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Research Article

Growth and fatty acid profiles of microalgae species isolated from the Baja California Peninsula, México

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ABSTRACT. The growth rate, fatty acid profile and toxicity of 21 microalgae strains (Cyanophyceae (3), Chlorodendrophyceae (1), Xanthophyceae (1) and Bacillariophyceae (16)), isolated from coastal waters of Baja California (NW México), were determined. Cyanophytes *Komvophoron* sp. had the highest growth rates (μ = 2.98 divisions day^{-1}) and *Aphanocapsa marina* had the highest cell concentration (11.92x10^6 cell mL^{-1}). xanthophyte *Heterococcus* sp. had the largest cell size (193.12 µm) with lower growth rates (μ = 0.67 divisions day^{-1}). Fatty acid content differed according to taxonomic group. Diatoms contained the highest percentages of polyunsaturated fatty acids (n-3 and n-6 PUFAs), ranging from 23.4 to 60.7%, and eicosapentaenoic acid (EPA) was the most abundant. None of the residual media from the isolated microalgae strains were toxic to *Artemia franciscana* nauplii. We conclude that based on their growth rate, fatty acid profile, and lack of toxicity, all the isolated microalgae strains have the potential to be used alone or as part of a mixed diet for the culture of marine organisms.

Keywords: microalgae, growth rate, fatty acids profile, Baja California, México.

Crecimiento y perfiles de ácidos grasos de especies de microalgas aisladas de la Península de Baja California, México

RESUMEN. Se determinó la tasa de crecimiento, perfil de ácidos grasos y toxicidad de 21 cepas de microalgas (Cyanophyceae (3), Chlorodendrophyceae (1), Xanthophyceae (1) y Bacillariophyceae (16)), aisladas de aguas costeras de Baja California, NE de México. La cianófita *Komvophoron* sp. presentó la mayor tasa de crecimiento (μ = 2,98 divisiones día^{-1}) y *Aphanocapsa marina* tuvo la mayor densidad celular (11,92x10^6 cél mL^{-1}). La xantofícea *Heterococcus* sp. presentó el mayor tamaño celular (193,12 µm) con baja tasa de crecimiento (μ = 0,67 divisiones día^{-1}). El perfil de ácidos grasos difirió acorde al grupo taxonómico. Las diatomeas contienen los mayores porcentajes de ácidos grasos poliinsaturados (n-3 y n-6 PUFAs), variando de 23,4 a 60,7%, el ácido eicosapentaenoico (EPA) fue el más abundante. Ninguno de los medios residuales de las especies de microalgas aisladas resultó ser tóxico para nauplios de *Artemia franciscana*. Se concluye que basados en la tasa de crecimiento, perfil de ácidos grasos y su falta de toxicidad, todas las especies de microalgas aisladas tienen potencial para ser usadas como dietas monoespecíficas o mixtas para el cultivo de organismos marinos.

Palabras clave: microalgas, tasa de crecimiento, perfil de ácidos grasos, Baja California, México.

INTRODUCTION

Microalgae are used as live food in the early stages (larval and juvenile) of most cultured aquatic species (Renaud *et al.*, 1994; Brown, 2002). These microalgae must be supplied at the appropriate quantity, have the proper quality, shape, and size, and be non-toxic to the target organism (Hemaiswarya *et al.*, 2011).

One fundamental aspect in nutrition of marine organisms is the proximate composition and quantity of polyunsaturated fatty acids (PUFAs) of microalgae (Xu *et al.*, 2008; Guedes *et al.*, 2011). Eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA: 22:6n-3), members of the PUFA family (n-3) are essential for marine organisms because they promote growth and high survival rates and are also components

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of membranes and storage organelles (Cardozo *et al.*, 2007; Guedes *et al.*, 2011).

Close to 5000 species of microalgae have been isolated worldwide, but only 50 or 60 are used commercially as live food for aquaculture (Kyle, 1989; Parvin *et al.*, 2007). In the Baja California Peninsula there are few data on fatty acid profile of the endemic microalgae that are used as live food for aquaculture (Mercado *et al.*, 2004; Correa-Reyes *et al.*, 2009). Additionally, the native microalgae strains are acclimated to local environmental conditions and avoid the introduction of allochthonous species (Hemaiswarya *et al.*, 2011; Ratha *et al.*, 2012). Thus, the aim of this study was to isolate and characterize the growth, fatty acid content and toxicity of native microalgae species from Baja California Peninsula, México, to evaluate their potential to be used as food for marine organisms.

MATERIALS AND METHODS

Sampling sites

The microalgae strains were isolated from the coastal waters of Ensenada and San Quintín (Baja California), and Mulegé (Baja California Sur), México (Fig. 1). The water samples collected from 2010 to 2012 were used for the isolation of microalgae cells (Throndsen, 1978; Arangúren *et al.*, 2002; Andersen & Kawachi, 2005).

Isolation technique

Strains were isolated by serial dilution using a micropipette and agar streaking in tubes with 10 mL of "f" medium (Guillard & Ryther, 1962) with 2% agar. The species were identified based on their morphological characteristics, using a compound microscope (Olympus CX31) and taxonomic keys (Round *et al.*, 1990; Moreno *et al.*, 1996; Tomas, 1997; Siqueiros-Beltrones, 2002; Wehr & Sheath, 2003; Komárek & Anagnostidis, 2005; Arora *et al.*, 2013), and classified according to the Integrated Taxonomic Information System (ITIS).

Culture conditions

The isolated strains were transferred from 10 mL tubes to 250 mL flasks and grown as non-axenic and monospecific batch cultures (Stein, 1973) "f" medium (100 mL). The cultures were maintained in duplicate in a climatic chamber at 20 ± 1ºC (temperature of most shrimp farms in México range from 20 to 22ºC as has been described by López-Elías *et al.*, 2004), continuous light at 100 μE m^{-2} s^{-1} was provided by a cool white fluorescent light.

Growth rate and cell concentration

The cell concentration of each strain was quantified by direct cell count every 48 h during 10 days, using a hematocytometer (0.1 mm depth), and a compound microscope. The benthic diatoms samples were sonicated (3-6 min at 50 Hz) prior to the cell count. Cell concentration of filamentous cyanophytes was obtained based on the total filament count and the xanthophytes was estimated based on cluster counts of each colony, using a Fuchs Rosenthal chamber (0.2 mm depth) (Huarachi *et al.*, 2013; Moheimani *et al.*, 2013). These data were used to generate growth curves and calculate the growth rate (μ), by the following equation (Fogg & Thake, 1987):

$$\mu = \frac{\log_2(N_2) - \log_2(N_1)}{t_2 - t_1}$$

where N_1 and N_2 are the cell concentrations at initial day (t_1) and final day (t_2) respectively, measured during the exponential growth.

Dry weight

Total dry weight, organic dry weight, and ash content were measured as described by Sorokin (1973). Samples of each culture were collected at day 5 and passed through 47 mm glass fiber filters (Whatman GF/C). The filters were washed with 5 mL ammonium formate (3%) to remove salt residues, and oven-dried at 60°C until constant weight. Ash content was obtained by incineration at 490°C with a muffle furnace.

Fatty acid analysis

The isolated microalgae strains were cultured as non-axenic and monospecific maintained in 1-L Erlenmeyer flasks with 700 mL "f" medium and 100 mL of inoculum of each strain under the same culture conditions as for the growth assay. The cells were collected at day 5 and concentrated by centrifugation at 4000 rpm and freeze-dried at -50°C and 0.110 bar with a lyophilizer (Labconco Freezone 2.5).

The total lipid content from lyophilized samples (50 mg) of each microalgae strain was extracted according to Folch *et al.* (1957). Fatty acid methyl esters (FAMEs) were measured following to Metcalfe *et al.* (1966) and analyzed by a gas chromatograph (GC Agilent Technologies 7890A) with a 30 m length, 0.320 mm inner diameter, 0.25 μm film thickness capillary column (Agilent J&W, 123-3232 DB-FFAP), and flame ionization detector. Hydrogen was used as the carrier gas. The injection volume was 1 μL, and the initial temperature was 120ºC, which was increased to 230ºC and maintained for 4 min more.

FAMEs were identified, based on a comparison of their retention times with those of a commercial stan-

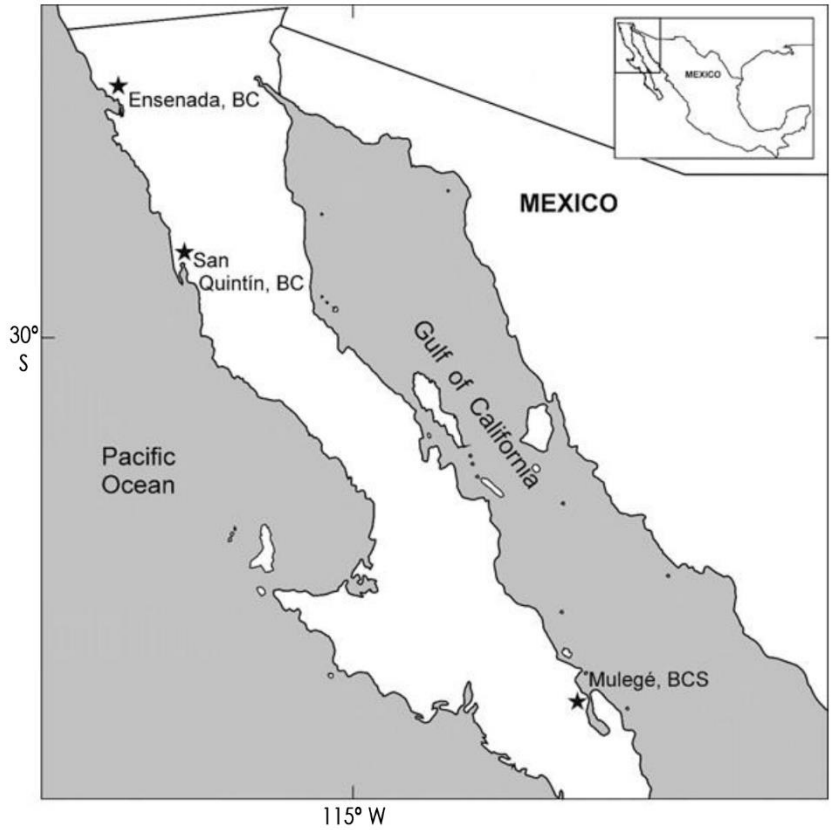


Figure 1. Map of sampling sites in Baja California (BC) and Baja California Sur (BCS), México.

dard (37 Component Supelco FAME Mix Sigma). The concentration of each fatty acid was calculated using ChemStation, B.04.01 software (Agilent, USA).

Aqueous extract and test toxicity test

To determine what microalga has potential as food in aquaculture, its toxicity to the organism for which it will be used as food must be measured. To this end, monospecific cultures of the isolated microalgae were maintained in duplicate 15 mL test tubes with 10 mL of "f" medium using the same culture conditions as described above. The cultures were harvested at stationary growth phase (day 12), to collect the extracellular products that accumulated and were passed through 47 mm glass fiber filters (Whatman GF/C) to obtain the cell-free liquid fraction (residual medium).

The toxicity of each microalgal strain was estimated by bioassay using *Artemia franciscana* nauplii (Vanhaecke *et al*., 1980) (Argentemia Golden, Grade 1). Nauplii were obtained, by cyst inducing of hatching and eliminating the alveolar layer (lipoproteins, chitin and haematin) by oxidation with 6% sodium hypochlorite bath for 5 min and rinsed with freshwater to remove the hypochlorite. The cysts were placed inside of a hatching cone with seawater (22‰) at 28ºC and constant aeration until hatching (22 h). Nauplii were collected by positive phototropism.

The toxicity assay was performed by quadruplicate for each aqueous extract using a 96 well plate with a lid. We placed 7 to 10 nauplii in each well in a final volume of 200 µL of each aqueous extract. Two controls (filtered seawater and "f" medium) were also established. All treatments were maintained for 24 h at 20ºC under continuous light (100 µE m^{-2} s^{-1}) without the addition of food. The initial and final number of the nauplii were used in each treatment to determine survival rate.

Statistical analysis

Differences in growth rate (µ), dry weight and fatty acids among microalgae species were examined by one-way analysis of variance (Zar, 1984). When significant differences were obtained a Tukey *a posteriori* test was used. All statistical analyses were performed using Statistica®, version 10.0 (Stat Soft Inc., 2011), and the level of significance was set to $P < 0.05$.

RESULTS

Isolation of strains

One-hundred and fifteen water samples were collected, and then 36 microalgae strains from Ensenada and San Quintín, Baja California, and Mulegé, Baja California Sur, México were initially isolated, from which 21 microalgae strains were finally chosen for this work, *i.e.*, those that grow in "f" medium, under the culture conditions previously described. The highest number of isolated species (11) was obtained from Ensenada samples (Table 1). The isolated species had various shapes and sizes and belonged to Cyanophyceae, Chlorodendrophyceae, Xanthophyceae, and Bacillariophyceae (Table 1). Most isolated species were Bacillariophyceae (16) (Table 1). The cell size of the isolated microalgae species ranged from 0.75 to 193.13 µm (Table 2). The cyanophytes had a cell size that ranged from 0.75 to 2.26 µm, the chlorophyte *Tetraselmis suecica* showed a cell size of 6.90 µm wide and 10.80 µm long, while the xanthophyte *Heterococcus* sp. had cell size of 13.14 µm wide and 193.13 µm long. The cell size among diatoms ranged from 2.80 to 13.64 µm wide and 3.65 to 16.85 µm long (Table 2).

Growth rate and cell concentration

Komvophoron sp. had the highest growth rate (µ) (2.98 divisions day^{-1}) ($P < 0.05$), while the diatom *Grammatophora angulosa* had the slowest growth (0.58 divisions day^{-1}) (Table 2, Fig. 2). The cyanophyte *Aphanocapsa marina* had the highest final cell concentration ($11.92x10^6$ cell mL^{-1}) ($P < 0.05$), while the diatom *Navicula* sp. (strain 1) had the slowest cell concentration ($0.13x10^6$ cell mL^{-1}) (Table 2, Fig. 2).

Dry weight

The total dry weight ($P < 0.05$), organic dry weight ($P < 0.05$) and ash content ($P < 0.05$) were significantly different among strains (Table 3). The diatom *Amphora* sp. (strain 5) had the highest values of total dry weight (395.6 pg $cell^{-1}$), organic dry weight (156.3 pg $cell^{-1}$) and ash content (239.3 pg $cell^{-1}$) (Table 3). The cyanophyte *Aphanocapsa marina* had the lowest values of total dry weight (1.6 pg $cell^{-1}$), organic dry weight (1.2 pg $cell^{-1}$) and ash content (0.4 pg $cell^{-1}$) (Table 3).

Fatty acid analysis

Among cyanophytes, which had higher percentages of saturated fatty acids, *Phormidium* sp. had a significantly higher ($P < 0.05$) percentage of 16:0 (47.8%), whereas *Aphanocapsa marina* contained the highest levels of 14:0 (29.5%) ($P < 0.05$) (Table 4). In the chlorophyte *Tetraselmis suecica* the higher percentages of fatty acids were 16:0 (30.3%), 18:3n-3 (24.1%), and 20:5n-3 (13.8%) (Table 4). The xanthophyte *Heterococcus* sp. had the following fatty acids as the most abundant: 16:0 (23.3%), 18:3n-3 (42.4%), 20:4n-6 (3.2%) (Table 4).

From all the diatoms, *Amphora* sp. (strain 4) had the highest ($P < 0.05$) 16:0 saturated fatty acid content (30.0%). The highest monounsaturated fatty acid levels ($P < 0.05$) were found in *Navicula* sp. (strain 4) in the form of 16:1n7 cis (46.8%). The principal polyunsaturated fatty acid ($P < 0.05$) was 20:5n-3 (39.6%) in *Amphora* sp. (strain 5) (Table 4).

The n-3 and n-6 PUFAs levels were elevated among the diatoms (23.4% to 60.7%). *Synedra* sp. had a high DHA: EPA ratio (0.2) and *Tetraselmis suecica* had a high EPA: ARA ratio (5.2) (Table 4).

Toxicity

The survival rate of *Artemia* nauplii in the residual media of all of the microalgae strains exceeded 94% (Table 5).

DISCUSSION

Growth rate and cell concentration

Brown (2002) recommended that microalgae cell size used as live food should be from 1 to 100 µm; therefore, the cell sizes of the isolated strains (except for *Heterococcus* sp.) were adequate for ingestion by filter feeders, fish and crustacean larvae. The isolated cyanobacteria *Aphanocapsa marina*, might be used to feed shrimp larvae of *Litopenaeus vannamei* as the cyanobacteria *Synechococus elongatus*, which forms chains of cells, that previously was used with this purpose (Moreno-Pérez & Sánchez-Saavedra, 2009), or to feed zooplankton like *Brachionus plicatilis*, that typically is fed with the Eustigmatophyceae *Nannochloropsis oculata* (Campa-Ávila & Sánchez-Saavedra, 2002).

The filaments of *Komvophoron* sp. consisted of small cells (1.29 µm by 2.26 µm), they had the highest growth rate (2.98 divisions day^{-1}) among the 21 microalgae isolated strains. The growth rate of *Komvophoron* sp. was higher compared with *Spirulina platensis* (0.11 to 0.92 divisions day^{-1}), which is a cyanobacteria that is commonly used in aquaculture, biotechnology, pharmaceutical and nutraceutical industries (Mexia-Bernal, 2011; Borowitzka, 2013).

The highest cell concentration ($11.92x10^6$ cell mL^{-1}) measured for the cyanophyte *Aphanocapsa marina* can be attributed to its cell size (0.75 µm of diameter). Reynolds (2006) proposed that cell concentration and growth rate depend on cell size and shape, *i.e.*, small cells have high surface: volume ratio and less complex

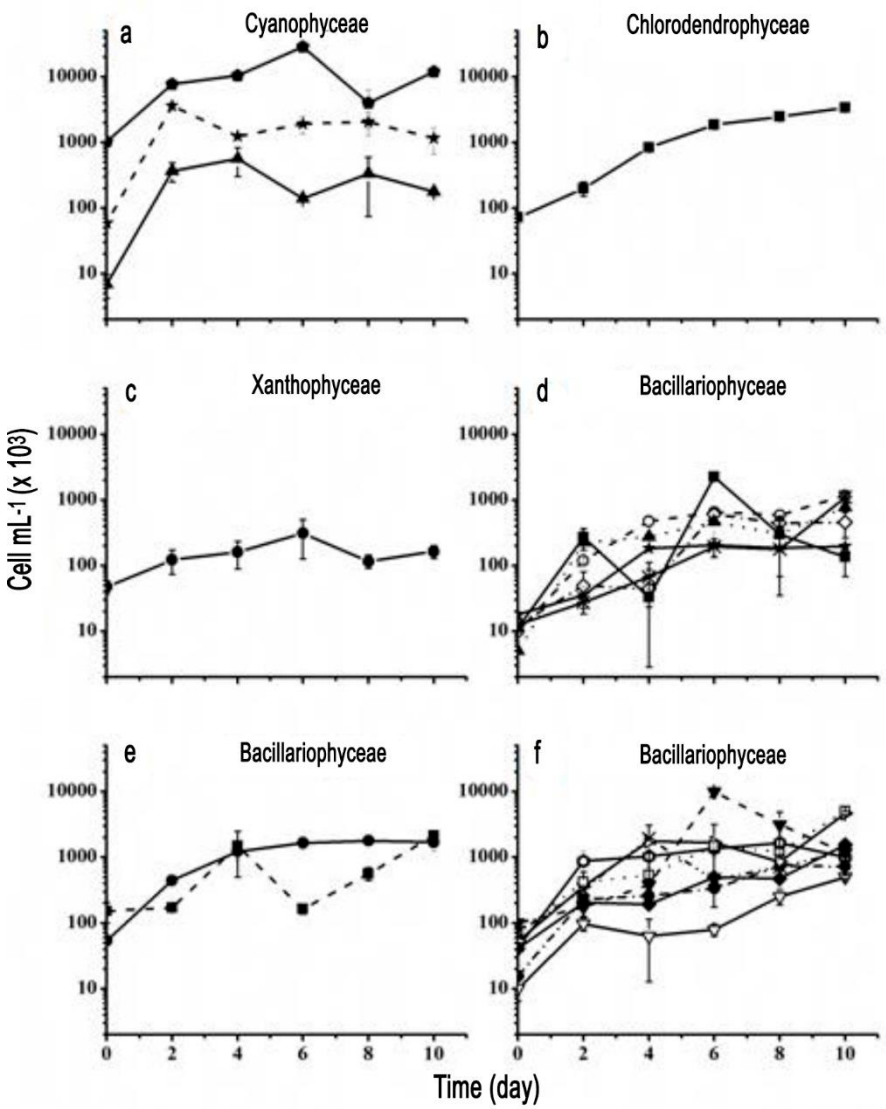
Table 1. Distribution of isolated microalgae species by taxonomic class.

Class	Species	Isolation site
Cyanophyceae	*Aphanocapsa marina* Hansgirg, 1890	Ensenada
Cyanophyceae	*Komvophoron* sp. Anagnostidis and Komárek, 1988	Ensenada
Cyanophyceae	*Phormidium* sp. Kützing ex Gomont, 1892	Ensenada
Chlorodendrophyceae	*Tetraselmis suecica* (Kylin) Butcher, 1959	Ensenada
Xanthophyceae	*Heterococcu*s sp. Chodat, 1908	Ensenada
Bacillariophyceae	*Amphora* sp. (strain 1) Ehrenberg ex Kützing, 1844	Mulege
Bacillariophyceae	*Amphora* sp. (strain 2) Ehrenberg ex Kützing, 1844	Mulege
Bacillariophyceae	*Amphora* sp. (strain 3) Ehrenberg ex Kützing, 1844	Mulege
Bacillariophyceae	*Amphora* sp. (strain 4) Ehrenberg ex Kützing, 1844	Mulege
Bacillariophyceae	*Amphora* sp. (strain 5) Ehrenberg ex Kützing, 1844	Ensenada
Bacillariophyceae	*Amphora* sp. (strain 6) Ehrenberg ex Kützing, 1844	Mulege
Bacillariophyceae	*Amphora* sp. (strain 7) Ehrenberg ex Kützing, 1844	Ensenada
Bacillariophyceae	*Cymbella* sp. (strain 1) C. Agardh, 1830	Mulege
Bacillariophyceae	*Cymbella* sp. (strain 2) C. Agardh, 1830	Mulege
Bacillariophyceae	*Navicula* sp. (strain 1) Bory de Saint-Vicent, 1822	Ensenada
Bacillariophyceae	*Navicula* sp. (strain 2) Bory de Saint-Vicent, 1822	Mulege
Bacillariophyceae	*Navicula* sp. (strain 3) Bory de Saint-Vicent, 1822	San Quintín
Bacillariophyceae	*Navicula* sp. (strain 4) Bory de Saint-Vicent, 1822	Ensenada
Bacillariophyceae	*Diploneis* sp. Ehrenberg ex Cleve, 1894	Ensenada
Bacillariophyceae	*Grammatophora angulosa* Ehrenberg, 1841	Ensenada
Bacillariophyceae	*Synedra* sp. Ehrenberg, 1830	San Quintín

Table 2. Cell size (µm), growth rate (µ: divisions day^{-1}), days in exponential growth (ED), and cell concentration (1x10^6 cell mL^{-1}) of isolated microalgae maintained in batch cultures.

Class and species	Cell size (µm)				µ	ED	Cell concentration	
	Lenght	Width	Diameter	Total length			Initial	Final
Cyanophyceae								
Aphanocapsa marina			0.75 ± 0.11		0.83 ± 0.19 b	0 - 6	1.03 ± 0.34	11.92 ± 0.91a
Komvophoron sp.	1.60 ± 0.37	1.62 ± 0.33		58.00 ± 13.51	2.98 ± 0.30 a	0 - 2	0.06 ± 0.02	1.16 ± 0.50def
Phormidium sp.	1.27 ± 0.29	2.26 ± 0.39		42.50 ± 20.98	2.32 ± 0.73 ab	0 - 2	0.01 ± 0.00	0.18 ± 0.02f
Chlorodendrophyceae								
Tetraselmis suecica	10.80 ± 0.88	6.90 ± 0.82			0.59 ± 0.05 b	0 - 6	0.07 ± 0.01	3.37 ± 0.40c
Xanthophyceae								
Heterococcus sp.	193.13 ± 41.69	13.14 ± 1.40			0.67 ± 0.13 b	0 - 6	0.05 ± 0.01	0.16 ± 0.04f
Bacillariophyceae								
Amphora sp. (strain 1)	14.37 ± 1.42	5.69 ± 1.17			0.66 ± 0.02 b	0 - 2	0.04 ± 0.01	1.00 ± 0.09def
Amphora sp. (strain 2)	14.36 ± 0.68	5.56 ± 1.11			0.88 ± 0.10 b	0 - 2	0.02 ± 0.01	0.73 ± 0.15ef
Amphora sp. (strain 3)	3.65 ± 0.20	2.84 ± 0.27			1.65 ± 0.09 ab	0 - 2	0.01 ± 0.00	0.50 ± 0.03ef
Amphora sp. (strain 4)	14.94 ± 1.22	5.58 ± 0.59			1.18 ± 0.03 ab	0 - 2	0.07 ± 0.01	1.20 ± 0.24def
Amphora sp. (strain 5)	15.55 ± 1.62	6.69 ± 1.60			0.63 ± 0.04 b	0 - 6	0.01 ± 0.00	1.10 ± 0.19def
Amphora sp. (strain 6)	5.70 ± 1.03	3.68 ± 0.39			0.68 ± 0.24 b	0 - 2	0.04 ± 0.03	1.55 ± 0.04de
Amphora sp. (strain 7)	13.83 ± 0.67	3.99 ± 0.58			2.04 ± 1.11 ab	0 - 2	0.01 ± 0.00	0.77 ± 0.10ef
Cymbella sp. (strain 1)	6.48 ± 0.45	3.00 ± 0.45			0.98 ± 0.06 ab	0 - 4	0.05 ± 0.00	4.67 ± 0.29bc
Cymbella sp. (strain 2)	12.59 ± 1.37	2.95 ± 0.67			0.74 ± 0.13 b	0 - 2	0.07 ± 0.03	5.00 ± 0.68b
Navicula sp. (strain 1)	6.77 ± 0.42	3.56 ± 0.54			2.16 ± 1.24 ab	0 - 2	0.01 ± 0.00	0.13 ± 0.07f
Navicula sp. (strain 2)	6.69 ± 0.74	2.80 ± 0.41			1.10 ± 0.04 ab	0 - 6	0.10 ± 0.01	1.17 ± 0.13def
Navicula sp. (strain 3)	6.61 ± 0.78	3.73 ± 0.40			0.79 ± 0.13 b	0 - 4	0.15 ± 0.05	2.14 ± 0.17cd
Navicula sp. (strain 4)	16.85 ± 1.64	4.93 ± 0.76			0.85 ± 0.21 b	0 - 4	0.01 ± 0.00	1.17 ± 0.07def
Diploneis sp.	10.21 ± 0.72	4.90 ± 0.37			1.79 ± 1.38 ab	0 - 2	0.01 ± 0.01	0.46 ± 0.19ef
Grammatophora angulosa	14.92 ± 2.76	13.64 ± 2.21			0.58 ± 0.04 b	0 - 4	0.02 ± 0.00	0.20 ± 0.00f
Synedra sp.	14.94 ± 1.22	5.58 ± 0.59			1.02 ± 0.21 ab	0 - 4	0.05 ± 0.00	1.69 ± 0.46de

structure, therefore do not need to invest resources in producing organelles, which allows them to have high rates of specific biomass production. *Komvophoron* sp. showed the highest growth rate (2.98 divisions day^{-1}), but not the highest cell concentration. That was because *Komvophoron* sp. was counted as the number of filaments grown, instead of the individual cells (1.60 µm long and 1.62 µm wide) by filaments (58.00 µm long).



a: Cyanophyceae: *Aphanocapsa marina* (−●−), *Komvophoron* sp. (- ★ -) and *Phormidium*sp. (−▲−).
b: Chlorodendrophyceae: *Tetraselmis suecica* (−■−).
c: Xanthophyceae: *Heterococcus* sp. (−●−).
d: Bacillariophyceae from Ensenada Bay: *Grammatophora angulosa* (−★−), *Navicula* sp. (strain 4) (- ○ -), *Diploneis* sp. (- ◇ -), *Amphora* sp. (strain 5) (- ■ -), *Navicula* sp. (strain 1) (−✳−) and *Amphora* sp. (strain 7) (· ▲ ·).
e: Bacillariophyceae from San Quintín Bay: *Navicula* sp. (strain 3) (- ▪ -) and *Synedra* sp. (−●−).
f: Bacillariophyceae from Mulege: *Amphora* sp. (strain 1) (−◇−), *Amphora* sp. (strain 2) (- ● -), *Cymbella* sp. (strain 1) (−▷−), *Amphora* sp. (strain 3), (· □ ·), *Cymbella* sp. (strain 2) (−▽−), *Amphora* sp. (strain 4) (- × -), *Amphora* sp. (strain 6) (−◆−) and *Navicula* sp. (strain 2) (- ▼ -).

Figure 2. Mean values and standard deviation of cell concentration (log_{10}) of isolated microalgae maintained in batch cultures.

Dry weight

The highest values of total dry weight, organic dry weight and ash content corresponded to *Amphora* sp. (strain 5), which is attributed to their silica cell walls. The lowest values of dry weight obtained for *Aphanocapsa marina* were attributed to the small cell size of this strain (0.75 µm of diameter).

The ash content of the some isolated species was higher than that reported in other studies for species that belong to the same taxonomic group (Lynn *et al*., 2000; Courtois de Viçose *et al*., 2012a, 2012b). Two reasons could account for this: 1) some authors indicated that certain microalgae accumulated minerals because of specific requirements (such as Cd, Cr, Cu and Fe), cau-

Table 3. Total dry weight, organic dry weight (pg cell^{-1}), and ash content of isolated microalgae species maintained in batch cultures. Letters indicate significant differences among strains (Tukey *a posteriori* test, $\alpha = 0.05$: a>b>c>d>e).

Class and species	Total dry weight	Organic dry weight	Ash content
Cyanophyceae			
Aphanocapsa marina	1.6 ± 0.1 d	1.2 ± 0.1 e	0.4 ± 0.1 b
Komvophoron sp.	39.8 ± 5.9 bcd	17.9 ± 6.6 e	21.9 ± 0.7 b
Phormidum sp.	64.6 ± 34.3 bcd	31.0 ± 19.8 de	33.6 ± 14.4 b
Chlorodendrophyceae			
Tetraselmis suecica	23.0 ± 0.7 cd	15.9 ± 0.7 e	7.1 ± 0.0 b
Xanthophyceae			
Heterococcus sp.	121.3 ± 26.5 bcd	90.2 ± 26.2 abcde	30.4 ± 0.2 b
Bacillariophyceae			
Amphora sp. (strain 1)	13.8 ± 0.4 cd	8.7 ± 1.8 e	5.2 ± 2.3 b
Amphora sp. (strain 2)	56.4 ± 13.1 bcd	34.6 ± 6.5 de	21.8 ± 6.6 b
Amphora sp. (strain 3)	185.6 ± 142.1 b	112.8 ± 94.2 abc	72.7 ± 48.0 b
Amphora sp. (strain 4)	14.7 ± 0.4 cd	4.5 ± 0.1 e	10.2 ± 0.5 b
Amphora sp. (strain 5)	395.6 ± 19.6 a	156.3 ± 13.4 ac	293.3 ± 6.2 a
Amphora sp. (strain 6)	163.4 ± 7.5 bc	131.9 ± 6.0 bc	31.5 ± 1.4 b
Amphora sp. (strain 7)	48.9 ± 20.3 bcd	29.6 ± 12.6 de	19.3 ± 7.7 b
Cymbella sp. (strain 1)	18.1 ± 11.0 cd	7.0 ± 4.2 e	11.1 ± 6.8 b
Cymbella sp. (strain 2)	21.1 ± 10.6 cd	12.1 ± 4.6 c	9.0 ± 6.0 cd
Navicula sp. (strain 1)	102.5 ± 37.1 bcd	47.9 ± 17.3 bcde	54.6 ± 19.8 b
Navicula sp. (strain 2)	84.2 ± 45.4 bcd	33.8 ± 6.7 de	50.4 ± 38.7 b
Navicula sp. (strain 3)	27.0 ± 15.2 cd	10.8 ± 5.2 e	16.2 ± 10.0 b
Navicula sp. (strain 4)	70.7 ± 20.6 bcd	37.1 ± 2.4 de	33.6 ± 18.2 b
Diploneis sp.	361.8 ± 57.2 b	138.1 ± 2.2 ab	223.7 ± 59.4 a
Grammathophora angulosa	79.9 ± 6.4 bcd	43.4 ± 2.7 ce	36.5 ± 3.7 b
Synedra sp.	101.6 ± 37.2 bcd	23.5 ± 8.8 de	78.2 ± 28.4 b

sing an increased ash content (31% to 71%) (Roger *et al.*, 1986; Roger, 2005; Vonshak, 1986) and, 2) the salts from the culture medium accumulated in the cells that were not removed by the ammonium formate solution (Zhu & Lee, 1997).

Fatty acid content

The fatty acid content varied among taxonomic groups, even within the same genus, such in the 7 species of *Amphora*, which had different fatty acids profiles. This can be attributed to species-specific responses to culture conditions, as previously described for other microalgae strains (Ying & Kangsen, 2005; Lang *et al.*, 2011; Chen, 2012).

The palmitic acid (16:0) is one of the first fatty acids that are synthesized by microalgae during the early stationary phase, in which the synthesis of reserve metabolites, such as lipids, begins (Go *et al*., 2012). In our study all isolated strains showed high palmitic acid content (5.8 to 45.4%) at early stationary phase.

The fatty acid profile in cyanophytes -high 16:0, 16:1n-7 cis, and 18:3n-3 content- was similar to that previously reported (Guedes *et al*., 2011; Lang *et al*., 2011; Scholz & Liebezeit, 2013). Fatty acid content differed between cyanophyte groups -the filamentous group had high α-linolenic acid content (13.4 to 37.5%), consistent with other studies (Zepka *et al*., 2007; Sharathchandra & Rajashekhar, 2011; Opris *et al*., 2013). Whereas *Aphanocapsa marina* (colonies of spherical cells) had a high percentage of palmitoleic acid (44.1%), this might be linked to their membrane lipid composition (Tedesco & Duerr, 1989; Sato & Wada, 2009).

The filamentous cyanobacteria had high content (30.9 to 43.9%) of polyunsaturated fatty acids, therefore this group can be used as food for juvenile shrimp such as *Penaeus monodon*, which has been previously fed with *Phormidium* sp., another filamentous cyanobacteria, that increased the growth rate and survivability of *Penaeus monodon* (Sivakumar *et al*., 2011). Additionally, the isolated strains of cyanobacteria could be used to obtain pigments, antioxidant compounds, and animal food supplements (Belay *et al.*, 1996; Abed *et al*., 2009; Varshney *et al*., 2015).

Table 4. Fatty acid composition (as percentage of total fatty acids) found in isolated microalgae maintained in batch cultures. Letters indicate significant differences among species by fatty acid (α = 0.05, Tukey test: a>b>c>d>e>f>g>h>i>j).

Fatty acids	Cyanophyceae			Chlorodendrophyceae	Xanthophyceae
	Aphanocapsa marina	*Komvophoron* sp.	*Phormidum* sp.	*Tetraselmis suecica*	*Heterococcus* sp.
Saturated					
14:0	29.5 ± 0.2 a			1.2 ± 0.0 i	6.7 ± 0.4 g
15:0					1.1 ± 0.1 c
16:0	21.5 ± 0.2 fg	32.0 ± 0.4 b	47.8 ± 2.0 a	30.3 ± 1.0 bc	23.3 ± 1.5 ef
18:0	2.0 ± 0.1 e	3.8 ± 0.1 b	2.3 ± 0.1 d	3.3 ± 0.1 c	
24:0					2.9 ± 0.3 b
Sum	53.0	35.8	50.1	34.8	34.1
Monounsaturated					
14:1n-5 cis	1.5 ± 0.1				
15:1n-5 cis					
16:1n-7 cis	44.1 ± 0.1 a	15.3 ± 0.1 gh	5.8 ± 0.1 ij	9.7 ± 0.2 hi	4.3 ± 3.4 j
18:1n-9	1.4 ± 0.1 j	4.9 ± 0.1 cd	13.2 ± 0.6 a	7.5 ± 0.2 b	3.2 ± 0.6 fg
22:1n-9					
Sum	47.0	20.3	19.0	17.2	7.4
Polyunsaturated					
18:2n-6 tra		6.4 ± 0.1 c	17.5 ± 0.6 a	6.4 ± 0.2 c	9.9 ± 0.6 b
18:3n-6					1.4 ± 0.2 bc
18:3n-3 (ALA)		37.5 ± 0.4 a	13.4 ± 0.5 c	24.1 ± 0.5 b	42.4 ± 6.3 a
20:2n-6				1.1 ± 0.0	
20:4n-6 (ARA)				2.6 ± 0.1 i	3.2 ± 0.1 i
20:5n-3 (EPA)				13.8 ± 0.4 fg	1.5 ± 0.4 h
22:6n-3 (DHA)					
Sum		43.9	30.9	48.0	58.5
Sum n-3 PUFA		37.5	13.4	37.9	43.9
Sum n-6 PUFA		6.4	17.5	10.1	14.5
Total		100	100	100	100
DHA:EPA					
EPA:ARA				5.2	0.5

Fatty acids	Bacillariophyceae				
	Amphora sp. (strain 1)	*Amphora* sp. (strain 2)	*Amphora* sp. (strain 3)	*Amphora* sp. (strain 4)	*Amphora* sp. (strain 5)
Saturated					
14:0	9.6 ± 0.3 f	10.3 ± 0.2 f	5.7 ± 0.5 g	6.4 ± 0.1 g	13.9 ± 0.1 de
15:0	8.5 ± 0.1 a	9.5 ± 0.2 a		4.4 ± 0.1 b	1.1 ± 0.1 c
16:0	23.0 ± 0.1 efg	22.3 ± 0.4 fg	27.0 ± 1.0 cd	30.0 ± 0.2 bc	13.7 ± 0.1 i
18:0			4.3 ± 0.3 a	1.5 ± 0.1 fg	1.1 ± 0.1 i
24:0			6.4 ± 0.1 a		
Sum	41.0	42.1	43.5	42.3	29.8
Monounsaturated					
14:1n-5 cis					
15:1n-5 cis	2.1 ± 0.1 bc	2.6 ± 0.1 ab			
16:1n-7 cis	22.3 ± 0.1 def	22.0 ± 0.4 defg	20.3 ± 0.4 efg	30.4 ± 0.2 bc	5.7 ± 0.1 ij
18:1n-9	4.3 ± 0.1 ce	3.6 ± 0.1 ef	5.2 ± 0.4 c	3.9 ± 0.1 def	3.7 ± 0.1 ef
22:1n-9					
Sum	28.6	28.1	25.5	34.4	9.4
Polyunsaturated					
18:2n-6 tra	1.7 ± 0.1 de	1.9 ± 0.1 d		2.0 ± 0.1 d	1.2 ± 0.1 f
18:3n-6	1.6 ± 0.1 bc	1.4 ± 0.1 bc		1.4 ± 0.1 bc	1.7 ± 0.1 b
18:3n-3 (ALA)			1.2 ± 0.1 d		
20:2n-6					

Continuation

Fatty acids	Bacillariophyceae				
	Amphora sp. (strain 1)	*Amphora* sp. (strain 2)	*Amphora* sp. (strain 3)	*Amphora* sp. (strain 4)	*Amphora* sp. (strain 5)
20:4n-6 (ARA)	5.7 ± 0.1 gh	6.0 ± 0.2 gh		5.1 ± 0.1 h	16.4 ± 0.1 a
20:5n-3 (EPA)	20.3 ± 0.2 df	19.5 ± 0.6 df	29.9 ± 0.5 b	14.8 ± 0.2 fg	39.6 ± 0.1 a
22:6n-3 (DHA)	1.0 ± 0.1 d	1.0 ± 0.1 d			1.8 ± 0.1 bc
Sum	30.3	29.7	31.1	23.4	60.7
Sum n-3 PUFA	21.3	20.4	31.1	14.8	41.4
Sum n-6 PUFA	9.0	9.3		8.6	19.3
Total	100	100	100	100	100
DHA:EPA	0.1				
EPA:ARA	3.5	3.3		2.9	2.4

Fatty acids	Bacillariophyceae				
	Amphora sp. (strain 6)	*Amphora* sp. (strain 7)	*Cymbella* sp. (strain 1)	*Cymbella* sp. (strain 2)	*Navicula* sp. (strain 2)
Saturated					
14:0	7.0 ± 0.7 g	12.4 ± 0.8 e	23.1 ± 1.4 b	15.6 ± 0.1 cd	5.8 ± 0.4 g
15:0	9.0 ± 0.7 a	1.1 ± 0.1 c	4.3 ± 0.7 b	4.6 ± 0.1 b	1.0 ± 0.1 c
16:0	20.0 ± 0.9 gh	14.3 ± 0.3 i	5.8 ± 0.9 j	7.2 ± 0.1 j	20.2 ± 0.1 gh
18:0	1.1 ± 0.1 i	1.7 ± 0.1 f	1.3 ± 0.1 gh	1.6 ± 0.1 f	2.1 ± 0.1 de
24:0					5.9 ± 0.3 a
Sum	37.1	29.5	34.6	29.1	35.1
Monounsaturated					
14:1n-5 cis					
15:1n-5 cis	2.1 ± 0.2 bc		2.9 ± 0.5 a	1.5 ± 0.1 c	1.9 ± 0.1bc
16:1n-7 cis	18.6 ± 1.5 fg	25.8 ± 1.0 cdef	24.8 ± 2.7 cdef	20.6 ± 0.1 efg	25.8 ± 0.1 cde
18:1n-9	5.0 ± 0.2 cd	4.5 ± 0.1 ce	1.6 ± 0.1 ij	2.4 ± 0.1 gi	2.2 ± 0.5 hi
22:1n-9					
Sum	25.7	30.3	29.3	24.5 ± 0.1	29.9
Polyunsaturated					
18:2n-6 tra	1.7 ± 0.1 de	1.2 ± 0.1 f		1.0 ± 0.1 f	
18:3n-6	1.2 ± 0.1 c	1.8 ± 0.1 b			
18:3n-3 (ALA)					
20:2n-6					
20:4n-6 (ARA)	6.9 ± 1.0 fgh	13.0 ± 0.7 bc	7.5 ± 1.6 eg	11.2 ± 0.1 cd	8.4 ± 0.2 ef
20:5n-3 (EPA)	25.8 ± 3.9 bd	24.2 ± 1.3 bde	28.6 ± 6.7 bc	31.8 ± 0.2 ab	24.6 ± 0.9 bde
22:6n-3 (DHA)	1.5 ± 0.2 c			2.3 ± 0.1 b	2.1 ± 0.2 b
Sum	37.1	40.2	36.1	46.4	35.0
Sum n-3 PUFA	27.3	24.2	28.6	34.1	26.7
Sum n-6 PUFA	9.8	16.0	7.5	12.3	8.4
Total	100	100	100	100	100
DHA:EPA	0.1			0.1	0.1
EPA:ARA	3.7	1.9	3.8	2.8	2.9

Fatty acids	Bacillariophyceae				
	Navicula sp. (strain 3)	*Navicula* sp. (strain 4)	*Diploneis* sp.	*Grammathophora angulosa*	*Synedra* sp.
Saturated					
14:0	4.0 ± 0.2 h	7.1 ± 0.1 g	15.1 ± 0.1 cd	17.1 ± 0.6 c	4.0 ± 0.2 h
15:0			4.2 ± 0.1 b		
16:0	26.1 ± 1.0 de	17.5 ± 0.3 h	13.3 ± 0.1 i	20.7 ± 1.1 fg	22.2 ± 0.3 fg
18:0	2.2 ± 0.1 de	2.1 ± 0.1 de	3.2 ± 0.1 c	1.2 ± 0.1 hi	1.4 ± 0.1 gh
24:0	5.3 ± 0.7 a				2.6 ± 0.1 b
Sum	37.7	26.6	35.8	38.9	30.2

Continuation

Fatty acids	Bacillariophyceae				
	Navicula sp. (strain 3)	*Navicula* sp. (strain 4)	*Diploneis* sp.	*Grammathophora angulosa*	*Synedra* sp.
Monounsaturated					
14:1n-5 cis					
15:1n-5 cis					
16:1n-7 cis	29.5 ± 1.1 bd	46.8 ± 1.1 a	20.2 ± 0.1 efg	9.6 ± 0.5 hi	34.5 ± 0.6 b
18:1n-9	2.5 ± 0.3 gh	1.4 ± 0.1 j	5.0 ± 0.1 cd	11.5 ± 0.7 a	3.5 ± 0.1 ef
22:1n-9		1.0 ± 0.1			
Sum	32.0	49.2	25.2	21.1	38.0
Polyunsaturated					
18:2n-6 tra			1.3 ± 0.1 ef	9.1 ± 0.6 b	1.2 ± 0.1 f
18:3n-6				4.4 ± 0.3 a	
18:3n-3 (ALA)	3.3 ± 0.1 d				
20:2n-6					
20:4n-6 (ARA)			14.1 ± 0.1 ab	14.6 ± 0.3 ab	9.6 ± 0.2 de
20:5n-3 (EPA)	27.0 ± 1.3 bd	24.2 ± 0.8	21.9 ± 0.1 cde	11.9 ± 0.2 g	18.0 ± 0.4 efg
22:6n-3 (DHA)			1.6 ± 0.1 c		3.0 ± 0.3 a
Sum	30.3	24.2	39.0	39.9	31.8
Sum n-3 PUFA	30.3	24.2	23.6	11.9	20.9
Sum n-6 PUFA			15.4	28.1	10.9
Total	100	100	100	100	100
DHA:EPA			0.1		0.2
EPA:ARA			1.6	0.8	1.9

The chlorophyte *Tetraselmis suecica* had a high level of palmitic acid (16:0), α-linolenic acid (ALA) (18:3n-3), and eicosapentaenoic acid (EPA) (20:5n-3), which are essential for nutrition of animals and humans (Brown, 2002; Patil *et al*., 2007; Guedes *et al*., 2011). In our study, the EPA content in *Tetraselmis suecica* was higher (13.8%) than that reported by Lourenço *et al*. (2002) for *Tetraselmis gracilis* (10.7%) during early stationary growth phase, which makes it a good source of EPA.

The xanthophyte *Heterococcus* sp. had a fatty acid profile similar to that observed in other xanthophytes by Patil *et al.* (2007) and Lang *et al.* (2011). *Heterococcus* sp. contained a low percentage of EPA (1.5%) and ARA (3.2%), and high levels of ALA (42.4%). ALA is important for animal nutrition, because it is a structural component of membrane lipids (Cardozo *et al*., 2007; Guedes *et al.*, 2011). There are few studies on fatty acids in xanthophytes group, making difficult the comparison of our results.

The high content of 14:0, 16:0, 16:1n-7, 20:4n-6, and 20:5n-3 fatty acids observed in diatoms is consistent with those reported for the diatom group during early stationary growth (Ying & Kangsen, 2005; Chen, 2012).

Diatoms had a high percentage of PUFAs (n-3 and n-6), particularly *Amphora* sp. (strain 5) had high levels of eicosapentaenoic acid (EPA) (39.6%), which were higher than the percentages obtained by Correa-Reyes *et al.* (2009) for various benthic diatoms that were used to feed red abalone postlarvae. The PUFA content varied among species, possibly because of differences in the distributions and accumulation of lipid classes in their intracellular structures (Ying & Kangsen, 2005; Lv *et al*., 2010; Chen, 2012).

Toxicity

Because of their easy hatching from dry cysts and their year-round availability, nauplii of the brine shrimp *Artemia salina* are the most convenient organisms for toxicity testing (Sorgeloos *et al*., 1978), therefore these organisms were used in this work. The residual media used from each strain evaluated here, did not contain toxic or inhibitory compounds for *Artemia* nauplii, thus none of the isolated microalgae residual media were considered toxic.

The production of toxic compounds depends on the taxonomic group (Ortega *et al*., 2007; Van Apeldoorn *et al*., 2007). In our study, we isolated cyanobacteria species that can produce toxins, but the toxicity tests performed on *Artemia* nauplii, showed that none of the isolated species were toxic. The production of toxic compounds from microalgae also depends on environmental factors such as temperature, pH, salinity, light intensity, and nutrient concentrations (Carballo *et al*., 2003; Scholz & Liebezeit, 2012). Ross *et al.* (2006)

Table 5. Survival of *Artemia franciscana* nauplii after toxicity test and cells concentration of species of microalgae used for aqueous extracts.

Strains and treatments	Cell concentration x10^5 by mL	Survival (%)
Medium "f"		100
Sea water		97
Cyanophyceae		
Aphanocapsa marina	147.38	100
Komvophoron sp.	9.13	100
Phormidium sp.	0.49	94
Chlorodendrophyceae		
Tetraselmis suecica	18.44	100
Xanthophyceae		
Heterococcus sp.	2.51	100
Bacillariophyceae		
Amphora sp.(strain 1)	9.38	100
Amphora sp. (strain 2)	6.13	100
Amphora sp. (strain 3)	0.69	100
Amphora sp. (strain 4)	6.00	100
Amphora sp. (strain 5)	2.75	100
Amphora sp. (strain 6)	11.06	100
Amphora sp. (strain 7)	153.06	100
Cymbella sp. (strain 1)	0.79	100
Cymbella sp.(strain 2)	8.94	100
Navicula sp. (strain 1)	0.30	100
Navicula sp. (strain 2)	3.19	95
Navicula sp. (strain 3)	3.63	97
Navicula sp. (strain 4)	2.63	100
Diploneis sp.	2.50	97
Grammatophora angulosa	1.70	97
Synedra sp.	6.03	100

reported that the toxicity of *Microcystis aeruginosa* incremented to 90% when the cyanophyte was stressed as the salinity increased. However, the culture conditions that were used to grow the isolated microalgae strains were the standard used for the production of microalgae cultures under controlled environments, as described by Andersen (2005).

Therefore, the growing conditions were adequate, since did not stress the microalgae isolates, and did not promote production of toxic compounds. In addition to the fatty acid profiles and high growth rates reported here, these microalgae can be easily grown, making them good candidates for applications in aquaculture from an economic perspective.

Aquaculture applications

The isolated strains obtained on this work offer to local aquaculture farms the possibility to use native microalgae strains with potential to be used as food alone or in mixed diets for the farmed organisms. The isolated strains are acclimated to the local environmental conditions of Baja California and therefore offer the opportunity of avoiding the introduction of allochthons strains.

In general, the fatty acids profiles in the isolated microalgae strains were similar to what has been reported in previous studies (Lourenço, 2002; Correa-Reyes *et al.*, 2009; Guedes *et al*., 2011). All isolated strains had a high content of PUFAs, which are essential for zooplankton, fish larvae, and crustaceans (StØttrup & Lesley, 2003; Patil *et al.*, 2007; Guedes *et al*., 2011; Hemaiswarya *et al*., 2011).

Data reported here is a preliminary study for the selection of microalgae species, and to evaluate their potential uses in aquaculture. The nutritional value of microalgae isolated and ingested by the organisms under culture, needs to be considered in a second part of the work.

In conclusion, the growth of the microalgae strains that we isolated differed among species in the same taxonomic class, based on their capacity to adapt to the culture conditions and the culture medium. The differences in fatty acid composition among species were affected by species-specific responses to the culture conditions and the type of lipid in their structure. Most of the isolated microalgae strains had proper DHA, ARA and EPA levels, which are important to the aquaculture of marine organism, thus the microalgae isolated and partially characterized in this work, can be used alone or as part of a mixed diet to feed invertebrate larvae or mollusk.

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