RESEARCH PAPER
www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences
DOI: 10.4194/1303-2712-v18_10_06

Influence of Media and Temperature on the Growth and the Biological Activities of *Desmodesmus protuberans* (F.E. Fritsch & M.F. Rich) E. Hegewald

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Abstract

The aim of this study is to determine different culture media and temperature values on the growth rate of *Desmodesmus protuberans* (F.E. Fritsch & M.F.Rich) E. Hegewald, and to investigate antimicrobial and antioxidant potency of the crude extracts. The microalga isolates from Eğirdir Lake in Isparta (Turkey) was identified as *D. protuberans* and included in Ege University Microalgae Culture Collection. *D. protuberans* was cultivated at two temperature regimes (22 and 28ºC) and growth media (BBM, BG-11 and RD medium) under the light intensity of 75 µmol $m^{-2}s^{-1}$ for testing the impact of different environmental conditions on the growth rate. All of the productions of *D. protuberans* biomass were extracted with methanol and investigated the antimicrobial and antioxidant activity. Antimicrobial activities of the extracts of alga were tested by disc diffusion assay against five Gram-positive and four Gram-negative bacterial strains and against the yeast *Candida albicans*. The methanol extract of the biomass from BBM medium (28°C) showed the highest growth inhibition effect on *C. albicans*, while RD medium (22°C) extract showed the highest antioxidant activity. These results demonstrated that the differences in the bioactivity of biomasses are stemmed from different temperature and media conditions.

Keywords: Antimicrobial activity, antioxidant activity, cultivation, crude extract, *Desmodesmus protuberans*.

Introduction

Microalgae (photosynthetic microorganisms) that found all over the world high in fresh, sea water and soil habitats are known to have many uses (Ghasemi, Moradian, Mohagheghzadeh, Shokravi, & Morowvat, 2007). Moreover, they allow the production of marketable products such as proteins, pharmaceuticals, crop protection products, bio-removal of heavy metals and feed for aquaculture (Mann, Schlegel, Schumann, & Sakalauskas, 2009). Algae products are also increasingly being used in medical and biochemical research. They are able to produce active compounds with antibacterial, antiviral, enzyme inhibiting, cytotoxic and antiplasmodial activities.

The important microalgae growth factors for indoor cultivation are nutrients, light regime, pH, temperature, aeration rate, CO_2 concentration in the indoor cultivation. However, microalgae are subjected to a number of environmental parameters (chemical and physical conditions) in the environment (Benavente-Valdés, Aguilar, Contreras-Esquivel, Méndez-Zavala, & Montañez, 2016; Karło, Wilk, Ziembińska-Buczyńska, & Surmacz-

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ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v18_10_06

Gorska, 2015). Microalgae can achieve adaptation in a broad range of culture conditions and temperatures. Especially, nitrogen densities in algal cultures have an impact on the cell growth and biomass yield (Rios, Klein, Luz, Maciel Filho, & Maciel, 2015). Temperature is important environmental parameter for inhibition of microalgae because the thermal energy influences cellular enzymatic activity, uptake of nutrients, CO_2 fixation (Karło et al., 2015). The cultivation of *Desmodesmus communis* (E.Hegewald) E.Hegewald was studied the effect of variation in temperature (21-29 °C) and in light intensity (200-600 µmol $m^{-2}s^{-1}$) in BG-11 medium (pH:7) using the flask experiments. The maximum biomass concentration was indicated as the cultivation temperature (25 °C) and light intensity (300 µmol $m^{-2}s^{-1}$) (Vanags, Kunga, Dubencovs, Galvanauskas, & Grīgs, 2015). Microalgal biomass also contains other potentially valuable antioxidants and antimicrobials, such as carotenoids, tocopherols (vitamin E), ascorbic acid (vitamin C) or phenolic compounds (Goîris et al., 2015). New compounds with antibacterial, antiviral, and antifungal properties have been densely researched in microalgae for the last years.

The main objectives of this research were to 1) isolate microalga *D. protuberans* from Lake Eğirdir in Turkey and identify them using morphological and molecular characterization, 2) determine suitable media (BBM, BG-11 and RD medium) and temperature (22 and 28ºC) regimes and 3) investigate the antimicrobial and antioxidant activity of methanol extracts obtained from algal biomass.

Materials and Methods

Isolation and Identification Microalga

Collected water samples (1 mL) from Lake Eğirdir, Isparta-Turkey in 2003 were inoculated into 9 mL of appropriate sterilized medium in a 15-mL tube. The tube was incubated for 7 days at 25°C at the light intensity of 30 µmol $m^{-2}s^{-1}$. The isolation was accomplished by streaking the water sample across the agar surface. Isolated colonies were picked from the agar plate by a loop and then both re-streaked on new agar plates and rinsed in appropriate liquid medium to suspend the cells. Isolates of the cells were incubated at 22°C, at the light intensity of 40 µmol $m^{-2}s^{-1}$ in 250 mL flasks for 14 days.

Morphological examinations were made using bright field (Olympus BX53, Japan) with 60X and green microalga was identified by partial 18S rRNA sequencing. The specie was classified morphologically according to reference print book by Bourelly (1962, 1972).

Genomic DNA was extracted from the strains using the ZR Fungal/Bacterial DNA MiniPrep (ZymoResearch) following the instructions provided by the manufacturer. The SSU was amplified in PCR reactions using the Helix Amp™ Hypersense DNA polymerase (Nannohelix) with the universal primers Forward 5'-TGGTTGATCCTGCCAGTAG-3' and Reverse 5'-TGATCCTTCCGCAGGTTCAC-3' (Shoup & Lewis, 2003). The PCR conditions were included with an initial denaturation step at 95 °C for 2 min followed by 35 cycles of 95 °C for 30 s, 54 °C for 40 s and 72 °C for 40 s, and a final extension step at 72 °C for 5 min (Yıldırım et al. 2014). The PCR products were analyzed by 1% agarose gel electrophoresis in Tris–boric acid–EDTA (TBE) buffer 1× and stained with SYBR safe and visualized under UV illumination.

Sequence analysis of the PCR products were performed by Izmir Institute of Technology, Biotechnology and Bioengineering Central Research Laboratories, Turkey, with the Applied Biosystems 3130XL (16-capillary) Genetic Analyzer. The nucleotide sequences are available in the NCBI sequence databases under the accession number (JQ726704).

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ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v18_10_06

Cultivation of Microalga

The freshwater alga *D. protuberans* was cultivated in Bold's Basal Medium (BBM) (https://www.ccap.ac.uk/media/documents/BB.pdf), Blue-Green medium (BG-11) (https://www.ccap.ac.uk/media/documents/BG11.pdf) and RD medium (Rudic & Dudnicenco, 2000) during this period the temperature of the culture was maintained nearly at 22±2ºC and 28±2ºC for 14 days, pH was averaged 7. The cells were incubated aseptically in a 2 L sterile bottle of airing under continuous illumination (75 µmol $m^{-2}s^{-1}$). Air was supplied to the culture at a flow rate of 2 L min^{-1}.

Analytical Procedure

Samples from the cultures were taken at indicated times, and the following growth parameters were measured immediately; the cell concentration was determined by counting of samples triplicate in a Neubauer hemocytometer. The cellular optical density was measured at 685 nm using UV/VIS spectrophotometer (Ultrospec 1100 pro, UK).

The specific growth rate (µ) of the cells was calculated from the initial logarithmic phase of growth for at least 48 h, as $\mu=(\ln X_2 - \ln X_1)/dt$, where X_2 is the final cell concentration, X_1 is the initial cell concentration and dt is the time required for the increase in concentration from X_1 to X_2. Cell count was used as an indicator for the specific growth rate. Doubling time (DT) was also calculated as DT =ln 2/µ (Imamoglu, Demirel, & Dalay, 2014).

The biomass was harvested by filtration. The filter cake was washed with distilled water and freeze-dried (Christ, Alpha 1–2 LD plus, Germany) for storage. The biomass was kept in -20 ºC until assayed.

Extraction of microalga

5 g freeze-dried alga sample was extracted with methanol in a Soxhlet extractor at 60ºC for 24 h. The solvent in the extract was then evaporated at 60ºC by means of a rotary evaporator and the dried extract kept in the dark at -86 ºC until further use.

Antimicrobial Activity Assay

Test Microorganisms and Growth Media

In vitro antibacterial studies were carried out against nine bacteria strains (*Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloaceae* ATCC 13047, *Escherichia coli* ATCC 29908, *Salmonella typhimurium* CCM 544, *Streptococcus faecalis* ATCC 29212) and one yeast strain (*Candida albicans* ATCC 10239) which were obtained from the Microbiology Department of Ege University, Faculty of Science. The bacteria strains were inoculated on nutrient broth and incubated for 24 h at 30±0.1 °C, while the yeast strain was inoculated on malt extract broth and incubated for 48 h. Adequate amounts of autoclaved Mueller Hinton Agar and Malt Extract Agar were dispensed into sterile plates, and allowed to solidify under aseptic conditions. The counts of bacteria strains and yeast strain were adjusted to yield approximately 1.0×10^7-1.0×10^8 mL^{-1} and 1.0×10^5-1.0×10^6 mL^{-1}, respectively, using the standard McFarland counting method. 0.1 mL of

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the suspensions of test organisms were inoculated with a sterile swab on the surface of appropriate solid medium plates (Koz, Yavasoglu, Demirel, Sukatar, & Ozdemir, 2009).

Determination of Inhibition Zones by Disc Diffusion Method

Antimicrobial activity of the *D. protuberans* extracts was tested by the paper disc diffusion method (Collins & Lyne, 1987; Bradshaw, 1992; Ozdemir, Ulku Karabay, Dalay, & Pazarbasi, 2004). Briefly, sterile filter paper discs of 6 mm were impregnated with 20 and 30 µL of the *D. protuberans* extract then placed onto the inoculated agar plates. The plates were incubated at 35±0.1°C for 18-24 h while the yeast plates were incubated at 25±0.1°C for 24-48 h. At the end of the incubation time, diameters of the growth inhibition zones were measured in millimeters. Also, the antimicrobial test was confirmed by the positive and negative controls. All tests were performed under aseptic conditions in three times.

Antioxidant Activity Assays

DPPH Assay

The DPPH radical is considered to be a model of a stable lipophilic radical. A chain reaction in lipophilic radicals was initiated by the lipid oxidation. The extracts were tested by measuring of bleaching purple colored methanol solution of DPPH (1,1-diphenyl-2-picryl hydroxyl) radical, spectrophotometrically (515 nm). Every sample was done in triplicate, and the percentage of DPPH scavenging activity was estimated with the following calculation. BHT (butylated hydroxytoluene) and α-tocopherol were used as positive control. Antioxidant activity was expressed as the percentage of DPPH decrease using the equation,

$AA\ \% = [1 - (A_1 / A_0)] \times 100$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample (Zhang & Hamauzu, 2004).

Total Phenolic Content

The Folin–Ciocalteu reagent assay was used to determine the total phenolics content (Zubia, Robledo, & Freile-Pelegrin, 2007; Demirel, Yilmaz-Koz, Karabay-Yavasoglu, Ozdemir, & Sukatar, 2011). The phenolic content was calculated and expressed as gallic acid equivalent (GAE) in milligram per 1 g algal extract. Total phenolics were quantified by calibration curve obtained by measuring the absorbance of the known concentrations of gallic acid standard solutions (10-100 mg L^{-1}).

□-Carotene Bleaching Assay

The □-carotene bleaching assay is mainly based on preserving the yellow color of the □-carotene-linoleate system in case of antioxidants present in the test material (Velioglu, Mazza, Gao & Oomah, 1998; Demirel, Yilmaz-Koz, Karabay-Yavasoglu, Ozdemir, & Sukatar, 2009). The absorbance of the samples was measured at 470 nm using a spectrophotometer. Absorbance measurements were continued until the color of □-carotene disappeared in the control reaction (t = 120 min).

Antioxidant activity (AA) was also calculated as percent inhibition relative to control using the following equation,

AA=[(Rcontrol-Rsample)/Rcontrol)]x100,

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ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v18_10_06

The second method of expression based on the oxidation rate ratio (ORR) was calculated using the equation,

ORR=Rsample/Rcontrol,

the antioxidant activity coefficient (AAC) was calculated as described by Velioglu et al. 1998

$AAC=[(As_{120} – Ac_{120} / Ac_0-Ac_{120})]x1000$

where As_{120} was the absorbance of the antioxidant mix at t=120 min, Ac_{120} the absorbance of the control at t=120 min, and Ac_0 the absorbance of the control at t= 0 min.

Statistics

Results were reported as mean values with standard deviations (n=3) unless otherwise indicated.

Results and Discussion

The green microalga of *D. protuberans* was isolated from the Eğirdir Lake, Isparta (Turkey). The taxonomy of *Scenedesmus* and *Desmodesmus* genera has been the center of contradictive in microalga systematics over decades because there are scarcely open diagnostic differentiate phenotypic features (Akgül, Kizilkaya, Akgül, & Erduğan, 2017). Species of *Scenedesmus* and *Desmodesmus* are widespread in freshwater ecosystems. The morphological characteristics and metabolic capacities are taken into account not merely enough to explicitly distinguish the different *Scenedesmus* and *Desmodesmus* taxa. However, the molecular phylogenetic analysis was recently implemented to solve several this taxonomic complexities (Akgül et al., 2017). Therefore, the isolate was identified both morphologically (Bourrelly, 1962, 1972) and molecular (18S rRNA) characterization as a non-axenic unialgal culture of *Desmodesmus protuberans* in the laboratory (Figure 1).

Figure 1. DNA isolation and PCR amplification results gel and light microscopic photography 60X.

D. protuberans (F.E. Fritsch & M.F. Rich) E. Hegewald: Cells cylindrical or spindle shaped in linear series, cells 5-7 µm broad and 10-25 µm long; spines 25-30 µm long, slightly truncated ends, joined for 2/3 of the total length into 2-8 celled coenobia.

D. protuberans was included to Ege University Microalgae Culture Collection, EGEMACC (EGEMACC10 http://www.egemacc.com/cultures.php) as NCBI (National Center for Biotechnology Information) access number JQ726704. The result of sequence analyzes was compared to other sequences deposited at GenBank using nucleotide BLAST search and indicated only 97% similarity with best relative being *Desmodesmus armatus* (Chodat) E. Hegewald, *D. pannonicus* (Hortobágyi) E. Hegewald, *D. communis* but with also nearly similar identities to other species of *Desmodesmus*. The species-level identification was made morphologically.

In a study from Tekirdağ, Turkey, Akgül (2017) isolated the algae *D. communis* (Sphaeropleales) and identified by molecular methods. Biomass from *D. communis* cultured in BG11 medium (7.5 pH, 24±2ºC, 500 mL min^{-1} aeration) was analyzed biochemically for total protein amount, total lipid amount and vitamin E amount and declared its importance as a source of single-cell protein.

Microalgae can be used for primary and secondary metabolites production (El-Sayed & Abdel-Maguid, 2010). A large number of microalgal extracts and extracellular products have been found to have antibacterial activity. However, pH of the medium, incubation period and temperature of incubation were significant important for the biosynthesis of antimicrobial agent products as secondary metabolites. Noaman, Fattah, Khaleafa & Zaky (2004) have reported that the antimicrobial metabolites from *Romeria leopoliensis* (Raciborski) Koczwara were obtained in the conditions at 35 °C, pH 8 and end of 15 days of incubation. It is at the end of the exponential phase that increased synthesis of secondary metabolites is clear, as all primary metabolites are diverted into

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ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v18_10_06

secondary metabolites. Thus, the highest amounts of secondary metabolites are accumulated in cultures at the stationary phase. In this study, biomass was harvested when the microalgae cultures reached stationary phase in different media and conditions. *D. protuberans* maximum cell concentration of $12.64x10^6$ cell mL^{-1} was obtained in the BBM at 28ºC on the 14th day of cultivation however the highest specific growth rate was found in the BG-11 at 28°C, as shown in Figure 2 and Table 1.

Figure 2. Growth curves of *D. protuberans* at 22°C and 28°C in BBM, BG-11 and RD medium under a light intensity of 75 µmol $m^{-2}s^{-1}$ according to cell number and optic density (685 nm)

Table 1. Culture conditions (cell concentration, specific growth rate and doubling time) of *D. protuberans*

Methanol is the most favored solvent for the extraction of polar compounds such as phenolic compounds and flavonoids. It was shown in a study that methanol extract of algae has more antioxidative power compared to the extracts obtained from other solvents and it was also claimed that methanol has disintegration ability on the cell membranes more than the others (Safafar, Van Wagenen, Møller, & Jacobsen, 2015). Maadane et al. (2015) was figured out that the yield of extracts is influenced by the extraction temperature, time, the type of solvents and the chemical composition of the sample. In the present study, the highest methanol extract yield from the culture of *D. protuberans* was achieved at 28ºC in BG-11 medium (30.723±0.041) whereas the lowest methanol extract yield was obtained in the same temperature in RD medium (20.224±0.038) (Table 2).

Table 2. Yields of methanol extracts of *D. protuberans* growth in three media under two different temperature

The results obtained from this study concerning the activity of antimicrobial agents produced by green microalga were summarized in Table 3. The effects of the methanol extract of *D. protuberans* were compared with standard antibiotic (tobramycin) and fungicide (nystatin) to verify this study. While methanol extracts of all the culture media inhibited *C. albicans*, the extract of BBM and RD (28 °C) of two different concentrations was found effective on *B. subtilis* and *B. cereus* (Table 3).

The cell extracts and active constituents of green algae, diatoms, dinoflagellates and cyanobacteria shown to have *in vitro* antibacterial and antifungal activities against Gram-positive, Gram-negative bacteria and fungi (Salem, Hoballah, Ghazi, & Hanna, 2014). This growing interest in algae and algal products has led to extraction of many important and novel compounds with promising bi ological activity from potentially useful organisms. Salem et al. (2014) which found that *Scenedesmus* methanol and acetone extracts had no antimicrobial effect against on *C. albicans*, in contrast with Abedin & Taha (2008) which reported that *Scenedesmus quadricauda* (Turpin) Brébisson inhibits the growth of the two fungal strains. This showed that the methanol extracts have significant antifungal activity.

Table 3. Test microorganisms and inhibition zone diameters (mm) obtained by the methanol extracts of *D. protuberans* and standard antibiotics

Najdenski et al. (2013) stated that ethanolic extract of *Tetradesmus obliquus* (Turpin) M.J. Wynne, *Chlorella* sp. and *Nostoc* sp. has antibacterial effect against *S. aureus* and *B. cereus*. In the same manner Sanmukh, Bruno, Ramakrishnan, Khairnar & Swaminathan (2014) explored bioactive compounds of a group of microalgae with emphasizing on the *Chlorella vulgaris* Beyerinck which revealed antibacterial effect against *Staphylococcus* sp.

Ozturk, Aslim & Beyatli (2006) indicated that *Scenedesmus* sp. S25 strain had the strongest effect (φ 12.6 mm) on *Bacillus cereus.* Extracts of *Scenedesmus* sp. S25, *Scenedesmus* sp. S39 and *Scenedesmus* sp. S59 were demonstrated inhibitor activity against *B. cereus* as strong as the effect of positive control antibiotic (30 □g vancomycin).

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Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v18_10_06

The presence of antimicrobial activity is affected by both algal species and the solvents use for their extraction (Radhika, Veerabahu, & Priya, 2012). The antimicrobial activity of algae extracts is generally assayed using various organic solvents which always provide a higher efficiency in extracting compounds for antimicrobial activity (Tüney, Çadirci, Unal, & Sukatar, 2006).

Some other potential (secondary or primary metabolites) applications of algae are the production of medicinal compounds for the pharmaceutical industry and both fertilizers and biocontrol agents for the agricultural (Ördög et al., 2004). For an initial step, searching of potential activity of crude algae could be more successful than the screening of pure substances isolated from natural products.

Antioxidant activity was also examined the extracts of *D. protuberans* through β-carotene bleaching assay by lineloic acid. The highest antioxidant activity was found in RD medium at 22°C (90.27±0.01 %) while BBM and BG-11 extracts had almost similar antioxidant activity around 84.84±0.043 % and 84.41±0.03 % (Table 4). As for the effect of growth temperature it was stated that 22 ºC higher than 28ºC using β-carotene bleaching assay. The method worked in an aqueous emulsion of linoleic acid and β-carotene, which was bleached by the radicals generated by the self-produced oxidation of the fatty acid at 50 °C (Prieto, Rodríguez-Amado, Vazquez, & Murado, 2012).

Table 4. Results of □-carotene bleaching assay

Figure 3. Total phenolic content and DPPH radical scavenging activity of methanol extracts from *D. protuberans* growth in BBM, BG-11, RD medium

Antioxidant activity of the methanol extracts was determined by DPPH assay. Percentages of DPPH radical scavenging activity are viewed in Fig. 3. According to the results; all tested microalga extracts possessed the scavenging ability of DPPH radical at 1 mg extract concentration. Extracts from 22°C cultures were found higher DPPH free radical scavenger than extracts of 28°C cultures. There are a number of studies on antioxidant capacity of commercial produced microalgae species belonging to *Dunaliella*, *Navicula*, *Isochrysis, Tetraselmis*, *Chlorella* and *Phaeodactylum*, etc. The antioxidant compounds of microalgae could have different properties especially polarities, thus the antioxidant capacity of microalgae are influenced by the solvent type in the extraction (Maadane e t al., 2015).

The total phenolic content of methanolic extracts was evaluated, using the Folin–Ciocalteu method and the results are shown in Fig. 3. The highest phenolic content was found in RD medium (22°C) extracts with 5000±0.023 mg/100g, followed by BBM (22°C) with 4300±0.04 mg/100g. Phenolic contents of the analyzed extracts in this study were higher than *Microchloropsis gaditana* (L.M. Lubián) M.W.Fawley, I. Jameson & K.P.Fawley, *Tetraselmis* sp. found by Goiris et al. (2015) in extracts of *Isochrysis* sp. (Maadane et al., 2015).

Several articles demonstrated a negligible correlation between carotenoids and phenolics in antioxidant activity (Li et al., 2007; Goh, Yusoff, & Loh, 2010). However, the other reports showed opposite of the results. Goiris et al. (2015) indicated that microalgae both carotenoid and phenolic components considerably associated with the antioxidant capacity. Nutrient stress results in the generation of free radical species in the cell and changes in the content of antioxidants. It is known that nutrient stress may induce accumulation of carotenoids in many species of microalgae (Goiris et al. 2015). Microalgae biomolecules could constitute natural antioxidant such as carotenoids, phenolics, polysaccharides, proteins and fatty acids.

Microalgae cultivated different temperatures and media afterwards extracts tested exhibited higher antioxidant despite lower antimicrobial activities. The higher antioxidant potentials were obtained in *D. protuberans* because

Erdoğan, Çağır, Conk Dalay, & Eroğlu, (2015) was reported that *Desmodesmus protuberans* (Chlorophyceae) was found in significant amounts of carotenoids especially including violaxanthin, all-trans-lutein, all-trans-α-carotene, all-trans-β-carotene, 9 or 9′-cis-β-carotene. A better understanding of the microalga is needed to have the possibility to take advantage of the potential antibacterial and antioxidant effect of microalga. Antimicrobial activity of alga will be beneficial economically and for aquaculture standpoint. Because of high total carotenoid, total phenolic and antimicrobial contents *D. protuberans* could be a potential, economic new source of natural antioxidants and antimicrobials. Further studies on *D. protuberans* would provide important and wide applications in the pharmaceutical and food industries.

References

Abedin, R.M.A., & Taha, H.M. (2008). Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett Burman design for antimicrobial activity of *Spirulina platensis*. *Global Journal of Biotechnology and Biochemistry*, 3(1), 22-31.

Akgül, R. (2017). Culture of *Desmodesmus communis* (E. Hegewald) E. Hegewald and Its Determination of the Biochemical Properties. *Turkish Journal of Agriculture-Food Science and Technology*, 5(4), 404-408. https://doi.org/10.24925/turjaf.v5i4.404-408.1254

Akgül, F., Kizilkaya, İ.T., Akgül, R., & Erduğan, H. (2017). Morphological and Molecular Characterization of *Scenedesmus*-Like Species from Ergene River Basin (Thrace, Turkey). *Turkish Journal of Fisheries and Aquatic Sciences*, 17(3), 609-619. https://dx.doi.org/10.4194/1303-2712-v17_3_17

Bradshaw, L. J. (1992). Laboratory Microbiology, Emeritus California State University, Fullerton.

Benavente-Valdés, J.R., Aguilar, C., Contreras-Esquivel, J.C., Méndez-Zavala, A., & Montañez, J. (2016). Strategies to enhance the production of photosynthetic pigments and lipids in chlorophycae species. *Biotechnology Reports*, 10, 117-125. https://dx.doi.org/10.1016/j.btre.2016.04.001

Bourrelly, P. (1962). Ulotricales d'eau douce rares ou nouvelles. Phykos, 1, 29-35.

Bourrelly, P. (1972). *Les algues d'eau douce. Initiation à la Systématique: Tome I. Les algues vertes*. Société Nouvelle des Éditions Boubée.

Collins C.M., & Lyne P.M. (1987): Microbiological Methods. London, Butterworths & Co. Ltd., 316 pp.

Demirel, Z., Yilmaz-Koz, F. F., Karabay-Yavasoglu, U. N., Ozdemir, G., & Sukatar, A. (2009). Antimicrobial and antioxidant activity of brown algae from the Aegean Sea. Journal of the Serbian Chemical Society, 74(6), 619-628. https://dx.doi.org/10.2298/JSC0906619D

Demirel, Z., Yilmaz-Koz, F. F., Karabay-Yavasoglu, N. U., Ozdemir, G., & Sukatar, A. (2011). Antimicrobial and antioxidant activities of solvent extracts and the essential oil composition of *Laurencia obtusa* and *Laurencia obtusa* var. *pyramidata*. *Romanian biotechnological letters*, 16(1), 5927-5936.

El-Sayed, A.B., & Abdel-Maguid, A.A. (2010). Immobilized-microalga *Scenedesmus* sp. for Biological Desalination of Red Sea Water: II. Effect on Macronutrients Removal. *Journal of American Science*, 6(9), 637-643.

Erdoğan, A., Çağır, A., Dalay, M. C., & Eroğlu, A. E. (2015). Composition of Carotenoids in *Scenedesmus protuberans*: Application of Chromatographic and Spectroscopic Methods. *Food Analytical Methods*, 8, 1970-1978. https://dx.doi.org/10.1007/s12161-015-0088-8

www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v18_10_06

Ghasemi, Y., Moradian, A., Mohagheghzadeh, A., Shokravi, S., & Morowvat, M.H. (2007). Antifungal and Antibacterial Activity of the Microalgae Collected from Paddy Fields of Iran: Characterization of Antimicrobial Activity of *Chroococcus dispersus*. *Journal of Biological Sciences*, 7(6), 904-9010.

Goh, S.H., Yusoff, F.M.,& Loh, S.P.A. (2010). A comparison of the antioxidant properties and total phenolic content in a diatom *Chaetoceros* sp. and a green microalga, *Nannochloropsis* sp. *Journal of Agricultural Science*, 2, 123-130. http://dx.doi.org/10.5539/jas.v2n3p123

Goiris, K., Colen, W.V., Wilches, I., León-Tamariz, F., Cooman, L.D., & Muylaert, K. (2015). Impact of nutrient stress on antioxidant production in three species of microalgae. *Algal Research*, 7,51-57. https://dx.doi.org/10.1016/j.algal.2014.12.002

Imamoglu, E., Demirel, Z., & Dalay, M.C. (2014). Evaluation of culture conditions of locally isolated *Dunaliella salina* strain EgeMacc-024. *Biochemical Engineering Journal*, 92, 22-27. https://dx.doi.org/10.1016/j.bej.2014.05.008

Karło, A., Wilk, A., Ziembińska-Buczyńska, A., & Surmacz-Gorska, J. (2015). Cultivation Parameters Adjustment for Effective Algal Biomass Production. *Rocznik Ochrona Środowiska*, *17*(cz. 1), 275-288.

Koz, F.F.Y., Yavasoglu, N.U.K., Demirel, Z., Sukatar, A., & Ozdemir, G. (2009). Antioxidant and Antimicrobial Activities of *Codium fragile* (Suringar) Hariot (Chlorophyta) Essential Oil and Extracts. *Asian Journal of Chemistry*, 21(2), 1197-1209.

Li, B., Cheng, K., Wong, C., Fan, K., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, 102, 771-776. https://dx.doi.org/10.1016/j.foodchem.2006.06.022

Maadane, A., Merghoub, N., Ainane, T., Arroussi, H.E., Benhima, R., Amzazi, S., Bakri, Y., & Wahby, I. (2015). Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. *Journal of Biotechnology*, 215, 13-19. https://dx.doi.org/10.1016/j.jbiotec.2015.06.400.

Mann, G., Schlegel, M., Schumann, R., & Sakalauskas, A. (2009). Biogas-conditioning with microalgae. *Agronomy Research* 7(1), 33-38.

Najdenski, H.M., Gigova, L.G., Ivan, I.I., Pilarski P.S., Lukavsky J., Tsvetkova, I.V., Ninova M.S., & Kussovski, V.K. (2013). Antibacterial and antifungal activities of selected microalgae and cyanobacteria. *International Journal of Food Science and Technology*, 48, 1533-1540. https://dx.doi.org/10.1111/ijfs.12122

Noaman, N.H., Fattah, A., Khaleafa, M., & Zaky, S.H. (2004). Factors affecting antimicrobial activity of *Synechococcus leopoliensis*. *Microbiological Research*, 159, 395-402. https://dx.doi.org/10.1016/j.micres.2004.09.001

Ozdemir, G., Ulku Karabay, N., Dalay, M. C., & Pazarbasi, B. (2004). Antibacterial Activity of Volatile Component and Various Extracts of *Spirulina platensis. Phytotherapy Research*, 18, 754-757. https://dx.doi.org/10.1002/ptr.1541

Ördög, V., Stirk, W.A., Lenobel, R., Bancirova, M., Strand, M., & Van Standen, J. (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, 16, 309-314. https://doi.org/10.1023/B:JAPH.0000047789.34883.aa

Ozturk, S., Aslim, B., & Beyatli, Y. (2006). Biological screening of microalgae isolated from different freshwaters of Turkey: Antimicrobial activity, viability and brine shrimp lethality. *Fresenius Environmental Bulletin*, 15(10), 1232-1237.

Prieto, M.A., Rodríguez-Amado, I., Vazquez, J.A., & Murado, M.A. (2012). β-Carotene Assay Revisited. Application To Characterize and Quantify Antioxidant and Prooxidant Activities in a Microplate. *Journal of Agricultural and Food Chemistry*, 60, 8983-8993. https://dx.doi.org/10.1021/jf302218g

Radhika, D., Veerabahu, C., & Priya, R. (2012). Antibacterial activity of some selected seaweeds from the Gulf of Mannar Coast, South India. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4), 89-90.

Rios, L. F., Klein, B. C., Luz, L. F., Maciel Filho, R., & Maciel, M. W. (2015). Nitrogen starvation for lipid accumulation in the microalga species *Desmodesmus* sp. *Applied biochemistry and biotechnology*, 175(1), 469-476. https://dx.doi.org/10.1007/s12010-014-1283-6

Rudic, V., & Dudnicenco, T. (2000). Process for cultivation of green alga *Haeamatococcus pluvialis* (Flotow), MD Patent, Nr. a 2000 0154.

Salem, O.M.A., Hoballah, E.M., Ghazi, S.M., & Hanna, S.N. (2014). Antimicrobial activity of microalgal extracts with special emphasize on *Nostoc* sp. *Life Science Journal*, 11(12), 752-758.

Safafar, H., Van Wagenen, J., Møller, P., & Jacobsen, C. (2015). Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Marine drugs*, *13*(12), 7339-7356. https://dx.doi.org/10.3390/md13127069

Sanmukh, S., Bruno, B., Ramakrishnan, U., Khairnar, K., & Swaminathan, S. (2014). Bioactive compounds derived from microalgae showing antimicrobial activities. *Journal of Aquaculture Research and Development*, 5(3), 224. http://dx.doi.org/10.4172/2155-9546.1000224

Shoup, S., & Lewis, L. A. (2003). Polyphyletic origin of parallel basal bodies in swimming cells of chlorophycean green algae (Chlorophyta). *Journal of Phycology*, 39(4), 789-796. https://dx.doi.org/10.1046/j.1529-8817.2003.03009.x

Tüney, I., Çadirci, B.H., Unal, D., & Sukatar, A. (2006). Antimicrobial Activities of the Extracts of Marine Algae from the Coast of Urla (İzmir, Turkey). *Turkish Journal of Biology*, 30, 171-175.

Vanags, J., Kunga, L., Dubencovs, K., Galvanauskas, V., & Grīgs, O. (2015). Influence of Light Intensity and Temperature on Cultivation of Microalgae *Desmodesmus Communis* in Flasks and Laboratory-Scale Stirred Tank Photobioreactor. *Latvian Journal of Physics and Technical Sciences*, 52(2), 59-70. https://doi.org/10.1515/lpts-2015-0012

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B.D. (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *Journal of Agricultural and Food Chemistry* 46, 4113-4117. https://dx.doi.org/10.1021/jf9801973

Yıldırım, A., Demirel, Z., İşleten-Hoşoğlu, M., Akgün, İ. H., Hatipoğlu-Uslu, S., & Conk-Dalay, M. (2014). Carotenoid and fatty acid compositions of an indigenous *Ettlia texensis* isolate (Chlorophyceae) under phototrophic and mixotrophic conditions. *Applied Biochemistry and Biotechnology*, 172, 1307-1319. https://dx.doi.org/10.1007/s12010-013-0599-y

Zhang, D., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry*, 88(4), 503-509. https://doi.org/10.1016/j.foodchem.2004.01.065

Zubia, M., Robledo, D., & Freile-Pelegrin, Y. (2007). Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. *Journal of applied phycology*, *19*(5), 449-458. https://dx.doi.org/10.1007/s10811-006-9152-5

Table 1. Culture conditions (cell concentration, specific growth rate and doubling time) of *D. protuberans*

	Max. cell con. (x10^6 cell/mL)	**Specific growth rate (μ day^{-1})**	**Doubling Time (Day)**
22°C			
BBM	8.3	0.1585±0.004	4.3741
BG-11	8.9	0.1979±0.023	3.5025
RD	7.1	0.1337±0.014	5.1877
28°C			
BBM	12.64	0.1627±0.024	4.2622
BG-11	10.56	0.2098±0.018	3.3034
RD	11.92	0.1511±0.021	4.5872

Table 2. Yields of methanol extracts of *D. protuberans* growth in three media under two different temperature

	Yield (%)	
Medium	**22º C**	**28º C**
BBM	20.862±0.012	28.074±0.026
BG-11	28.354±0.035	30.723±0.041
RD	27.554±0.026	20.224±0.038

Table 3. Test microorganisms and inhibition zone diameters (mm) obtained by the methanol extracts of *D. protuberans* and standard antibiotics

Microorganisms		**Inhibition Zones (mm)**													
		BBM 22°C		**BG-11 22°C**		**RD 22°C**		**BBM 28°C**		**BG-11 28°C**		**RD 28°C**		**Standard Tobramycin (10 µg/disc)**	**Standard Nystatin (30 µg/disc)**
	Gr	**20 µL**	**30 µL**	**20 µL**	**30 µL**	**20 µL**	**30 µL**	**20 µL**	**30 µL**	**20 µL**	**30 µL**	**20 µL**	**30 µL**		
Streptococcus faecalis ATCC 29212	+	-	0.5	-	-	-	0.5	0.5	1	0.5	1	0.5	1	9	nt
Bacillus subtilis ATCC 6633	+	-	0.5	-	-	0.5	1	1	2	0.5	1	0.5	2	24	nt
Staphylococcus	+	-	-	-	-	-	-	-	-	-	-	-	-	16	nt

aureus ATCC 6538P																
S. epidermidis ATCC 12228	+	-	-	-	-	-	-	-	-	-	-	-	-	7		nt
Bacillus cereus ATCC 7064	+	-	1	1	2	-	0.5	1	2	-	-	0.5	2	17		nt
Pseudomonas aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	-	-	-	-	-	12		nt
Enterobacter cloaceae ATCC 13047	-	-	-	-	-	-	-	-	-	-	-	-	-	13		nt
Escherichia coli ATCC 29908	-	-	-	-	-	-	-	-	-	-	-	-	-	10		nt
Salmonella typhimurium CCM 544	-	-	0.5	-	-	-	0.5	-	-	-	-	-	0.5	10		nt
Candida albicans ATCC 10239		4	5	2	4	1	2	5	6	2	3	3	4	nt		18

Zone of inhibition, including the diameter of the filter paper disc (6 mm); mean value of three independent experiments; Gr, Gram; nt, not tested; –, no activity

Table 4. Results of□□-carotene bleaching assay

	AA %	**ORR**	**AAC**
BHT	93.71±0.036	929.89±0.029	0.073±0.017
Vitamin E	85.12±0.026	1008.6±0.054	0.149±0.013
22°C			
BBM	84.84±0.043	1165.95±0.092	0.152±0.026
BG-11	84.41±0.03	1158.80±0.05	0.139±0.029
RD	90.27±0.01	972.44±0.01	0.097±0.01
28°C			
BBM	82.69±0.03	1181.49±0.022	0.173±0.03
BG-11	82.69±0.020	1194.56±0.04	0.173±0.026
RD	85.41±0.045	1078.88±0.036	0.146±0.014

AA: Antioxidant activity, ORR: Oxidation rate ratio, AAC: Antioxidant activity coefficient, mean ± SD: standard deviation

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ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v18_10_06

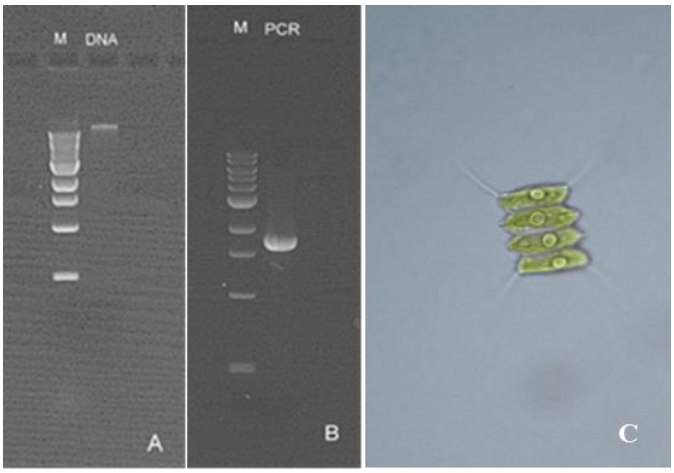

Figure 1. A-Genomic DNA, B- PCR amplification results gel (M: 1 kb ladder BioLabs, England) and C- light microscopic photography 60X.

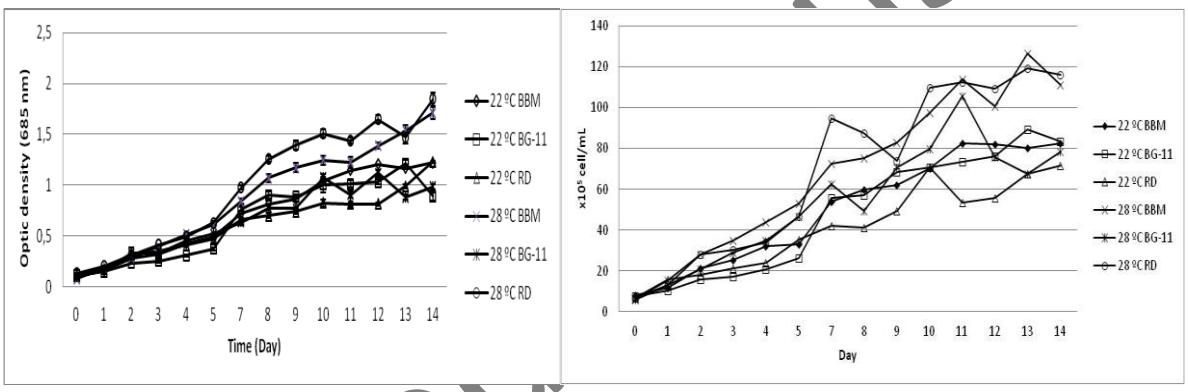

Figure 2. Growth curves of *D. protuberans* at 22°C and 28°C in BBM, BG-11 and RD medium under a light intensity of 75 µmol $m^{-2}s^{-1}$ according to cell number and optic density (685 nm)

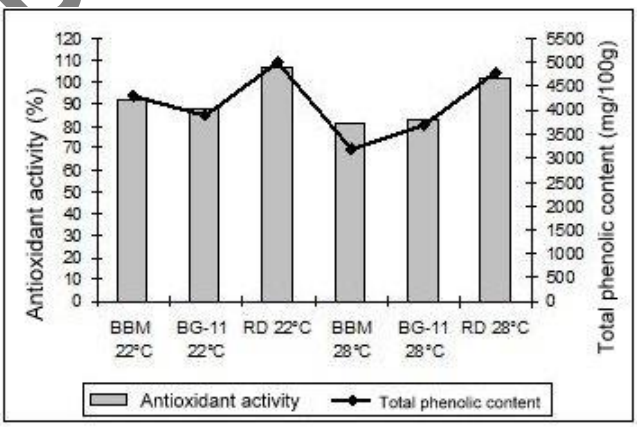

Figure 3. Total phenolic content and DPPH radical scavenging activity of methanol extracts from *D. protuberans* growth in BBM, BG-11, RD medium