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Biotechnological potential of microalgae from lake Chapala, Mexico

Potencial biotecnológico de microalgas del lago de Chapala, México

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Abstract

During the months of January, May and September 2012, samples of microalgae and cyanobacteria were obtained at 10 stations located in Lake Chapala, to isolate and maintain axenic monoalgal cultures and achieve sufficient biomass to perform an approximate chemical analysis of total lipids and proteins. Various nutrient media were used for the cultures, based on inexpensive formulations and others of reagent grade. To obtain the algal biomass, a Millipore equipment and GF/C filters with a 45 µm pore and 25 mm diameter were used. Lipids were extracted using the Folch technique (1957) and proteins using the Bradford method (1976). Of 10 isolated species, five were cyanobacteria and five chlorophytes; lipid production was higher in this last group, with *Monoraphidium tortile* having the highest percentage of this metabolite (22.9%), while Cyanobacteria *Phormidium* sp. outperformed all cultivated species with 17% protein. Likewise, the modified culture medium of RM6 was efficient in the production of biomass for cyanobacteria and the CHU10 medium for chlorophytes; Both media were prepared with commercial salts to reduce production costs.

Algal biomass, Proteins, Lipids

Resumen

Durante los meses de enero, mayo y septiembre de 2012, se obtuvieron muestras de microalgas y cianobacterias en 10 estaciones ubicadas en el de Lago de Chapala, para aislar y mantener cultivos monoalgales axénicos y lograr suficiente biomasa para efectuar un análisis químico aproximado de lípidos totales y proteínas. Se emplearon diversos medios nutritivos para los cultivos, basados en formulaciones económicas y otras de grado reactivo. Para obtener la biomasa algal se empleó un equipo Millipore y filtros GF/C de 45 µm de poro y 25 mm de diámetro. Los lípidos se extrajeron mediante la técnica de Folch (1957) y las proteínas utilizando el método de Bradford (1976). De 10 especies aisladas, cinco fueron cianobacterias y cinco clorofitas; la producción de lípidos fue mayor en este último grupo teniendo a *Monoraphidium tortile* con el mayor porcentaje de este metabolito (22.9%), mientras que la Cyanobacteria *Phormidium* sp., superó a todas las especies cultivadas con 17% de proteínas. Así mismo, el medio de cultivo modificado de RM6, fue eficiente en la producción de biomasa para cianobacterias y el medio CHU10 para clorofitas; ambos medios fueron preparados con sales comerciales, para abatir costos de producción.

Biomasa algal, Proteínas, Lípidos

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Introduction

Mass cultures of microalgae and cyanobacteria in the world are a promising source of renewable energy, being highly efficient and fast-growing microorganisms, they produce biomass with proteins rich in essential amino acids and lipids of high value in polyunsaturated fatty acids, besides also containing pigments, carbohydrates, minerals and vitamins (Acién Fernández *et al*. 2018; Galarza, 2019).

Microalgae and cyanobacteria possess an incredible biochemical system, which is why they have been used for several decades, as a commercial basis for value-added products. Currently, other applications are being explored as promising solutions for environmental bioremediation, food, pharmaceutical industry, and biofuel production, among many others (Montero *et al*. 2012; Camacho Aguilar and Flórez-Castillo, 2020; Rojo Gómez, 2022). These organisms are the primary link in the food chain and, by photosynthesizing, provide 50% of the oxygen on the earth. An important characteristic of microalgae is that they are capable of growing in any environment rich in nitrogen and phosphorus, these, plus CO_2, being the main source of nutrients for their growth (Rojo Gómez, 2022).

The world production of algal biomass exceeds four thousand tons per year and tends to increase, on the one hand, because they do not compete with arable land and can be obtained with different sources of nutrients such as wastewater and agricultural fertilizers (Bitog *et al*. 2009), in addition to the various applications they have in the biotechnological area (Jiménez Escobedo and Castillo Calderón, 2021). Microalgae are also used in improving the environmental and economic sustainability of certain processes due to their ability to mitigate CO_2 emissions (Chisti, 2007; Galarza, 2019; Méndez Ancca *et al*., 2022). In short, microalgae and cyanobacteria could satisfy, in a more natural way, many of the human needs of today's globalized society.

Among the various studies carried out in our country to take advantage of phytoplankton from inland waters and study their biotechnological potential is that of Garduño-Solórzano *et al*. (2011) in Lake Catemaco, Veracruz, and in Laguna de Términos, Campeche.

Likewise, fertilizer-based crops have been experimented to reduce production costs, taking advantage of the nutrient absorption capacity of microalgae (Nieves-Soto, 1994; Valenzuela-Espinoza *et al*., 2005; Piña *et al*., 2007; Ortega-Salas and Reyes-Bustamante, 2012). The purpose of the present study was to isolate and culture phytoplanktonic species from Lake Chapala, determine the total lipid and protein content to define their potential exploitation.

Materials and method

Study area

Lake Chapala is the largest lake in Mexico and is considered a polymictic tropical lake, with a certain degree of eutrophication (Lind, *et al*., 1992). It is located in western Mexico at 1524 masl, between 20° 7' - 20° 21' LN and 102° 40' 45"-103° 25' 30" LW (Estrada *et al*., 1983). Its depth varies according to the interannual rainfall cycle, with the greatest depth in September and October (Guzmán and Orbe, 2002). The climate of the region is warm-sub-humid with summer rains, corresponding to the Awo(e) subtype, according to the Köppen classification modified by García (1989). Total annual precipitation is 875.2 mm. The average annual temperature is 19.9° C, the maximum between 27° and 30° C (May to June) and the minimum from December to February between 9° and 12° C. Total annual evaporation is 1912 mm (INEGI, 2010).

Field work

During the months of January, May and September 2012, microalgae and cyanobacteria samples were obtained from ten sites in Lake Chapala (Figure 1). Surface and two-meter depth trawls were conducted in a boat with an outboard motor, for one minute, using a conical net of 30 µm mesh size.

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Figure 1 Location of sampling stations in Lake Chapala, Mexico

Samples were stored in one-liter containers. 250 ml were fixed with lugol for taxonomic determination and the rest was kept in a cooler until analysis at the Laboratory of Marine Ecosystems and Aquaculture (LEMA) of the University of Guadalajara.

Isolation and monoalgal culture

To obtain a monoalgal and axenic culture as far as possible, the method of successive dilutions (Figure 2) and agar-agar plates was used (Richmond, 1986; Andersen, 2005).

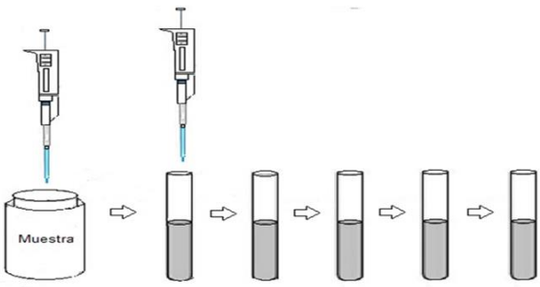

Figure 2 Isolation technique by serial dilutions

Different alternative media (modified RM6, CHU10 and Zarrouk), and formulated with reagent grade salts (F/2 and Bristol) were used. Ambient temperature was maintained between 25 and 28 °C. Illumination of the cultures was constant for 24 h of the day at an intensity of approximately 45 µmol m-2.s-1. Samples were collected from bulk cultures of 18 l capacity carboy, harvested at their exponential phase. They were filtered from 2 to 10 l, using Whatman GF/C filters of 0.45 µm pore size and 25 mm diameter on a Millipore system. The biomass was extracted from the filter, placed in 1.5 ml Eppendorf tubes and frozen at -20 °C for subsequent proximate chemical analysis.

Proximate chemical analysis (AQA)

Protein Determination

Proteins were obtained using the method of Bradford (1976). Fifty mg of biomass was weighed in 2 ml Eppendorf tubes; adding 500µl of 0.1N NaOH as buffer. It was then homogenized in an Ultraturrax (ultrahomogenizer) for 3 minutes at 10,000 rpm. The standard protocol suggested by BIO-RAD brand was used to determine protein concentrations in the range of 20-150 µg protein, 2 stock samples were made, to make 5 replicates of each with 5 µL of sample in microplates and 250 µL of Coomassie Brilliant Blue G-250 dye was added to each, the samples were read in a THERMO Multiskan Ascent Elisometer for reading with a 595 nanometer filter, the elisometer program shows results by correlating the amount of known protein and its absorbance value, it is calculated by the absorbance value of the samples to know the amount of protein present in this.

Lipid Determination

Lipids were extracted using the Folch (1957) technique. We weighed 150 µg of algal biomass, in 13 x 100 tubes with bakelite stopper in an analytical balance (Sartorius capacity of 250g and precision of 0.0001g), adding 5 ml of Chloroform-Methanol (C:M) 2:1 solution with BHT (Butyl hydroxytoluene) at 0. 01 % (C:M BHT), then they were homogenized with an Ultraturrax at 20,000 rpm for 4 min, rinsing the blade of the ultrahomogenizer with 5 ml of C:M, to this homogenate was added 2 ml of KCl at 0. 88 % and was centrifuged at 2000 rpm in a centrifuge (Hermle Z233 Mk-2 brand) for 6 min. 2 phases were obtained, the upper one is removed and the lower one is extracted with a long-stemmed pipette, filtered with blotting paper soaked with chloroform and potassium chloride (KCl) powder (to sequester excess water in the extract) to 13 x 100 mm glass tubes previously labeled and weighed. They were allowed to stand for 24 h in the freezer at a temperature of -4 °C. The extract was immersed in a water bath (34 °C) and evaporated to dryness with nitrogen. The tubes with lipids were transferred to a desiccator with silica gel for 1.5 h to eliminate moisture and were subsequently weighed and quantified with respect to the sample obtained. Three replicates were made per species.

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Moisture

Moisture was determined according to the method proposed by AOAC (1980). The wet weight was obtained by gravimetry using an analytical balance (Sartorius). The weights of the empty glass slides were recorded, then 1 g of the wet sample was placed on the slide and the weights were noted, finally the samples were kept at 100°C for 24 h, after which time they were removed from the oven and placed for one hour in a silica gel desiccator, the moisture content was calculated as the weight lost from the sample during drying with the following formula:

Where:

Pi = Initial weight.

Pf = Final weight.

Ash

The ash content indirectly indicates the amount of minerals present in the sample. The method to obtain the percentage of dry ash was carried out according to the methods proposed by the AOAC (1980), the porcelain crucibles were weighed and recorded separately in an analytical balance that remained 24 h in a silica gel desiccator and later the crucible was weighed together with the sample. Approximately 0.1 g of sample was carbonized in the crucible and subjected to 550° C for a period of 8 h, in a muffle furnace model SX2 -2.5 - 12N, removed from the furnace and cooled in a silica gel desiccator for a couple of hours, then weighed on the analytical balance to record, the weight and the percentage of ash obtained using the following formula:

Where:
%CbH= Percent Ash on Dry Basis.

Mc = Weight of ash sample.

Mh = Weight of wet sample.

Data Analysis

Results

Ten species with biotechnological potential were isolated, five cyanobacteria and five chlorophytes. In general, cyanobacteria recorded a higher biomass production according to absorbance values than chlorophytes. In *Chlorella vulgaris*, the culture media with the highest efficiency in algal biomass production according to absorbance values were CHU10 and Zarrouk, showing no significant differences between them, according to the ANOVA test p >0.05 (Table 1). The culture media with the lowest efficiency in biomass production were F/2 and Bristol.

Days	F/2	Bristol	CHU 10	Zarrouk	RM6 modified	P
1	0.199 ±0.09^{b}	0.189±0.02^{b}	0.486±0.07^{a}	0.156±0.06^{b}	0.156±0.04^{b}	<0.001
3	0.295±0.08^{b}	0.236±0.05^{b}	0.628±0.03^{a}	0.42±0.05^{c}	0.419±0.06^{c}	<0.014
4	0.317±0.03^{b}	0.262±0.03^{b}	0.661±0.04^{a}	0.438±0.08^{c}	0.431±0.02^{c}	<0.001
6	0.347±0.05^{b}	0.317±0.02^{b}	0.705±0.07^{a}	0.539±0.09^{c}	0.486±0.01^{c}	<0.001
9	0.351±0.07^{b}	0.261±0.05^{b}	0.700±03.09^{a}	0.629±0.07^{c}	0.502±0.04^{c}	<0.001
10	0.404±0.05^{b}	0.293±0.09^{b}	0.873±0.08^{a}	0.795±0.05^{a}	0.661±0.03^{b}	<0.001
12	0.457±0.08^{b}	0.296±0.04^{b}	0.806±0.07^{a}	0.864±0.07^{a}	0.669±0.07^{b}	<0.001
15	0.499±0.02^{b}	0.291±0.06^{b}	0.848±0.05^{a}	0.905±0.04^{a}	0.703±0.08^{b}	<0.001
16	0.518±0.06^{b}	0.268±0.03^{b}	0.902±0.09^{a}	0.68±0.06^{a}	0.68±0.09^{b}	<0.001
17	0.583±0.03^{b}	0.258±0.08^{b}	0.995±0.03^{a}	1.071±0.04^{a}	0.774±0.02^{b}	<0.001
	0.3970	0.2671	0.7604	0.6497	0.5481	

Table 1 Absorbance of *Chlorella vulgaris* biomass tested on five culture media, F/2, Bristol, CHU 10, Zarrouk and modified RM6. Subscripts with the same letter are not different

The most appropriate media for biomass production, macronutrient content, salts and low cost were CHU10 and modified RM6, since these were prepared by replacing the technical and reactive grade salts with fertilizers, observing very good results in terms of biomass production, probably due to the availability of nutrients and better adaptation of microalgae and cyanobacteria to the salts of the new medium, where it is observed as in the case of green algae such as *Chlorella vulgaris* (Table 1), the comparison between the growth of the different culture media Zarrouk and CHU10 are very similar for biomass development and have no statistical differences in the final phase of exponential growth.

Table 2 shows the results of proximal analysis of lipids and proteins in the 10 isolated species. In the Chlorophyta Division, the highest amount of lipids can be seen for the species *Monoraphidium tortile* with a percentage of 22.9% followed by *Chlorella vulgaris* (20.5%), *Desmodesmus quadricaudatus* and *S. obliquus* with a similar amount of lipids (17.7%), the lowest amount being *Desmodesmus acutudesmus* with 14.8%.

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Regarding proteins, the species with the highest amount was *Desmodesmus quadricaudatus*, with 14.5%, and those with the lowest amount were *Chlorella vulgaris* with 10.5% and *Monoraphidium tortile* with 2.78%. The highest moisture content was found in *Monoraphidium tortile* and the lowest in *Desmodesmus quadricaudatus*. The ash content was highest for *Chlorella vulgaris* and lowest for *Monoraphidium tortile.*

Group	Parameters			
Cyanobacterias	Lipids	Proteins	Humidity	Ashes
Aphanomenon flos-aquae	7.6 ± 1.11	5.4±0.16	86.6±1.54	3.2±0.2
Pseudanabaena cf *papillaterminata*	10.5±1.0	6.0±0.03	84.9±1.38	3.4±0.28
Planktolyngbya cf *limnetica*	4.4±0.81	8.7±4.3	87.1±3.26	4.6±0.19
Leptolyngbya sp.	10.0 ±1.93	13.4±0.09	77.1±1.94	1.7±0.05
Phormidium sp.	10.4±7.6	16.9±0.39	71.9±0.97	4.02±0.33
Chlorophyta				
Desmodesmus acutudesmus	14.8±3.66	13.8±0.3	72.9±0.09	2.9±0.09
Desmodesmus quadricaudatus	17.7±2.29	14.5±0.04	67.7±0.98	4.4±0.04
Scenedesmus obliquus	17.1±1.70	13.0±0.11	70.2±1.35	2.9±0.65
Chlorella vulgaris	20.5±0.63	10.5±0.69	69.3±0.17	4.5±0.89
Monoraphidium tortile	22.9±0.72	2.7±0.28	75.2±0.11	2.2±0.28

Table 2 Approximate chemical analysis of microalgae and cyanobacteria isolated from Lake Chapala in percent dry weight

According to the percentages of proteins and lipids obtained in the present study, a decrease in the values could be observed with respect to other authors. In the case of *Leptolyngbya sp*. and *Phormidium sp*. species, they registered the highest percentage of proteins (13.4%) with respect to the other cyanobacteria studied, being a low value compared to that reported by Taton *et al*. (2012) of 35.4% in microalgae cultivated in conventional media. Even so, our results are considered acceptable and are associated with the stress caused by lack of photoperiod in the cultures, since they were maintained under continuous light conditions 24 hours a day. The same case is handled for *Phormidium sp*., which obtained a total of 16.9%, compared with other studies that have reported percentages of 29.9 to 40.7% (Jonte *et al*., 2003). Regarding the species *Pseudanabaena cf papillaterminata*, there is little biochemical characterization data regarding its lipid and protein content, so as in the case of lipids we will take its synonym, of the genus *Phormidium*, mentioned above, where the amount of proteins reported is very high compared to that obtained of 6.02% for this study.

Table 3 shows the potential use of some species isolated in this study.

Species	Biotechnological potential	Bibliographic reference
Chlorella vulgaris	Obtaining proteins, lutein, and immunonutrition, obtaining long chain lipids, bioremediation.	Bich *et al.*, 1999; Cleber *et al.*, 2006 ; Morris *et al.*, 2009; Chader *et al.*, 2011
Scenedesmus sp.	Live feed in aquaculture, wastewater bioremediation and lipid production.	Abalde *et al.*, 1995; Chisti, 2007; Badwy *et al.*, 2008
Monoraphidium tortile	Biodiesel production Aquaculture feedstock	Bogen *et al.*, 2013
Aphanomenon flos-aquae	Nitrogen fixer	Mayz-Figueroa, 2004
Pseudonabaena sp.	Potential source of pigments phycocyanin, chlorophyll-a and carotenoids, exopolysaccharides, phycobiliproteins protect against liver damage and oxidative stress caused by Hg2+, Fertilizer, Biohydrogen	Moreno, 2000, Gallardo *et al.*, 2010
Leptolyngbya sp.	Beauty *et al.*, 2003	Belleza *et al.*, 2003
Planktolyngya sp.	Biofuel production	Chinnasamy, 2010
Phormidium sp.	Antibiotics, chlorophyll-a, phycocyanin and protein production	Torres-Ariño, 2004; Jonte, *et al.*, 2013

Table 3 Biotechnological applications of the isolated microalgae and cyanobacteria

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Conclusions

The culture media with the highest biomass production for cultured cyanobacteria and chlorophytes and the lowest formulation cost were modified RM6 and CHU10.

The content of primary metabolites such as total lipids and proteins in cyanobacteria and chlorophytes are within the range reported by other authors.

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The species *Monoraphidium tortile* (Chlorophyta) is suggested for the production of total lipids and for proteins the species *Phormidium sp*. (cyanobacteria).

Desmodesmus quadricaudatus culture is suggested as a balanced microalgae for lipid and protein production.

The environmental conditions of cultivation during the experiments are the minimum necessary to obtain biomass, total lipids and proteins susceptible to be used in the biotechnological area.

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