Food Chemistry 116 (2009) 129–136

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

PCR amplification of 18S rRNA, single cell protein production and fatty acid evaluation of some naturally isolated microalgae

Sara Rasoul-Amini [a], Younes Ghasemi [b,*], Mohammad Hossein Morowvat [b], Abdolali Mohagheghzadeh [b]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, P.O. Box 71345-1583, Shiraz, Iran
[b] Department of Pharmacognosy and Pharmaceutical Biotechnology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, P.O. Box 71345-1583, Shiraz, Iran

ARTICLE INFO

Article history:
Received 5 November 2008
Received in revised form 25 January 2009
Accepted 11 February 2009

Keywords:
SCP
PCR amplification
18S rRNA
Microalgae
Fatty acids

ABSTRACT

Microalgae were isolated during a screening program from soil samples collected from paddy-fields of Fars province, south of Iran. The protein content was assayed by the Kochert method. Total genomic DNA were isolated and used for PCR amplification of the 18S rRNA gene. The sequences were determined for 12 species of microalgae. Some bioinformatic tools were used for more investigation on these biologic data. Total lipids from five microalgal species were extracted and used for determination of different types of fatty acids by gas chromatography–mass spectrometry method. In our experiments the green algae yielded a maximum protein of about 42% ± 1.64. The DNA sequences were published in the NCBI under specific accession numbers. The composition of fatty acids was mainly, myristic acid, palmitic acid, oleic acid, α-linolenic acid, and γ-linolenic acid.

1. Introduction

Algae are important constituents of many ecosystems ranging from marine and fresh water environments to desert sands and from hot springs to snow and ice. They account for more than half of the total primary production at the base of the food chain worldwide (Guschina & Harwood, 2006). Since the early 1950s intense efforts have been made to explore new alternate protein sources as food supplements, primarily in anticipation of a repeatedly predicted insufficient future protein supply. For these, i.e. yeasts, fungi, bacteria and microalgae, the name single cell protein (SCP) was coined to describe the protein production from biomass, originating from different microbial sources (Becker, 2007). The production of SCP from various microbes, particularly from fungi and bacteria has received considerable attention, in contrast, only a few studies have dealt with the feasibility of using SCP from microalgae (Mahasneh, 1997). Comprehensive analysis and nutritional studies have demonstrated that these algal proteins are of high quality and comparable to conventional vegetable proteins. However, due to high production costs as well as technical difficulties to incorporate the algal material into palatable food preparations, the propagation of algal proteins is still in its infancy.

Fatty acids are primarily metabolites of acetyl CoA pathway which is generally determined, evolutionary very old, and therefore conservative (Petkov & Garcia, 2007). Fatty acids are becoming increasingly important in the pharmaceutical industry and therapeutic agent for numerous health disorders such as heart disease, Parkinson disease, multiple sclerosis, inflammatory disease, premenstrual syndrome, plasma cholesterol level and other disorders (Vargas et al., 1998). Microalgae may contain significant quantities of fats and oils (lipids) with compositions similar to those of vegetable oils. Under certain conditions, microalgae have been reported to contain up to 85% of the dry weight as lipids (Borowitzka & Borowitzka, 1998). This exceeds the lipid content of most terrestrial plants (Borowitzka & Borowitzka, 1998). The major dietary sources of docosahexaenoic acid (DHA) are oils from marine fish and microalgae. Fish obtain most of their long-chain ω3-PUFAs (polyunsaturated fatty acids), by consumption of marine microalgae, which are considered to be the primary producers of it (Pereira, Leonard, Huang, Chuang, & Mukerji, 2004). The lipids of microalgae are generally esters of glycerol and fatty acids with a chain length of C_{14} to C_{22}. They may be either saturated or unsaturated (Borowitzka & Borowitzka, 1998). To date, the majority of microalgal preparations are marketed as health food, as cosmetics or as animal feed (Becker, 2007). Nutritional supplements produced from microalgae have been the primary focus of microalgal biotechnology for many years. Dried biomass or cell extracts produced from *Chlorella*, *Dunaliella* and *Spirulina* have dominated the commercial opportunities. These products are directed mainly at the nutraceutical or health food market and collectively are like worth many hundred of million dollars (Apt & Behrens, 1999).

* Corresponding author. Tel.: +98 711 242 5305; fax: +98 711 242 6070.
E-mail address: ghasemiy@sums.ac.ir (Y. Ghasemi).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.02.025

In this study the protein and lipid content of some environmentally isolated microalgae and possibility of large scale cultivation of them to produce SCP and lipid for human or animal food and feed was determined. Furthermore, the quality of fatty acids from these naturally isolated microalgal species were studied. Moreover, the sequence of encoding ribosomal RNA genes, which their product are essential in protein synthesis were studied to confirm the identification of the microalgal species with the other identification methods.

2. Materials and methods

2.1. Collection, preservation and identification of the microalgae

Microalgae were isolated during a screening program from soil samples collected from paddy-fields of Fars province, south of Iran from April to December 2004. Soil samples were prepared from 15 different habitats from surficial or 5 cm of top of soil. Every sample was specified by a unique code. Soil samples were suspended in specific volume of distilled water. Surficial part (100 μL) was transferred to BG-11 solid culture medium and plates were kept in culture room (Ghasemi et al., 2007). *Dunaliella salina* was isolated from water samples collected from Maharlu Salt Lake, 30 km southeast of Shiraz, Iran. Culturing was done in Johnson medium (Borowitzka & Borowitzka, 1998). Preserved specimens were prepared and the living specimens were incubated in 50 mL conical flasks, under unlimited carbon dioxide condition. Constant illumination was used at 60 $\mu E\ m^{-2}s^{-1}$ intensity with white fluorescent lamps. Temperature was 25 ± 2 °C. The identification was done using bacteriological and botanical approaches (Castenholz, 2001; John, Whitton, & Brook, 2003; Prescott, 1962), and confirmed using molecular markers. Each strain was deposited in Microalgal Culture Collection of Shiraz University of Medical Sciences (MCCS) with a specific code.

2.2. Protein content analysis

The number of cells in normal media was determined by placing the cells in a 0.1 mm deep counting chamber (Neubauer improved) using a light microscope (magnification ×10) in 14 days (Freshney, 1992). The cells was harvested at stationary growth phase ($2.8–3.0 \times 10^6$ cells mL^{-1}) by centrifuge and dried to a constant weight by freeze-drier. Total protein of the dried samples was determined after hydrolysis in 1 M NaOH for 1 h at 100 °C. The protein content of each sample were assayed with the dye Coomassie Brilliant Blue G 250 by comparing their absorbances at 595 nm with a bovine serum-albumin standard curve and calculated as the percentage of dry weight (Kochert, 1978).

2.3. DNA extraction and PCR amplification

An aliquot of cultured cells (1 mL) were harvested in mid to late exponential phase (10–14 days) by centrifugation (13,000*g* for 3 min at room temperature) in a sterile 1.5 mL microcentrifuge tube. Pelleted cells were resuspended in 0.5 mL of TBE buffer. The mixture was shaken and then the genomic DNA was extracted using the heat shock method and used for the PCR analysis. DNA samples were electrophoresed in a 1% (w/v) agarose gel using TBE electrophoresis buffer containing 1 μg/mL ethidium bromide. The molecular weight of the PCR amplified product was calculated and confirmed using Major Sciences gel documentation system. The gel was photographed under UV light. The two oligonucleotide primers used for amplification of microalgal 18S rRNA gene were: The universal eukaryotic primers 5′-GTCAGAGGTGAAATTCTTGGATTTA-3′ as forward primer and 5′-AGGGCAGGGACGTAATCAACG-3′ as reverse primer, which amplify a ~700-bp region of the 18S rRNA gene (prepared by Aran Sanat Yekta, Tehran, Iran). PCR reaction was performed in a total volume of 50 μL containing 10 μL of chromosomal DNA in TBE buffer pH 8.0 and 2 μL of each primer. The amplification was performed in a Bioflux thermo-cycler. The amplification conditions were as described before (Ghasemi et al., 2008).

2.4. Extraction of total lipids

Freshly algal pellets (2 g) were boiled in 5 mL of isopropanol for 2 min to inhibit the lipase activity and were then dried under nitrogen gas. The dried pellet was homogenised in chloroform–methanol (1:2) in the presence of BHT (Butylated hydroxytoluene) added as an antioxidant agent. A total of 0.8 mL distilled water was added to the supernatant of the centrifuged extract, followed by 5 mL of chloroform and 5 mL of 0.88% aqueous solution of potassium chloride. The mixture was shaken vigorously and the solvent phase was collected and concentrated under nitrogen gas. The concentrated extract redissolved in 5 mL of chloroform was used for qualitative determination of different classes of fatty acids (Mooney, Nichols, De Salas, & Hallegraeff, 2007).

2.5. Esterification of fatty acids

Extracted crude lipids of microalgae (0.5 g) were dissolved in 3 mL of methanol in the presence of catalytic amount of sulphuric acid. The mixture was heated to reflux using Dien–Stark apparatus. After cooling, the reaction mixture was washed twice with 4 mL of saturated sodium hydrogen carbonate aqueous solution, dried over anhydrous sodium sulphate and the solvent was removed by distillation under reduced pressure to give an oily substance. Then the thin-layer chromatography (TLC) was applied to monitor the

Table 1
Protein percent (Pr% ± SD), the accession numbers, the length base pair of DNA published sequences at NCBI and the similarity between amplified sequences and the nearest recorded sequence in NCBI using BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi), for 11 strains of environmentally isolated microalgae.

Microalga	Pr% ± SD	Accession number	Length (base pair)	Similarity with the nearest recorded sequence
Chlorella vulgaris MCCS 011	24 ± 2.48	EU374169	630	100% with *Chlorella vulgaris* NJ-7
Chlorella vulgaris MCCS 012	32 ± 6.59	EU374170	620	100% with *Chlorella vulgaris* CCAP 211/82
Chlorella vulgaris MCCS 013	31 ± 2.81	EU374171	617	100% with *Chlorella vulgaris* CCAP 211/80
Chlorella vulgaris MCCS 014	23 ± 1.84	EU375837	628	100% with *Chlorella vulgaris* SAG 211-11b
Chlorella vulgaris MCCS 015	36 ± 2.15	EU379938	602	100% with *Chlorella vulgaris* CCAP 211/81
Dunaliella salina MCCS 001	20 ± 0.92	EF682841	685	100% with *Dunaliella salina* CCAP 19/30
Dunaliella salina CCAP 19/18	23 ± 1.11	EF682843	415	100% with *Dunaliella salina* SAG 42.88
Scenedesmus rubescens MCCS 018	26 ± 6.56	EU621364	620	100% with *Scenedesmus rubescens* CCAP 232/1
Scenedesmus obliquus strain 019	21 ± 1.58	EF564131	578	100% with *Scenedesmus obliquus* UTEX 1450
Desmodesmus communis MCCS 010	42 ± 1.64	EF564130	635	99% with *Scenedesmus subspicatus* UTEX 2532
Chlamydomonas reinhardtii MCCS 002	24 ± 1.86	EF682842	556	100% with *Chlamydomonas reinhardtii* CC124
Chlorococcum sp. MCCS009	–[a]	EU374168	643	99% with *Chlorococcum* sp. KNU-F-2002-C1

[a] Protein percent is not determined.

progress of methyl esterification on the extracted fatty acids. The TLC was performed using silica gel 60 F_{254} plates (20 × 20 cm, 250 μm layer thickness, Merck, Darmstadt, Germany) and pure chloroform as solvent system. The obtained oily substance was injected in GC/MS for analysis.

2.6. GC/MS analysis protocol

The GC/MS analysis was carried out using a Hewlett–Packard 6890. The gas chromatograph was equipped with a HP-5 M capillary column (phenyl methyl siloxan, 25 m × 0.25 mm i.d., Hewlett–Packard Part No. 190915.433, Palo Alto, CA, USA). The oven temperature was programmed from 85 °C (5 min) to 265 °C at the rate of 7 °C /min and finally held at 265 °C for 10 min. The carrier gas was helium with the flow rate of 1.2 mL/min. The mass spectrometer (Hewlett–Packard 5973, USA) was operated in EI (Electron ionisation) mode at 70 eV. The interface temperature was 265 °C and the mass range was 15–650 m/z. The identification of fatty acids was performed comparing the obtained mass spectra with Wiley (275) libraries

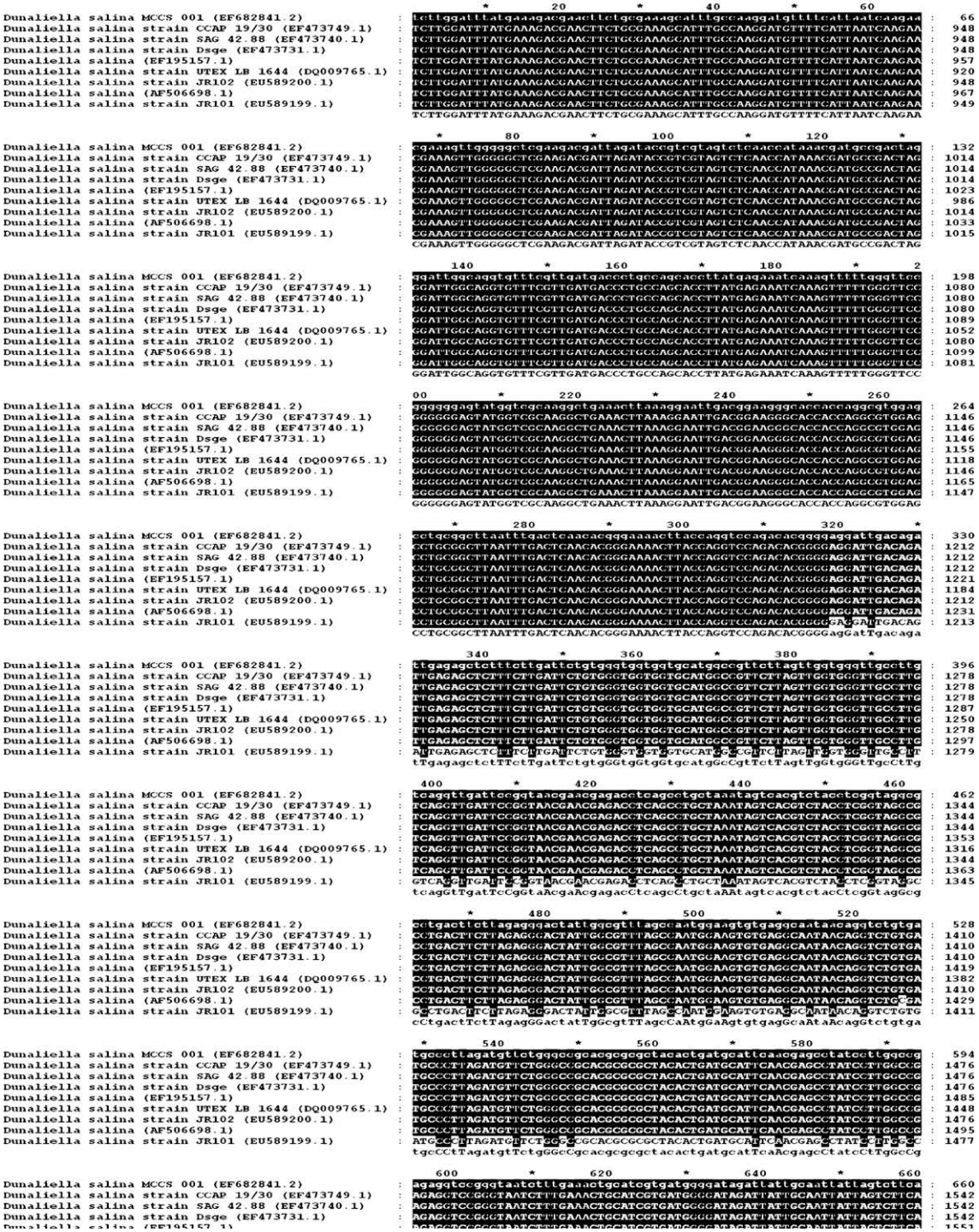

Fig. 1. The similarity between 18S rRNA gene of *Dunaliella salina* MCCS 001 with nearest published sequences in the NCBI using GeneDoc, version 2.6.002, a bioinformatic tool for alignment editing, shading and annotating multiple sequence alignment. Black colour: 100% similarity between sequences, grey colour: 80% similarity between sequences, and white colour: 60% similarity between sequences. The accession numbers are shown in parenthesis.

(Mohagheghzadeh, Faridi, & Ghasemi, 2007; Rodriguez-Garcia & Guil-Guerrero, 2008).

3. Results and discussion

3.1. Protein content analysis

The SCP amount in 11 studied strains of microalgae is given in protein percent (%dry weight) in Table 1.

The range of protein count was 20–42% (Table 1), whilst the maximum and minimum were found in *Desmodesmus communis* MCCS 010 and *Dunaliella salina* MCCS 001 respectively. The mean of SCP for all strains was 27.45% ± 6.93 dry weight after reaching the stationary phase of growth under controlled conditions. The mean value and thus the ranges obtained in this investigation on SCP production were similar to the values reported by other authors, 36–50% dry weight for *Sprulina maxima* (Oleguin et al., 1994), 30.9% dry weight for *Chlorella stigmatophora* (Ben-Amotz, Fisher, & Schneller, 1987), and 31% dry weight for *Chlorella* spp. (Mahasneh, 1997).

This is the first study on the feasibility of using SCP from the environmentally isolated microalgae in Iran. In previous studies, mainly, *Chlorella*, *Dunaliella,* and *Spirulina* strains were reported as a source of SCP production, for the first time, in this work, it is shown that *Desmodesmus* might be a good candidate to be used as SCP.

3.2. 18S rRNA gene amplification

The PCR amplification of chromosomal DNA of the algae with forward and reverse primer revealed efficient amplification. A single band of amplified DNA product of ~700-bp was recorded. The DNA sequences were published in the NCBI databases under the specific accession numbers. The lengths of the 18S rRNA region of 12 species of microalgae and their specific accession numbers are shown in Table 1.

The result of PCR blasted with other sequenced microalgae in NCBI showed similarity to the 18S small subunit rRNA of other microalgae. Edited sequences were used as queries in BLASTN searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi), to determine the nearest identifiable match present in the complete GenBank nucleotide data base (Table 1).

A bioinformatic tool, GeneDoc software, version 2.6.002, was used for more investigation of these sequences (Fig. 1). A total of 408 of the nucleotides of the partial sequence of *D. salina* MCCS 001 were 100% similar to the 18S ribosomal RNA genes in eight recorded strains of *D. salina* in database sequences.

The PCR amplification of chromosomal DNA of the algae with forward and reverse primer revealed efficient amplification. A single band of amplified DNA product of ~700-bp was recorded by gel documentation system. The amplified sequences were cut and sent for sequencing analysis and then were published in NCBI databases (Table 1).

In the present study, using the universal primer, 18S rRNA, we amplified a fragment of the 18S rRNA region from the genomic DNA of several strains of the environmentally isolated microalgae. This fragment was between 415 base pairs (in *D. salina* CCAP 19/18) to 685 base pairs (in *D. salina* MCCS 001).

3.3. Fatty acid identification

Fatty acids in the five strains of microalgae (*Chlorella vulgaris* MCCS 013, *D. salina* MCCS 001, *D. salina* CCAP 19/18, *Scenedesmus obliquus* strain 019, *Scenedesmus rubescens* MCCS 018) were primarily esterified and then identified through GC/MS analysis. The GC/MS analyses were carried out on the product of esterification (fatty acids (FAs)/fatty acid methyl esters (FAMEs)) in the above mentioned microalgae strains. The total fatty acid content of each strain was about 25% of dry weight of biomass.

The identification of FAs/FAMEs was performed through the comparison of their mass spectra with those in Wiley libraries.

Table 2
Chlorella vulgaris MCCS 013 fatty acids (FAs)/fatty acid methyl esters (FAMEs), they are classified on the base of their profiles identified in comparison to the Wiley library (Rodriguez-Garcia & Guil-Guerrero, 2008).

Systematic name of identified FA	Common name	Chemical formula	No. of carbon atoms	No. of double bond(s) in FA	Fatty acid content (% total)	Family
2-Methyl-2-propenoic acid	Methacrylic acid	$C_4H_6O_2$	4	1	0.4	Monounsaturated FA
Hexanoic acid	Caproic acid	$C_6H_{12}O_2$	6	0	0.6	Saturated FA
Octanoic acid	Caprylic acid	$C_8H_{16}O_2$	8	0	0.6	Saturated FA
Undecanoic acid		$C_{11}H_{22}O_2$	11	0	30.5	Saturated FA
Methyl dodecanoate	Methyl laurate	$C_{13}H_{26}O_2$	13	0	2.4	Saturated FAME
Pentadecanoic acid		$C_{15}H_{30}O_2$	15	0	4.3	Saturated FA
Methyl tetradecanoate		$C_{15}H_{30}O_2$	15	0	1.7	Branched saturated FAME
Methyl 9-Methyl tetradecanoate		$C_{16}H_{32}O_2$	16	0	3.1	Branched saturated FAME
7,10,13-Hexadecatrienoic acid		$C_{16}H_{26}O_2$	16	3	2.4	Polyunsaturated (ω3) FA
Heptadecanoic acid	Margarinic acid	$C_{17}H_{34}O_2$	17	0	2.3	Saturated FA
10-Octadecenoic acid		$C_{18}H_{34}O_2$	18	1	0.6	Monounsaturated FA
16-Octadecanoic acid		$C_{18}H_{34}O_2$	18	1	5.2	Monounsaturated FA
9,11-Octadecadiynoic acid		$C_{18}H_{30}O_2$	18	2 (triple bonds)	3.8	Polyunsaturated (alkynoic) FA
9,12,15-Octadecatrienoic acid	α-Linolenic acid or ALA	$C_{18}H_{30}O_2$	18	3	3.6	Polyunsaturated (ω3) FA
Eicosanoic acid	Arachidic acid	$C_{20}H_{40}O_2$	20	0	28.2	Saturated FA
11,14,17-Eicosatrienoic acid	Eicosatrienoic acid or ETE	$C_{20}H_{34}O_2$	20	3	1.2	Polyunsaturated (ω3) FA
Heneicosanoic acid		$C_{21}H_{42}O_2$	21	0	0.4	Saturated FA
Docosanoic acid	Behenic acid	$C_{22}H_{44}O_2$	22	0	0.7	Saturated FA
Tricosanoic acid		$C_{23}H_{46}O_2$	23	0	4.5	Saturated FA
Tetracosanoic acid	Lignoceric acid	$C_{24}H_{48}O_2$	24	0	1.9	Saturated FA

The results are given in the profiles including systematic name, common name, chemical formula, number (No) of carbon atoms, number (No) of double bond(s), position of double bond(s) and fatty acid's family according to un/saturated) in Tables 2–6 (*C. vulgaris* MCCS 013 (Table 2), *D. salina* MCCS 001 (Table 3), *D. salina* CCAP 19/18 (Table 4), *S. rubescens* MCCS 018 (Table 5) and *S. obliquus* strain 019 (Table 6)).

As shown in these tables (Tables 2–6), different types of fatty acids are detected in the five sample strains. Esterification of the studied microalgal fatty acids is the choice to identify those using GC/MS (Butte, 1983; Müller, Husamann, & Nalik, 1990). GC/MS analysis allowed us to discover several types of fatty acids, such as chained, branched, monounsaturated, polyunsaturated, alkynoic and dioic fatty acids in some naturally isolated microalgal strains and showed the presence of at least 50 fatty acids in them.

GC/MS analysis of fatty acids requires the conversion of fatty acids to fatty acid methyl esters, which is often done by saponification and esterification with methanol (Müller et al., 1990). This method seems to be the choice for the evaluation of fatty acid profiles using a one-step transesterification procedure (Butte, 1983). It is known that specific groups of organisms can be characterised by particular fatty acids, which can be used as their biological markers (Řezanka, Dor, Prell, & Dembitsky, 2003).

Considering the importance of presence of polyunsaturated FAs in microalgae the results of this study approved that our environmentally isolated microalgae have a vast variety of polyunsaturated FAs which warrants a great potential for large scale production.

The major fatty acids in our five studied strains of microalgae were shown to be undecanoic acid, myristic acid, palmitic acid, and eicosanoic acid (Tables 2–6).

The shortest identified fatty acids was propenoic acid with 3 carbon atoms in *D. salina* MCCS 001 and the longest was octatriacontanoic acid with 38 carbon atoms in *D. salina* MCCS 001 (Table 3). Furthermore *D. salina* MCCS 001 showed the most diverse strain in the studied microalgae which had 34 types of fatty acids. Undecanoic acid was found in all of the studied strains but some fatty acids were found only in one strain such as 2-propenoic acid

Table 3
Dunaliella salina MCCS 001 fatty acids (FAs)/fatty acid methyl esters (FAMEs), they are classified on the base of their profiles identified in comparison to the Wiley library (Rodriguez-Garcia & Guil-Guerrero, 2008).

Systematic name	Common name	Formula	No. of carbon atoms	No. of double bond(s)	Fatty acid content (% total)	Family
2-Propenoic acid	Acrylic acid	$C_3H_4O_2$	3	1	0.4	Monounsaturated FA
Butanoic acid	Butyric acid	$C_4H_8O_2$	4	0	0.3	Saturated FA
Pentanoic acid	Valeric acid	$C_5H_{10}O_2$	5	0	0.4	Saturated FA
2-Pentenoic acid		$C_5H_8O_2$	5	1	0.2	Monounsaturated FA
4-Pentenoic acid		$C_5H_8O_2$	5	1	0.3	Monounsaturated FA
Hexanoic acid	Caproic acid	$C_6H_{12}O_2$	6	0	1.6	Saturated FA
3-Hexenoic acid		$C_6H_{10}O_2$	6	1	0.8	Monounsaturated FA
5-Hexenoic acid		$C_6H_{10}O_2$	6	1	0.1	Monounsaturated FA
Heptanedioic acid	Pimelic acid	$C_7H_{12}O_4$	7	0	0.2	Saturated (dioic) FA
2-Heptenoic acid		$C_7H_{12}O_2$	7	1	0.6	Monounsaturated FA
Octanoic acid	Caprylic acid	$C_8H_{16}O_2$	8	0	0.6	Saturated FA
3-Octenoic acid		$C_8H_{14}O_2$	8	1	1.4	Monounsaturated FA
Decanoic acid	Capric acid	$C_{10}H_{20}O_2$	10	0	1.2	Saturated FA
Undecanoic acid	Hendecanoic acid	$C_{11}H_{22}O_2$	11	0	24.1	Saturated FA
Methyl decanoate	Methyl caprinate	$C_{11}H_{22}O_2$	11	0	0.9	Branched saturated FAME
Dodecanoic acid	Lauric acid	$C_{12}H_{24}O_2$	12	0	1.7	Saturated FA
Tridecanoic acid		$C_{13}H_{26}O_2$	13	0	1.1	Saturated FA
Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	14	0	23.2	Saturated FA
Pentadecanoic acid		$C_{15}H_{30}O_2$	15	0	0.2	Saturated FA
Methyl tetradecanoate		$C_{15}H_{30}O_2$	15	0	0.2	Branched saturated FAME
Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	16	0	26.5	Saturated FA
9-Hexadecenoic acid	Palmitoleic acid	$C_{16}H_{30}O_2$	16	1	0.3	Monounsaturated (ω7)FA
Heptadecanoic acid	Margarinic acid	$C_{17}H_{34}O_2$	17	0	0.7	Saturated FA
Methyl hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	17	0	0.1	Branched saturated FA
Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$	18	0	2.3	Saturated FA
Methyl heptadecanoate		$C_{18}H_{36}O_2$	18	0	0.5	Branched saturated FAME
6-Octadecenoic acid	Petroselinic acid	$C_{18}H_{34}O_2$	18	1	0.2	Monounsaturated FA
15-Octadecenoic acid		$C_{18}H_{34}O_2$	18	1	0.9	Monounsaturated FA
16-Octadecenoic acid		$C_{18}H_{34}O_2$	18	1	0.4	Monounsaturated FA
9,12-Octadecadienoic acid	Linoleic acid	$C_{18}H_{32}O_2$	18	2	2.3	Polyunsaturated FA
Nonadecanoic acid		$C_{19}H_{38}O_2$	19	0	1.1	Saturated FA
Heneicosanoic acid		$C_{21}H_{42}O_2$	21	0	0.3	Saturated FA
Tricosanoic acid		$C_{23}H_{46}O_2$	23	0	0.5	Saturated FA
Tetracosanoic acid	Lignoceric acid	$C_{24}H_{48}O_2$	24	0	0.2	Saturated FA
15-Tetracosenoic acid	Nervonic acid or Selacholeic acid	$C_{24}H_{46}O_2$	24	1	0.4	Monounsaturated FA
Heptacosanoic acid	Carboceric acid	$C_{27}H_{54}O_2$	27	0	0.2	Saturated FA
Triacontanoic acid	Mellisic acid	$C_{30}H_{60}O_2$	30	0	0.5	Saturated FA
Octatriacontanoic acid		$C_{38}H_{76}O_2$	38	0	1.1	Saturated FA

Table 4
Dunaliella salina CCAP 19/18 fatty acids (FAs)/fatty acid methyl esters (FAMEs), they are classified on the base of their profiles identified in comparison to the Wiley library (Rodriguez-Garcia & Guil-Guerrero, 2008).

Systematic name	Common name	Formula	No. of carbon atoms	No. of double bond(s)	Fatty acid content (% total)	Family
2-Hexenoic acid		$C_6H_{10}O_2$	6	1	0.5	Monounsaturated FA
Decanoic acid	Capric acid	$C_{10}H_{20}O_2$	10	0	0.4	Saturated FA
Undecanoic acid	Hendecanoic acid	$C_{11}H_{22}O_2$	11	0	36.1	Saturated FA
Dodecanoic acid	Lauric acid	$C_{12}H_{24}O_2$	12	0	0.3	Saturated FA
Tridecanoic acid		$C_{13}H_{26}O_2$	13	0	0.2	Saturated FA
Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	14	0	16.1	Saturated FA
Pentadecanoic acid		$C_{15}H_{30}O_2$	15	0	0.3	Saturated FA
Methyl tetradecanoate		$C_{15}H_{30}O_2$	15	0	0.2	Branched saturated FAME
Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	16	0	23.4	Saturated FA
Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$	18	0	1.3	Saturated FA
6,9,12-Octadecatrienoic acid	γ-Linolenic acid or GLA	$C_{18}H_{30}O_2$	18	3	1.2	Polyunsaturated (ω6) FA
Nonadecanoic acid		$C_{19}H_{38}O_2$	19	0	3.2	Saturated FA
Eicosanoic acid	Arachidic acid	$C_{20}H_{40}O_2$	20	0	11.1	Saturated FA
Eicosenoic acid	Gondoic acid	$C_{20}H_{38}O_2$	20	1	0.3	Monounsaturated (ω9) FA
Heneicosanoic acid		$C_{21}H_{42}O_2$	21	0	1.6	Saturated FA
Heptacosanoic acid		$C_{27}H_{54}O_2$	27	0	0.4	Saturated FA
Triacontanoic acid	Mellisic acid	$C_{30}H_{60}O_2$	30	0	0.1	Saturated FA

Table 5
Scenedesmus rubescens MCCS 018 fatty acids (FAs)/fatty acid methyl esters (FAMEs), they are classified on the base of their profiles identified in comparison to the Wiley library (Rodriguez-Garcia & Guil-Guerrero, 2008).

Systematic name	Common name	Formula	No. of carbon atoms	No. of double bond(s)	Fatty acid content (% total)	Family
Butanoic acid	Butyric acid	$C_4H_8O_2$	4	0	1.1	Saturated FA
Hexanoic acid	Caproic acid	$C_6H_{12}O_2$	6	0	0.2	Saturated FA
Octanoic acid	Caprylic acid	$C_8H_{16}O_2$	8	0	0.5	Saturated FA
Decanoic acid	Capric acid	$C_{10}H_{20}O_2$	10	0	0.3	Saturated FA
Undecanoic acid		$C_{11}H_{22}O_2$	11	0	18.1	Saturated FA
Methyl decanoate	Methyl caprinate	$C_{11}H_{22}O_2$	11	0	1.7	Branched saturated FAME
Tridecanoic acid		$C_{13}H_{26}O_2$	13	0	1.9	Saturated FA
Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	14	0	42.3	Saturated FA
Pentadecanoic acid		$C_{15}H_{30}O_2$	15	0	0.8	Saturated FA
Methyl tetradecanoate		$C_{15}H_{30}O_2$	15	0	0.7	Branched saturated FAME
Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	16	0	13.6	Saturated FA
9,12-Hexadecadienoic acid		$C_{16}H_{28}O_2$	16	2	0.4	Polyunsaturated (ω4) FA
Heptadecanoic acid	Margarinic acid	$C_{17}H_{34}O_2$	17	0	0.4	Saturated FA
9-Octadecenoic acid	Oleic acid	$C_{18}H_{34}O_2$	18	1	0.1	Monounsaturated (ω9) FA
6,9,12-Octadecatrienoic acid	γ-Linolenic acid or GLA	$C_{18}H_{30}O_2$	18	3	4.1	Polyunsaturated (ω6) FA
Eicosanoic acid	Arachidic acid	$C_{20}H_{40}O_2$	20	0	12.1	Saturated FA

and pentanoic acid, in *D. salina* MCCS 001 (Table 3), nonanoic acid, 7-hexadecenoic acid, 9,15-octadecadienoic acid, 10,13-octadecadienoic acid and 12,15-octadecadienoic acid in *S. obliquus* strain 019 (Table 6), 9,12-hexadecadienoic acid and 9-octadecenoic acid in *S. rubescens* (Table 5), 7,10,13-hexadecatrienoic acid, 11,14,17-eicosatrienoic acid, and 9,12,15-octadecatrienoic acid (α-linolenic acid) in *C. Vulgaris* MCCS 013 (Table 2).

Furthermore, important fatty acids like oleic acid, a monounsaturated (ω9) fatty acid in *S. rubescens* MCCS 018 (Table 5), 6,9,12-octadecatrienoic acid (γ-linolenic acid), a polyunsaturated (ω6) fatty acid in *D. salina* CCAP 19/18 (Table 4) and *S. rubescens* MCCS 018 (Table 5), 7,10,13-hexadecatrienoic acid, a polyunsaturated (ω3) fatty acid in *C. vulgaris* MCCS 013 (Table 2), 11,14,17-eicosatrienoic acid, a polyunsaturated (ω3) fatty acid in *C. vulgaris* MCCS 013 (Table 2) and 9,12,15-octadecatrienoic acid (α-linolenic acid), a polyunsaturated (ω3) fatty acid in *C. vulgaris* MCCS 013 (Table 2) were also identified.

This is evident that our analysis method can effectively lead us to identify various types of fatty acids and fatty acid methyl esters such as chained, branched, saturated, unsaturated, dioic, alkynoic acid and polyunsaturated ones.

The data in the above paragraph show that our method to analyse fatty acids from microalgae is a useful tool for the analysis of the fatty acids of microalgae. Furthermore the fatty acids of microalgae appear to be a rather useful marker contributing to the other means of microalgal chemotaxonomy.

Further testing will be required to elucidate the present fatty acids in the other strains of isolated microalgae from different habitats in Iran. It can be said that the obtained profiles in this study can be a demonstrative profile for fatty acids from microalgae isolated from the natural habitats in Iran. Also more studies should be done, qualitatively and quantitatively on the other strains collected from other habitats in Iran, to confirm this.

Table 6
Scenedesmus obliquus strain 019 fatty acids (FAs)/fatty acid methyl esters (FAMEs), they are classified on the base of their profiles identified in comparison to the Wiley library (Rodriguez-Garcia & Guil-Guerrero, 2008).

Systematic name	Common name	Formula	No. of carbon atoms	No. of double bond(s)	Fatty acid content (% total)	Family
Hexanoic acid	Caproic acid	$C_6H_{12}O_2$	6	0	0.3	Saturated FA
2-Hexenedioic acid		$C_6H_8O_4$	6	1	1.4	Monounsaturated FA
2,4-Hexadienedioic acid	Muconic acid	C6H6O4	6	2	0.5	Polyunsaturated FA
Nonanoic acid	Pelargonic acid	$C_9H_{18}O_2$	9	0	0.7	Saturated FA
Decanoic acid	Capric acid	$C_{10}H_{20}O_2$	10	0	0.4	Saturated FA
Undecanoic acid		$C_{11}H_{22}O_2$	11	0	27.2	Saturated FA
Dodecanoic acid	Lauric acid	$C_{12}H_{24}O_2$	12	0	7.2	Saturated FA
Methyl undecanoate		$C_{12}H_{24}O_2$	12	0	0.9	Branched saturated FAME
Tridecanoic acid		$C_{13}H_{26}O_2$	13	0	0.8	Saturated FA
Methyl dodecanoate	Methyl laurate	$C_{13}H_{26}O_2$	13	0	0.1	Branched saturated FAME
Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	14	0	29.8	Saturated FA
7-Hexadecenoic acid		$C_{16}H_{30}O_2$	16	1	8.6	Monounsaturated FA
Heptadecanoic acid	Margarinic acid	$C_{17}H_{34}O_2$	17	0	0.1	Saturated FA
Methyl hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	17	0	0.2	Branched saturated FAME
Methyl heptadecanoate		$C_{18}H_{36}O_2$	18	0	0.5	Branched saturated FAME
6-Octadecenoic acid		$C_{18}H_{34}O_2$	18	1	0.1	Monounsaturated FA
8-Octadecenoic acid		$C_{18}H_{34}O_2$	18	1	0.6	Monounsaturated FA
9,15-Octadecadienoic acid	Mangiferic acid	$C_{18}H_{32}O_2$	18	2	2.3	Polyunsaturated FA
10,13-Octadecadienoic acid		$C_{18}H_{32}O_2$	18	2	0.2	Polyunsaturated FA
12,15-Octadecadienoic acid		$C_{18}H_{32}O_2$	18	2	0.4	Polyunsaturated FA
6,9,12-Octadecatrienoic acid	γ-Linolenic acid or GLA	$C_{18}H_{30}O_2$	18	3	5.1	Polyunsaturated (ω6) FA
Nonadecanoic acid		$C_{19}H_{38}O_2$	19	0	0.2	Saturated FA
Heneicosanoic acid		$C_{21}H_{42}O_2$	21	0	0.1	Saturated FA
Eicosanoic acid	Arachidic acid	$C_{20}H_{40}O_2$	20	0	6.1	Saturated FA
Tetracosanoic acid	Lignoceric acid	$C_{24}H_{48}O_2$	24	0	3.2	Saturated FA

4. Conclusions

Briefly, it is suggested that microalgae are a good and unstudied candidates to be used as SCP as human food or animal feed, because of their high content of protein, fatty acids. We can easily cultivate them, their growth rate is high, their productivity is high, there is no risk for pathogenicity, their culture media is simple and inexpensive, and finally there are different habitats with different resources for finding and screening other strains of naturally isolated microalgae for SCP and lipid production. These microalgae have potential important applications for the pharmaceutical and food industries.

Acknowledgement

This work was supported by a grant from the Management and planning organisation, Fars Province.

References

Apt, K. E., & Behrens, P. W. (1999). Commercial developments in microalgal biotechnology. *Journal of Phycology, 35*, 215–226.
Becker, E. W. (2007). Microalgae as a source of protein. *Biotechnology Advances, 25*, 207–210.
Ben-Amotz, A., Fisher, R., & Schneller, A. (1987). Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. *Marine biology, 95*, 31–36.
Borowitzka, M. A., & Borowitzka, L. J. (1998). *Microalgal Biotechnology*. Cambridge: Cambridge University Press.
Butte, W. (1983). Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulphonium hydroxide for transesterification. *Journal of Chromatography, 261*, 142–145.
Castenholz, R. W. (2001). Cyanobacteria. In W. R. Castenholz & D. Boone (Eds.), *Bergey's manual of systematic bacteriology, Oxygenic photosynthetic bacteria. Phylum BX*. Baltimore: Williams and Wilkins.
Freshney, R. I. (1992). *Culture of animal cells, a practical approach* (2nd ed.). Oxford: Oxford University Press.
Ghasemi, Y., Faramarzi, M. A., Arjmand Inalou, M., Mohagheghzadeh, A., Shokravi, Sh., & Morowvat, M. H. (2007). Side-chain cleavage and C-20 ketone reduction of hydrocortisone by a natural isolate of *Chroococcus dispersus*. *Annals of Microbiology, 57*(4), 577–581.
Ghasemi, Y., Rasoul-Amini, S., Morowvat, M. H., Raee, M. J., Ghoshoon, M. B., Nouri, F., et al. (2008). Dehydrogenation, C-20 ketone reduction and side-chain cleavage of hydrocortisone in *Chlamydomonas reinhardtii* cultures. *Molecules, 13*, 2416–2425.
Guschina, I. A., & Harwood, J. L. (2006). Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research, 45*, 160–186.
John, D. M., Whitton, B. A., & Brook, A. J. (2003). *The freshwater algal flora of the British Isles an identification guide to freshwater and terrestrial algae*. Cambridge: Cambridge University press. pp. 39–43.
Kochert, G. (1978). Protein determination by dye binding. In J. A. Hellbust & J. S. Craigie (Eds.), *Handbook of phycological methods – physiochemical and biochemical methods* (pp. 91–93). Cambridge: Cambridge University Press.
Mahasneh, I. A. (1997). Production of single cell protein from five strains of the microalga *Chlorella* spp. (Chlorophyta). *Cytobios, 90*, 153–161.
Mohagheghzadeh, A., Faridi, P., & Ghasemi, Y. (2007). *Carum copticum* Benth. and Hook., essential oil chemotypes. *Food Chemistry, 100*, 1217–1219.
Mooney, B. D., Nichols, P. D., De Salas, M. F., & Hallegraeff, G. M. (2007). Lipid, fatty acid and sterol composition of eight species of Kareniaceae (Dinophyta): Chemotaxonomy and putative lipid phycotoxins. *Journal of Phycology, 43*, 101–111.
Müller, K. D., Husamann, H., & Nalik, H. P. (1990). A new and rapid method for the assay of bacterial fatty acids using high resolution capillary gas chromatography and trimethylsulphonium hydroxide. *Zentralblatt für Bakteriologie, 274*, 174–182.
Oleguin, E. J., Hernandez, B., Araus, A., Camacho, R., Gonzalez, R., Ramirez, M. E., et al. (1994). Simultaneous high-biomass protein production and nutrient removal using *Spirulina maxima* in sea water supplements with anaerobic effluents. *World journal of microbiology and biotechnology, 10*, 576–578.
Pereira, S. L., Leonard, A. E., Huang, Y. S., Chuang, L. T., & Mukerji, P. (2004). Identification of two novel microalgal enzymes involved in the conversion of

the ω3-fatty acids, eicosapentaenoic acid, into docosahexaenoic acid. *The Biochemical Journal, 384*, 357–366.
Petkov, G., & Garcia, G. (2007). Which are fatty acids of the green alga *Chlorella*? *Biochemical Systematics and Ecology, 35*, 281–285.
Prescott, G. W. (1962). *Algae of the western great lake areas*. Dubuque, Iowa: Brown Company Publisher.
Řezanka, T., Dor, I., Prell, A., & Dembitsky, V. M. (2003). Fatty acid composition of six freshwater wild Cyanobacterial species. *Folia Microbiologica, 48*(1), 71–75.
Rodriguez-Garcia, I., & Guil-Guerrero, J. L. (2008). Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. *Food Chemistry, 108*, 1023–1026.
Vargas, M. A., Rodriguez, H., Moreno, J., Olivares, H., Del Campo, J. A., Rivas, J., et al. (1998). Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. *Journal of Phycology, 34*, 812–817.