	+	-
S20	Fresh	Agriculture soil	Jeddah	+	+	-	-
S21	Fresh	Agriculture soil	Yanbu	+	++++	-	-
S22	Fresh	Agriculture soil	Al-Taif	+	+++	-	-
S23	Fresh	Roadside soil	Al-Madina	+	++++	-	-
S24	Fresh	Greenhouse hydroponics	Thuwal	+	++++	-	-
S25	Marine	Thuwal beach	Thuwal	+	+	-	-

Strain (S1) *Chlamydomonas zebra*: Microscopic examination of S1 demonstrated green, motile, unicellular; oval shaped cells (4-5.5 µm long, 4-3 µm wide) showed morphology consistent with *Chlamydomonas* sp. features (Figure 3). Previous research described *Chlamydomonas* species as an ovoid-shaped unicellular, of (9-16 µm long, 5-12 µm wide) with two anterior isokont flagella and single cup-shaped chloroplast, single nucleus; two anterior contractile vacuoles. Reproduction by producing 2-8 zoospores; or by isogamous (Serediak and Huynh, 2011; Zhang et al., 2014). The phylogenetic tree revealed that the sequence of S1 strain aligned with other strains of *Chlamydomonas* genus described in previous studies (Luo et al., 2010; Hoshina, 2014). According to the BLAST analysis results, the S1 is homologous and is closely related to the *Chlamydomonas zebra*. Therefore, the isolated S1 strain was given the name *Chlamydomonas zebra* UJ (Figure 2 a).

Strain (S3) *Chlorococcum pamirum*: Microscopic observation of S3 showed green spherical cells, vary in size (4.5-6 µm in diameter), solitary and form temporary groups (Figure 4). This morphology was similar to *Chlorococcum* sp. as mentioned in Feng et al., (2014), vegetative cells range in diameter from 5-16 µm. The cells have a distinct pyrenoid surrounded by a sheath of biplate starch and are uninucleate. Reproduced asexually by zoospores and aplanospores or sexually by isogametes (Blackwell, Cox and Gilmour, 1991). Therefore, S3 was preliminarily hypothesized to belong to genus *Chlorococcum* sp. The BLAST analysis result

supported our hypothesis, as shown in phylogenetic tree (Figure 2 a). The sequence of S3 was located in the same clade with other *Chlorococcum* species, and therefore, it was given the name of *Chlorococcum pamirum* UJ.

Table 2. Identification results of isolated stains according to the BLAST hits.

E-value	Identity (%)	Nucleotide length	Coverage (%)	Name and accession of number of the most related strain in NCBI GenBank		Isolates
2E-114	90%	333	98 %	AF033294.1	*Chlamydomonas zebra*	S1
2E-99	98%	221	99 %	MF360000.1	*Dunaliella salina*	S2
7E-129	99%	269	98 %	KX147379.1	*Chlorococcum pamirum*	S3
3E-72	91%	221	96 %	JX456465.1	*Graesiella emersonii*	S4
0	100%	493	100 %	MH176127.1	*Coelastrella* sp.	S5
0	96%	442	99 %	MF360000.1	*Dunaliella salina*	S6
4E-75	95%	221	85 %	LR215790.1	*Chlorella sorokiniana*	S7

Figure 2: Neighbor-joining phylogenetic tree of ITS region sequences of isolated algae.

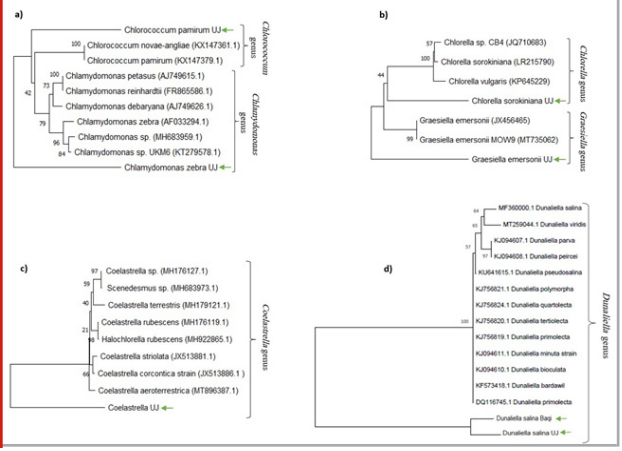
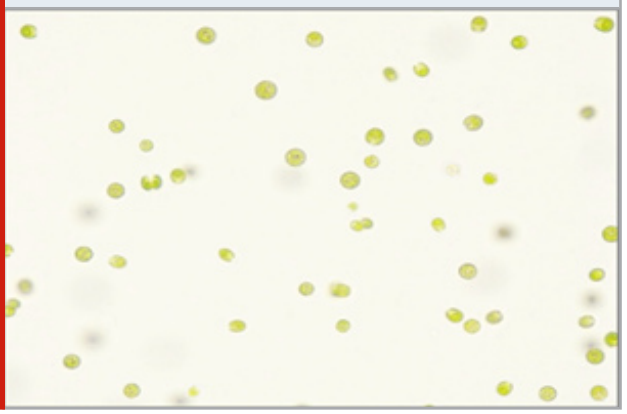


Figure 3: Microscopic photograph of *Chlamydomonas zebra* cells at a magnification of 400x.



Strain (S4) *Graesiella emersonii*: Under light microscope, the cells of S4 strain were giant (diameter ranging 9-22 µm), non- motile, unicellular, nearly globose to ellipsoidal shape with different sizes, enclosed by thick transparent sheath, and form auto-spores as a mean of asexual reproduction (Figure 5). These observations were similar to *Graesiella emersonii* features described by Nozaki et al., (1995) as a large globose to ellipsoidal cells (up to 5-17 µm in diameter), surrounded by double-layered cell wall. *Graesiella emersonii* also had a massive chloroplast containing a single pyrenoid and exhibiting several vacuoles and reproduce asexually by autospores. The molecular analysis of ITS regions of S4 showed a high similarity to *Graesiella emersonii* (Table 2) which supports the morphological characteristics; therefore, it was given the name of *Graesiella emersonii* UJ (Figure 2 b).

Strain (S5) *Coelastrella* sp.: Microscopic observation of isolated strain S5 shows there was similarity in morphological characteristic described in literature of *Coelastrella* sp. which is unicellular spherical cells, tend to aggerate in temporary groups (Figure 6 a,c). These characteristics were previously described in *Coelastrella* species (Wang et al., 2019; Goecke et al., 2020). Another important characteristic that has been reported in several *Coelastrella* species is the production of secondary pigments of carotenoids (Punčochářová and Kalina, 1981; Abe et al., 2007; Hu et al., 2013a; Kawasaki et al., 2020).

Changing of cell color in old and stressed cultures was noticed in S5 which indicating carotenoids production (Figure 6 b). Based on these finding, it is suggested that S5 to is a member of genus *Coelastrella*, and the molecular analysis supported this suggestion. Thus, the isolate S5 was given the name *Coelastrella* sp. UJ (Figure 2 c). *Coelastrella* species are widely distributed worldwide. It has been reported to be found in temporary waterbodies, birdbaths, and fountains (Neofotis et al., 2016). They found as single cell or in aggregations of few cells, these species are characterized by double layered cell wall, with distinct sculpture. The inner layer composed of cellulose and an outer one composed from (sporopollenin) which is acetolysis-resistant material (Tschaikner and Kofler, 2008). Previous research noticed the presence of small thickenings at the poles of the cells a citriforme in addition of longitudinal ribs considered as an important character

of *Coelastrella* species (Punčochářová and Kalina, 1981; Abe et al., 2007; Hu et al., 2013b; Kawasaki et al., 2020; Ciurli et al., 2021).

Coelastroideae subfamily members have been previously placed under family of Oocystaceae, *Chlorella*ceae, and Sctielloideae regarding to morphology and cellular structure (Kalina and Punčochářová, 1987). Later, the phylogenetic molecular studies suggested that *Coelastroideae* should be placed within the family Scenedesmaceae, order Sphaeropleales (Hanagata, 1998; Hegewald et al., 2010; Kaufnerová and Eliáš, 2013; Lee et al., 2016; Ancona-Canché et al., 2017). Nowadays, many species of this genus attract attention from researchers due to its ability of accumulation carotenoids and fatty acids, as well as for a potential use for bioremediation (Abe et al., 2007; Hu et al., 2013b; Kawasaki et al., 2013; Luo et al., 2016; Dimitrova et al., 2017; Thao et al., 2017; Goecke et al., 2020; Karm and Dwaish, 2021).

Figure 4: Microscopic photograph of *Chlorococcum pamirum* cells at a magnification of 400x.

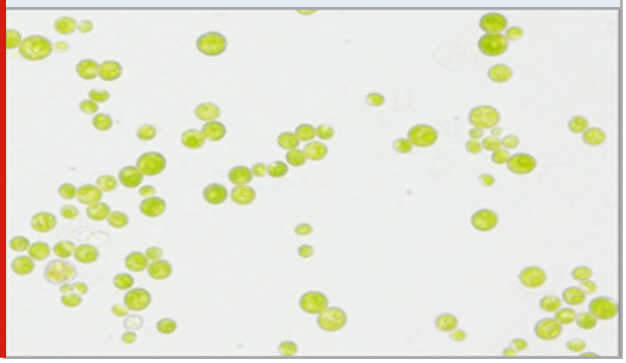
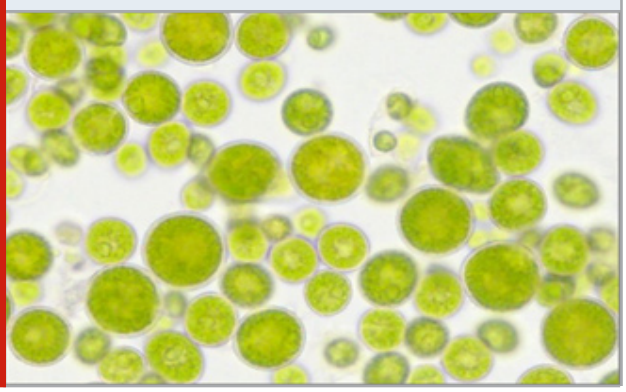


Figure 5: Microscopic photograph of Graesiella emersonii cells at a magnification of 400x.



Strains (S2 and S6) *Dunaliella salina*: Although S2 and S6 obtained from different environments (anthropogenic silt soil and saltmarshes), respectively, both of them were shows similarity in morphological and physiological characteristic of *Dunaliella* species. Under microscope, ovoid motile cells with cup shaped chloroplast were observed (Figure 7 a,b). It was also noticed that both strains change their cell color from green into orang under high light and salt stress (Figure 7 c). Previous works described *Dunaliella* sp. characteristic as following: ovoid to spherical motile cell with two equal flagella and cup-shaped chloroplast. Depending on different environmental conditions, the size and shape of the cell can vary within a species (between 2 to 28 µm and in width between 1 to 15 µm) (Hosseini et al., 2009). Although, *Dunaliella* cells are naked, they are surrounded by mucilaginous substance (Ben Amtoz et al., 2009).

Furthermore, it is well known that many species of *Dunaliella* are halotolerant and capable to grow in environment of high salinities, they were previously isolated from Dead Sea, and the Great Salt Lake, USA (Oren, 2014). *Dunaliella* response to such environmental stress through over-accumulation of beta-carotene pigment which is responsible for turn cell color into orange (Polle et al., 2017). Until now, 26 saltwater species and five freshwater species have been described for the genus *Dunaliella*. All freshwater species considered rare and its classification is still uncertain (Ben Amtoz et al., 2009; Gonzalez et al., 2001; Melkonian and Preisig, 1984). Molecular analysis of both isolated strains (S2 and S6) was confirmed the morphological and physiological findings. As our isolates were closely related to *Dunaliella salina*, they have been given the following names *Dunaliella salina* Baqi and *Dunaliella salina* UJ to distinguish its unique origin for further studies (Figure 2 d).

Figure 6: Microscopic photograph of *Coelastrella* sp. cells at a magnification of 400x. a) Cell's aggregation, b) Orange cells indicating carotenoids production, c) Single spherical cell.

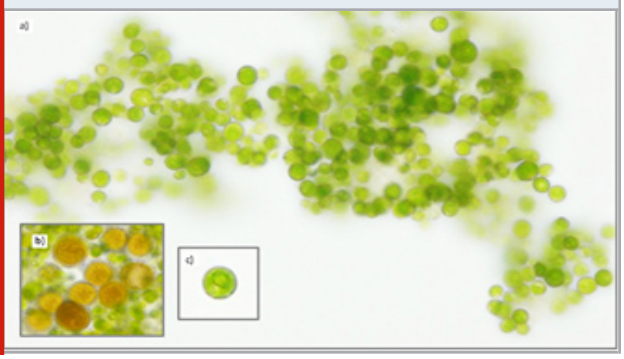
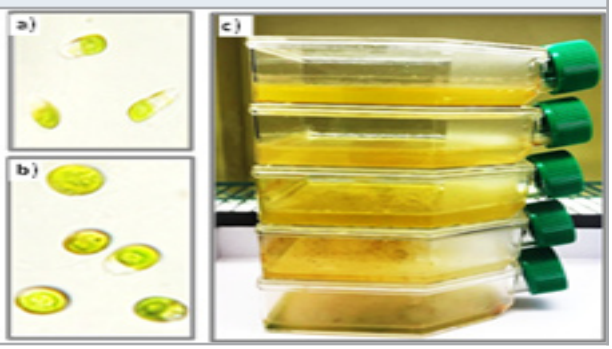


Figure 7: Light microscopic images (magnification of 400x) of *Dunaliella salina*. a) Strain isolated from saltmarshes, b) Strain isolated from anthropogenic silt soil, c) Orange color of *Dunaliella salina* stressed cultures.



Strain (S7) *Chlorella sorokiniana*: Microscopic observation of S7 shows a small (diameter 2-2.5 µm) spherical unicellular, emerald-green color alga (Figure 8). These morphological characteristics are similar to the morphological characteristics of *Chlorella* genus (Krienitz and Bock, 2012; Lizzul et al., 2018). The molecular identification confirmed this finding, therefore, S7 was designated as *Chlorella sorokiniana* UJ (Figure 2 b). *Chlorella* genus members are widely distributed in different habitat due to their rapid growth (Lizzul et al., 2018), *Chlorella* species have been used as model organisms for photosynthesis studies and biotechnological applications for decades (Béchet et al., 2013; Lizzul et al., 2018).

Figure 8: Microscopic photograph of *Chlorella sorokiniana* cells at a magnification of 400x.

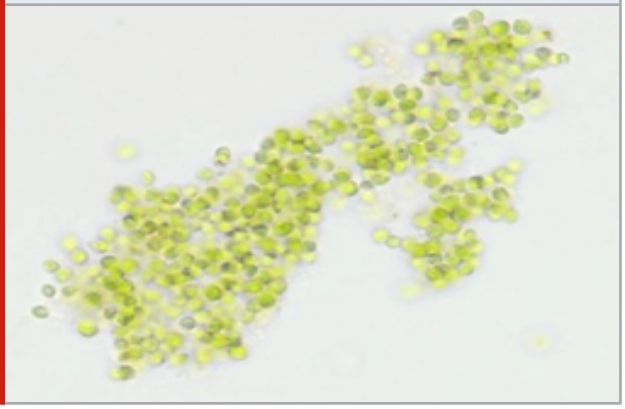


Figure 9: Growth curves of a) *Chlamydomonas zebra* UJ, b) *Dunaliella salina* Baqi, c) *Chlorococcum pamirum* UJ, d) *Graesiella emersonii* UJ, e) *Coelastrella* sp. UJ, f) *Dunaliella salina* UJ, g) *Chlorella sorokiniana* UJ, every point on the graph is showing the mean of three OD reading.

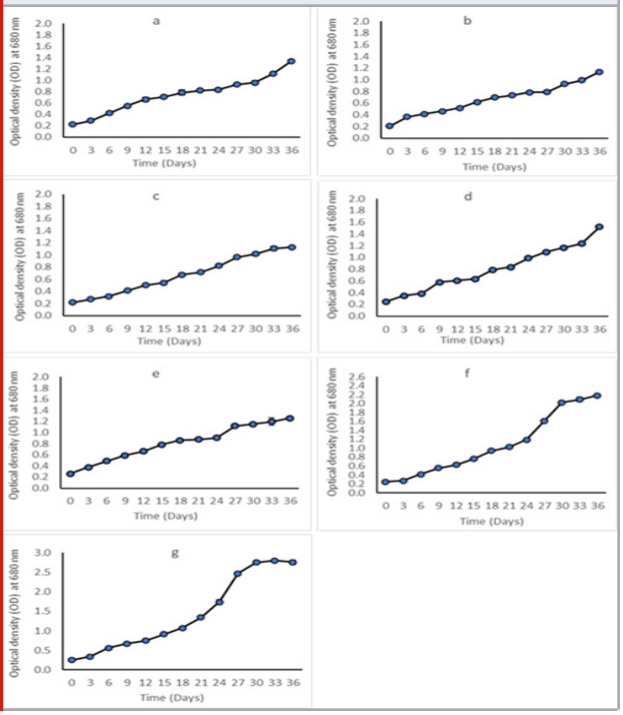


Table 3. Algae species, culture medium, specific growth rate, and biomass productivity

Isolates	Algal species	Culture medium	Specific ($mg.\ L^{-1}.day^{-1}$)	Biomass productivity growth rate (day^{-1})
S1	*Chlamydomonas zebra*	BG-11	0.158	68.3
S2	*Dunaliella salina Baqi*	Johnson	0.087	14.703
S3	*Chlorococcum pamirum UJ*	BG-11	0.169	113.9
S4	*Graesiella emersonii UJ*	BG-11	0.171	123.8
S5	*Coelastrella* sp. UJ	BG-11	0.169	128.9
S6	*Dunaliella salina UJ*	Johnson	0.173	111
S7	*Chlorella sorokiniana UJ*	BG-11	0.180	150.2

To date there are more than 20 characterized *Chlorella* species (Furnas, 1990; Krienitz et al., 2015). In the past few years, the classification of the genus of *Chlorella* has received a lot of attention and many species within the genus were re-classified (Luo et al., 2010; Lemieux et al., 2014; Krienitz et al., 2015). *Chlorella sorokiniana* is a sub-species first isolated in 1953 by Sorokin, and believed to be a thermotolerant mutant of *Chlorella pyrenoidosa* (Sorokin and Myers, 1953; Kunz, 1972; Lizzul et al., 2018). Later, this taxonomy was changed and re-classified *Chlorella sorokiniana* as a separate species (Kessler, 1985; Dörr and Huss, 1990; Kessler and Huss, 1992). Furthermore, it is worth to mention that this sub-species is unique and robust alga due to its ability to thrive under harsh conditions such as high salinities and temperature up to 40 °C. Therefore, *Chlorella sorokiniana* consider the subject of research in several major laboratories (de-Bashan et al., 2008; Lizzul et al., 2014, 2018; Krienitz et al., 2015; Neofotis et al., 2016; Jiang and Pei, 2021).

Growth and biomass productivity: In general, algal growth can be monitored by determining the changes in biomass directly using cell count, or using other parameters such as chlorophyll a, optical density and dry weight (Richmond, 2003). The growth response of our isolates was measured using optical density at 680 nm on regular basis throughout the span of cultivation. The data obtained from OD showed similar trend for all species under same

growth condition as presented in (Figure 9). The growth rate defined as the increasing of biomass over specific period of time (Richmond, 2003).

In this study, *Chlorella sorokiniana* had the highest growth rate followed by *Dunaliella salina* UJ and *Chlamydomonas zebra*. Whereas, *Chlorococcum pamirum, Coelastrella* sp. and *Graesiella emersonii* showed a comparable growth rate. The slowest growth rate was noted in *Dunaliella salina* Baqi. The growth parameters of all strains are shown in (Table 3).

As growth rate measures the cellular response to nutrients and growth conditions, different algal strains grown under a variety of culture conditions gives a variable response depending on cultivation mode, types of media, temperature, light intensity, photoperiod, and supplying of CO_2 (Enamala et al., 2018). Several studies performed previously to investigate the growth potential of different microalgae strains, however, these studies indicated that there is no standard species can be used to make precise comparisons, as well as the differences of cultivation conditions and methods (Richmond, 2003). Feng et al. (2014) found that growth rate of *Chlorococcum pamirum* was 1.88 day $^{-1}$ which is slightly higher than our result. The growth rates of *Dunaliella salina* and *Chlorella sorokiniana* were found to be 0.16 -0.20 day^{-1} and 0.19 - 0.20 day $^{-1}$ respectively (Pertumbuhan et al., 2017; Sajjadi et al., 2018; Khatoon et al., 2020; Karm and Dwaish, 2021).

Which are close to the growth rate obtained from our study (Table 3). Specific growth rate reflects the time required for cells to divide, however, some microalgae species grow by increasing their size rather than increasing their cell number (Zachleder et al., 2016) therefore, high growth rate does not necessary reflect high productivity. Thus, biomass productivity is usually used as a more reliable method to evaluate strain efficiency. Biomass productivity is generally calculated as the increase in biomass over a period of time. In this study, we compared the biomass productivity of all strains and found that *Chlorella sorokiniana* is the most productive strain, whereas *Dunaliella salina* Baqi was the least. In general, the productivity values obtained from this study (shown in Table 3) were approximately similar to the values mentioned in previous works (Khan et al., 2009; Rodolfi et al., 2009; Mata, Martins and Caetano, 2010; Park et al., 2012; Enamala et al., 2018; Sajjadi et al., 2018; Khatoon et al., 2020; Ciurli et al., 2021).

CONCLUSION

Identification of microalgae is considered a key step in microalgae-based industry. This work screened microalgae strains isolated from the local environment of Saudi Arabia. The best performing algal strains were selected to be identified and characterized to discover strains that can be utilized for mass cultivation. This study successfully isolated and identified seven local strains, most of them are already known with high biomass productivity (fast growers) and are considered as good candidate to serve as platform for many further applications. Among these strains are *Dunaliella* and *Coelastrella genera*, these two genera were reported to be capable to accumulate carotenoids and thus they could be exploited for carotenoid production. We also identified strain of Chlorella sorokiniana which is currently one of the most promising algal species that can be used as biofuel feedstocks. The results of this study can greatly enrich our knowledge of microalgae biodiversity in Saudi Arabia. To the best of our knowledge, this study is one of few reports focus on exploring the local microalgae isolates. Integrating phyco-prospecting and characterizing native isolates could contribute in supporting algae-based industry for future.

ACKNOWLEDGEMENTS

We would like to thank King Fahd Medical Research Center (KFMRC), for providing facilities for conducting the research.

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