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PROSPECTING THE FIRST CULTURE COLLECTION OF ALGAE FROM THE PERNAMBUCO SEMIARID, BRAZIL

PROSPECÇÃO DO PRIMEIRO BANCO DE CEPAS ALGAIS DO SEMIÁRIDO PERNAMBUCANO, BRASIL

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Resumo Os organismos fitoplanctônicos desempenham um papel importante nos ecossistemas aquáticos, principalmente por serem produtores primários e consequentemente a base da cadeia alimentar. O presente trabalho objetivou isolar e comentar as espécies das comunidades fitoplanctônicas nativas de quatro reservatórios no semiárido pernambucano, disponibilizando-os para experimentos que possam avaliar o potencial biotecnológico e suprir as demandas da região. As coletas ocorreram em corpos de água localizados no semiárido pernambucano e a identificação dos táxons ocorreu com base em caracteres morfológicos utilizando literatura específica. Foram identificados 21 táxons, sendo 8 da classe Cyanophyceae, 10 da classe Chlorophyceae e apenas 1 das classes Bacillariophyceae, Euglenophyceae e Dinophyceae. A bioprospecção de microalgas no semiárido pernambucano tem possibilitado a formação de um banco de cepas importante para estudos ficológicos e obtenção de biomassa de espécies com potencial biotecnológico.

Palavras-Chave: Biotecnologia, microalgas, isolamento.

Abstract Phytoplankton organisms play an important role in aquatic ecosystems, mainly by their primary production capacity in the food chain. The present study aimed at making a commented list and isolating species from the native phytoplankton communities of four reservoirs in Pernambuco's semiarid, making them available for experiments that can evaluate the biotechnological potential and meet the region's demands. The collections occurred in water bodies located in the Pernambuco semiarid and the taxa identification occurred based on morphological characters using specific literature. A total of twenty-one taxa were identified, eight of them Cyanophyceae, ten Chlorophyceae and only one of the following classes Bacillariophyceae, Euglenophyceae and Dinophyceae. The bioprospection of microalgae in the semiarid region of Pernambuco has made possible the formation of a bank of strains, important for fictional studies and obtaining biomass of species with biotechnological potential.

Keywords: biotechnology, microalgae, isolation.

Introduction

Phytoplankton organisms play an important role in aquatic ecosystems, mainly by their primary production capacity in the food chain. Microalgae are also potentially used as bioindicators of water quality, mainly because they have a short life cycle and respond quickly to the dynamics of the ecosystem in which they are (Singh & Gu, 2010).

Additionally, microalgae biotechnology has been developed to produce products with various commercial applications (food, nutraceuticals, medicines, biofuels, etc.) (Concas et al., 2013; Shuba & Kifle, 2018). However, for the aforementioned applications, these microalgae must be isolated, preferably from unpolluted waters, in order to avoid genetically adapted lineages (Pfleeger et al., 1991; Nascimento et al., 2002; Saranya et al., 2015).

In Brazil, the number of studies carried out in continental aquatic environments, aiming at the prospection of algal strains, has increased considerably in the last years; however, most of them are directed towards the South and Southeast regions (Pompêo, 2011; Moura et al., 2007), with little literature on this subject in reservoirs in the Brazilian semiarid region. Minhas et al. (2016) evaluating the production of lipids and carotenoids under stress conditions in the microalgae culture medium, considers the potential of microalgae to produce a variety of products and indicates that there is potential in future research for developing appropriate criteria for species selection through the bioprospection of microalgae obtained from various habitats and climatic conditions.

Bioprospecting native algal strains contributes directly to these demands as well as to the most diverse applications, among them the treatment of effluents and the feeding of animals with high commercial value (Mata et al., 2010; Bhatt et al., 2014). The formation of a culture collection contributes to the knowledge and maintenance of local biodiversity, since in the semiarid region, due to the high evaporation rates and the long periods of drought, many of the reservoirs tend to dry up.

This is the first paper to be published by members of the Microalgae Biotechnology Laboratory (LABIM/UAST). We intend to encourage and assist future studies on phytoplankton carried out in reservoirs from the Brazilian semi-arid region. The present study aimed at making a commented list and isolating species native of commercial and biotechnological interest from the phytoplankton communities of four reservoirs in Pernambuco's semiarid, making them available for experiments that can evaluate the biotechnological potential and meet the above-mentioned region's demands. Although most of the species recorded did not constitute our culture collection, we hope to assist in the identification of taxa in future studies in this region.

Materials and Methods

The study took place between February and October 2017. Samples for microalgae isolation were collected at the Cachoeira II (07º 58'23.1"S 038º 19'24.0"W) (Figure 1A), Saco I reservoirs (07º 56'53.1"S 038º 16'58.6"W) (Figure 1B), and Varzinha dam (08º 01'57.3"S 038º 07'11.4"W) (Figure 1D) both located in Serra Talhada, Pernambuco state and; Sítio dos Nunes Lake (08º 03'02.3"S 037º 50'15.4"W) (Figure 1C) located in district of Sítio dos Nunes, Flores city, Pernambuco state. Samples containing phytoplankton were collected during daytime (approx. at 10 a.m.). Horizontal hauls were carried out using a plankton net (20 µm) at an average depth of 0.5m (surface layer).

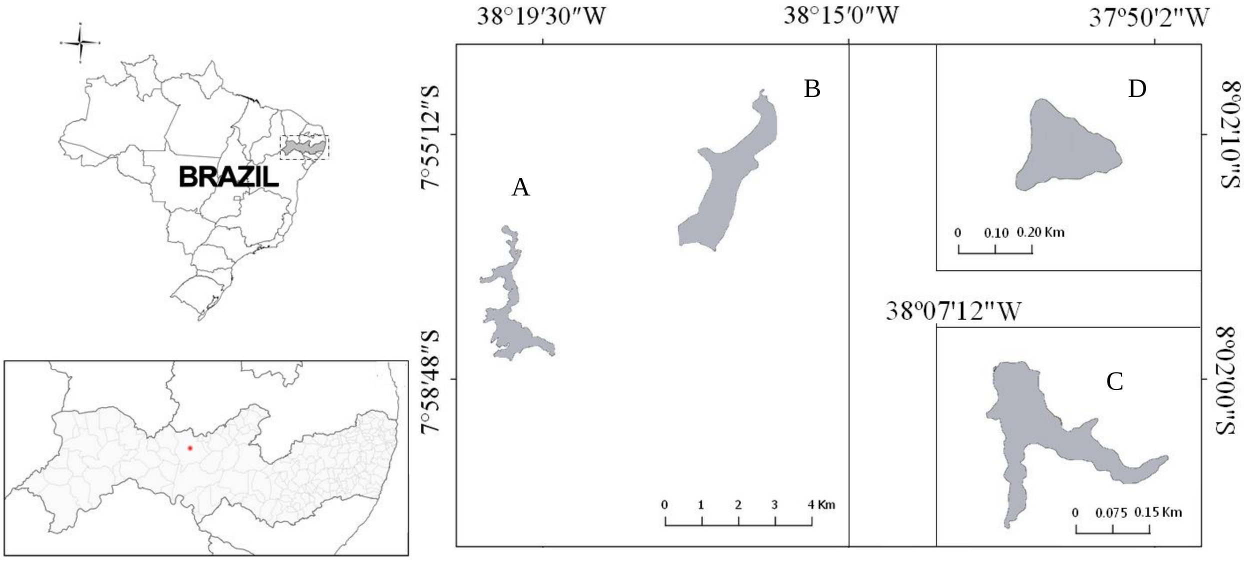

Figure 1. Collection place. Cachoeira II (A), Saco I (B) reservoir, and Varzinha Dam (D), located in Serra Talhada city. Sitio dos Nunes Lake (C) located in district of Sitio dos Nunes, Flores city, both in Pernambuco, Brazil.

Qualitative analysis was performed according to the method adapted from Utermöhl (1958), using samples fixed with aqueous formaldehyde (4%), an optical microscope binocular Motic® model BA300, and a Sedgwick-rafter chamber. In order to identify the taxa, they were classified based on morphological characters, with the following references: Comas González (1996), Bicuco & Menezes (2006), Cybis et al. (2006), Menezes & Bicudo (2008) using the formaldehyde-fixed samples.

The samples' remainders were cultured in different mediums: Provasoli, F2 Guillard, Bold's Basal Medium. Subsequently, attempts were initiated to isolate specimens that occurred by serial dilution, micropipetting techniques, and plating, following the algal isolation protocol, adapted from Arredondo Vega & Voltolina (2007), from the Microalgae Biotechnology Laboratory (LABIM), located at the Federal Rural University of Pernambuco, Unit Academic of Serra Talhada. These were kept in a refrigerated room at 20ºC and an integral photoperiod of 270 μmols m^2 s^{-1}.

On data collection days, all necessary care was taken in order to avoid crusted contaminations between the reservoirs studied. In addition, 70% alcohol was used to ensure the contamination occurred during sample disposal.

Results and Discussion

Taxa list

Formaldehyde-fixed samples collected in this study contained 21 taxa and its distribution by class is presented in Figure 2. The most representative class was Chlorophyta with ten species (47% of taxa), *Chlorella* sp., *Cosmarium bioculatum*, *Desmodesmus* spp., *Dictyosphaerium* sp., *Pediastrum duplex*, *Monactinus simplex*, *Raphidocelis subcapitata*, *Scenedesmus* sp., S*taurastrum leptocladum*, *Trochiscia* sp. Then, Cyanobacteria (blue algae) accounted for 38% of the taxa, *Anabaena* sp., *Aphanocapsa elachista*, *Chroococcus dispersus*, *Dolichospermum* sp., *Merismopedia* sp., *Microcystis aeruginosa*, *Pseudanabaena limnetica*, *Synechococcus* sp. and the lowest representative classes (only one taxon) were Diatoms (*Aulacoseira granulata*), Euglenoidea (*Trachelomonas volvocina*) and Dinoflagellate (*Ceratium furcoides*).

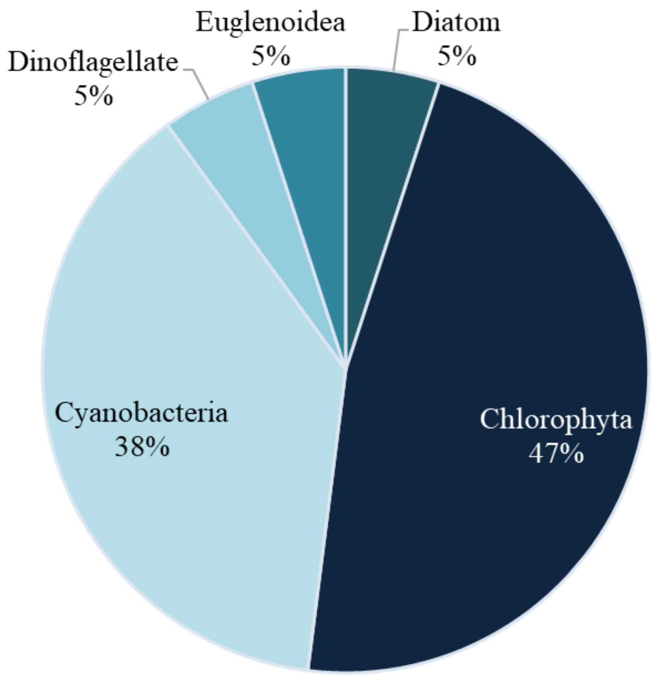

Figure 2. Percentage distribution of the planktonic classes identified in four reservoirs in Pernambuco's semiarid.

A commented list of species reported was made and is shown in Table 1.

Table 1. Commented list of species reported in the present study.

Taxa	Place	Description
Cyanobacteria		
Family Nostocaceae		
Anabaena sp.	B	Solitary trichomes, spiral, wrapped in a narrow sheath. Spherical cells with approximate length and diameter of 9µm and 10µm, respectively.
Family Merismopediaceae		
Aphanocapsa elachista West & G. S. West	A, B	Irregular and diffuse colonies surrounded by a mucilaginous sheath. Scattered and irregularly distributed; however, all with a spherical shape and a diameter of approximately 2µm.
Merismopedia sp.	B	Tubular colonies consisting of at least 4 cells arranged in rows with a diameter between 1.5 and 3µm.
Family Chroococcaceae		
Chroococcus dispersus (Keissler) Lemmermann	B	Regular colonies and quadrangles individually surrounded by sheath, spherical shape, and mucilage. Approximate diameter of 4µm.
Family Aphanizomenonaceae		
Dolichospermum sp.	B	Solitary trichomes, twisted and with a notorious presence of big akinetes (larger than other cells).

Family Microcystaceae		
Microcystis aeruginosa (Kützing) Kützing	B	Microscopic or macroscopic colonies, elongated, and irregular with dark green cells. Mucilage type hyaline.
Family Pseudoanabaenaceae		
Pseudanabaena limnetica (Lemmermann) Komárek	B	Solitary trichomes, straight, but slightly curved. Cylindrical shape cells with homogeneous cellular content.
Family Synechococcaceae		
Synechococcus sp.	B	Isolated cells, oval or cylindrical without mucilage. With homogeneous cellular content, and approximate diameter of 2µm.
Chlorophyta		
Family Oocystaceae		
Chlorella sp.	B, C, D	Solitary and free-living individuals. Spherical or ovoid cell with narrow cell wall. Diameter ranging from 1 to 10µm.
Trochiscia sp.	B	Solitary and free-living individuals. Spherical cell with relatively thick and ordered cell wall of pointed spines.
Family Desmidiaceae		
Cosmarium bioculatum Brébisson ex Ralfs	A	Cells generally isolated, most often slightly elongated. Frequently broader (approximately 6µm) than long (approximately 4µm).
S*taurastrum leptocladum* Nordstedt	A, B	Solitary cells with large variation in size. They have vertical and radial symmetry.
Family Scenedesmaceae		
Desmodesmus sp.	C, D	Solitary individuals, but usually with habit to form colonies of 2, 4 or 8 cells arranged horizontally. The cells may be ellipsoids or ovoid.
Family Dictyosphaeriaceae		
Dictyosphaerium sp.	A	Free-living colonies formed by four groups of four cells each. The cells have formed spherical or ellipsoidal.
Family Hydrodictyaceae		
Monactinus simplex Corda	B	Flat cenobios, hollow-centered, with cell numbers of two multiple. Cells are triangle-shaped.
Pediastrum duplex Meyen	B	Flat cenobios with cells numbers of two multiple. The cells are roughly shaped like a little flag. Radial length of approximately 8µm.
Family Selenastraceae		
Raphidocelis subcapitata (Korshikov) Nygaard, Komáred, J.Kristiansen & O.M.Skulberg	D	Lone and half-moon shaped cells with one pointed and one rounded. Vertical length of approximately 3µm.
Diatom		
Family Coscinodiscophyceae		
Aulacoseira granulata (Ehrenberg) Simonsen	C	Grouped elliptic and cylindrical cells. Ornate valves with a thorn at each end.
Euglenoidea		

Family Euglenaceae		
Trachelomonas volvocina (Ehrenberg) Ehrenberg	A, B	Individual with a generally spherical or semi-spherical free life. It has a pore in its anterior part, which emerges the flagellum.
Dinoflagellate		
Family Ceratiaceae		
Ceratium furcoides (Levander) Langhans	A, B	Solitary, free-naturing and asymmetric cells with tower format. Composed of 16 ornate plaques.

Species explanation

Some species recorded in this study are considered to be harmful. Whether by the production of toxins or by their flowering they affect aquatic ecosystems. This is worrying because these four bodies are used by the population both for supply and recreational activities. *Microcystis aeruginosa* is a potential producer of microcystin-LR (MC), the only cyanotoxin with water potability guidelines proposed by the World Health Organization (WHO), being tolerated up to 1 μg L-1 (WHO, 2003). *Anabaena genera* is also potentially known to produce MC and Anatoxin-a (ANTX); however, such a toxin is less likely to be found in the southern hemisphere due to climatic factors. ANTX was the first cyanobacterial toxin with established toxicological effects, and it is present in filamentous microalgae (Carmichael et al., 1975; Devlin et al., 1977). Recent studies aim at identifying the palatability of noxious algal species by zooplankton, in order to decrease these cells' density by direct predation (dos Santos Severiano et al., 2018).

Ceratium furcoides is an exotic dinoflagellate that was registered in Brazil in 2007 in the state of Minas Gerais, and only in 2016 its first occurrence was recorded in the Northeast region, state of Bahia (Santos-Wisniewski et al., 2007; Oliveira & Oliveira, 2018). *C. furcoides* can produce unpleasant taste and unpleasant smell in the water, resulting in serious problems for regions with supply problems, such as in the present study (Jati et al., 2014; Meichtry de Zaburlín et al., 2016).

However, some algae have a biotechnological potential already described in the literature and do not present risks to society, such as the genus *Chlorella*, *Desmodesmus*, and *Pseudokirchineriella* (Matos, 2017). *Chlorella* is one of the most productive in the world, being used as a functional food, available in capsules, powder or length. *Chlorella* and *Scenedesmus* also present a great potential to produce biofuels and several researches are done about increasing the lipid potential in order to obtain a higher yield.

We emphasize that not all species recorded in this study are isolated at the Biotechnology Microalgae Laboratory. This is due to some factors: absence of specific culture media for some species; not using silica for the diatom; or by the non-interest in the strain.

Conclusions

In the present study, 21 taxa were identified and up to the present moment our LABIM culture collection counts eight isolated species of commercial and biotechnological interest. The isolation of the sweet strains of Pernambuco's semiarid is in continuous process of tests in order to enable the conditioning of these species in the Biotechnology of Microalgae Laboratory, UFRPE/UAST. We intend to include new reservoirs in our studies, with the aim of expanding our culture collection and keeping it up to date.

Future studies can (and are) be developed to evaluate the biotechnological application of the isolated species, as well as the use of these as an auxiliary tool in the phycology studies from the analysis of live samples.

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