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Bioprospection of freshwater microalgae from Bonito, MS, Brazil

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The great biodiversity of the Serra da Bodoquena is the result of years of biological evolution. A complex combination of natural factors allows aquatic plants, fish and invertebrates to coexist in absolutely crystalline water springs. Together, organisms form an intricate web of life, connecting a single-celled microalga with large river predators. For better knowledge of the biodiversity of microalgae, an important tool is the bioprospecting and study of novel species, avoiding as much as possible the introduction of exotic species. Thus, the aim of this work was bioprospecting microalgae species from Bonito, MS, Brazil, in order to obtain more information about the local microbial biodiversity. Freshwater samples were collected from two lakes of the municipality. The samples were plated in Basic Basal medium added of bacteriological agar. After plating, the samples were kept in a biochemical oxygen demand (BOD) oven at 25ºC with photoperiod for growth. After isolation of the obtained colonies, the identification of the species was carried out according to the morphological characteristics of cells. Despite the long periods for adaptation, seven microalgae taxa were successfully isolated from the samples collected, four at the genus level and three at the species level; one from the Trebouxiophyceae class, five from the Chlorophyceae class and one from the Bacillariophyceae class.

Key words: Biodiversity, freshwater species, identification, morphology.

INTRODUCTION

Bonito, a city located in the Brazilian State of Mato Grosso do Sul integrates the tourist complex of the Serra da Bodoquena National Park. The dominant vegetation is characteristic of the Brazilian Savannah, contacted with seasonal forest and seasonal deciduous forest. The region belongs to the Paraguay River Basin, Miranda Sub-basin. In Bonito, the rivers have peculiar characteristics: they are transparent due to the crystalline waters as the rivers are originated from mainly from limestone rocks (Boggiani et al., 1999).

A long and complex combination of geological and evolutionary processes made the region's springs natural systems of high biodiversity, with a high degree of unicity (Boggiani et al., 1999). The rich biodiversity of

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Bodoquena allows aquatic plants, fish and all sorts of invertebrates to coexist in crystalline water springs. Together, organisms form an intricate net of life, which connects a single-celled microalga to large river predators, such as golden dorado (*Salminus brasiliensis*) and giant otters (*Pteronura brasiliensis*) (Sabino, 2002). Through the bioprospection and cultivation of microalgae, it is possible to evaluate the ecophysiology of species which could not be observed and monitored in the field, such as the life cycle, plasticity and response to different environmental variables, allowing also to help in the biological systematics of the groups (Lourenço, 2006).

Moreover, the concern with environmental issues has become increasingly evident due to the excessive use of natural resources in productive processes, thus, causing a high potential of pollution (Barcellos et al., 2009). Biological processes have become an interesting alternative against pollution and in the generation of new products, since these processes use microbial metabolism to degrade and remove pollutants (Gadd, 2009), generating products less harmful to the environment. In these processes, there is a range of active microorganisms, including microalgae. In this context, it underlines the importance of bioprospecting new strains and species of microalgae not only with environmental and ecological purposes, as the treatment of effluents and biosorption of toxic metals (Mezzomo et al., 2010; Schmitz et al., 2012) and CO_2 biofixation (Morais and Costa, 2007), but also applications of economic interest, as the productions of lipids (Xu et al., 2006) and biofuels (Xu and Mi, 2011). In addition, bioprospecting of species is a preponderant factor for the knowledge of the local biodiversity and for the local sustainable development, besides avoiding the introduction of exotic species. The bioprospecting generates inventories of microalgae, scarce in certain regions of Brazil, fomenting the creation of collections of microalgae for the these regions (Mendes et al., 2012).

Thus, the aim of this work was bioprospecting microalgae from lakes located in the municipality of Bonito, MS, Brazil, and identifying the species according to their morphological characteristics.

MATERIALS AND METHODS

The samplings were carried out in two lakes located at the coordinates 21°02'18.9"S 56°33'23.7"W and 21°02'21.0"S 56°33'04.3"W in the city of Bonito, MS, Brazil. Both lakes are surrounded by vegetation in their interior, including grasses, shrubs and trees. The soil of the region is limestone and stony, and the water ranging from crystalline to turbid. The samples were collected in 500-mL sterilized pet bottles and stored in a thermal box that was transported to the laboratory. The samples were then plated in Bold Basal Medium (Bischoff and Bold, 1963) with autoclaved bacteriological agar (121ºC, 15 min) and then placed in the BOD (MA 415 Marconi) at 25°C with 8 Klux illumination and aeration.

After growth, colonies were spiked into sterile plates for isolation. It was made by successive pealing with a platinum loop through the technique of stretch-out. An optical microscope (40x objective lens) was used to observe the microalgae and to verify the purity of the colonies. After complete isolation, the strains were cultured in Erlenmeyer flasks with liquid Bold Basal medium for identification. Flasks were maintained in biochemical oxygen demand (BOD) equipped with orbital rotatory stirrer (200 rpm) and illumination. The cultures were then placed in Petri dishes with culture medium and stored in BOD at 3°C.

The species were identified according to their morphological characteristics observed with the aid of an optical microscope, based on images and information from microalgae databases, e.g. Algae base, Culture Collection of Autotrophic Organisms, Online Bookstore, and literature references (Round et al., 1990; Komárek and Marvan, 1992; Comas, 1996; Menezes and Bicudo, 2006, 2008; Fanés et al., 2009; Godinho et al., 2010; Mendes et al., 2012; Ramos et al., 2012).

RESULTS AND DISCUSSION

As a result of the bioprospection, seven microalgae taxa were isolated from the collected samples: four at the genus level and three at the species level. The six first species isolated are representative of the Class Chlorophyceae while the last one belongs to the Class Bacillariophyceae. Their characteristics are described below:

(1) *Chlorella* sp. (Figure 1a) from family Oocystaceae, was identified from the ellipsoid format in the young cells and spherical in the adults. They cells were found isolated or in small transient clusters. The single chloroplast, parietal, do not always occupy the whole cell, with presence or absence of pyrenoid. The reproduction was of 2-4-8 elliptical autospores.

(2) *Chlorococcum* sp. (Figure 1b) from family Chlorococcaceae was identified by the presence of spherical cells with varying sizes, solitary or forming groups, without mucilage. The pituitary choroplast or in the form of a sphere internally fulfills the entire cell. As the genetic material increases, size increases and the chloroplast assumes the star shape. They had pyreneo with discontinuous starch coating, contractile vacuoles and reproduction by ellipsoidal zoospores.

(3) *Coelastrum* sp (Figure 1c) from family Scenedesmaceae was identified by the variety of spherical, ellipsoid, pyramidal, cuboid, tetrahedral to polygonal shaped cells, with rounded conical poles. The spherical domes had cells attached directly by their walls. The single chloroplast, parietal, presented pyrenoid. The asexual reproduction was presumed by a number of appendages more or less long that join cells together. The only chloroplastidium in each cell has the form of a cup (poculiform) and a more or less central pyrenoid.

(4) *Desmodesmus brasiliensis* (Figure 1d) from family Scenedesmaceae was identified by the elliptical cells with rounded poles. They had coenobies with four cells linearly aligned, cell wall with, sometimes, discontinuous longitudinal ribs and single chloroplast, parietal with pyrenoid. Tiny spines were found around the coenoby if not absent.

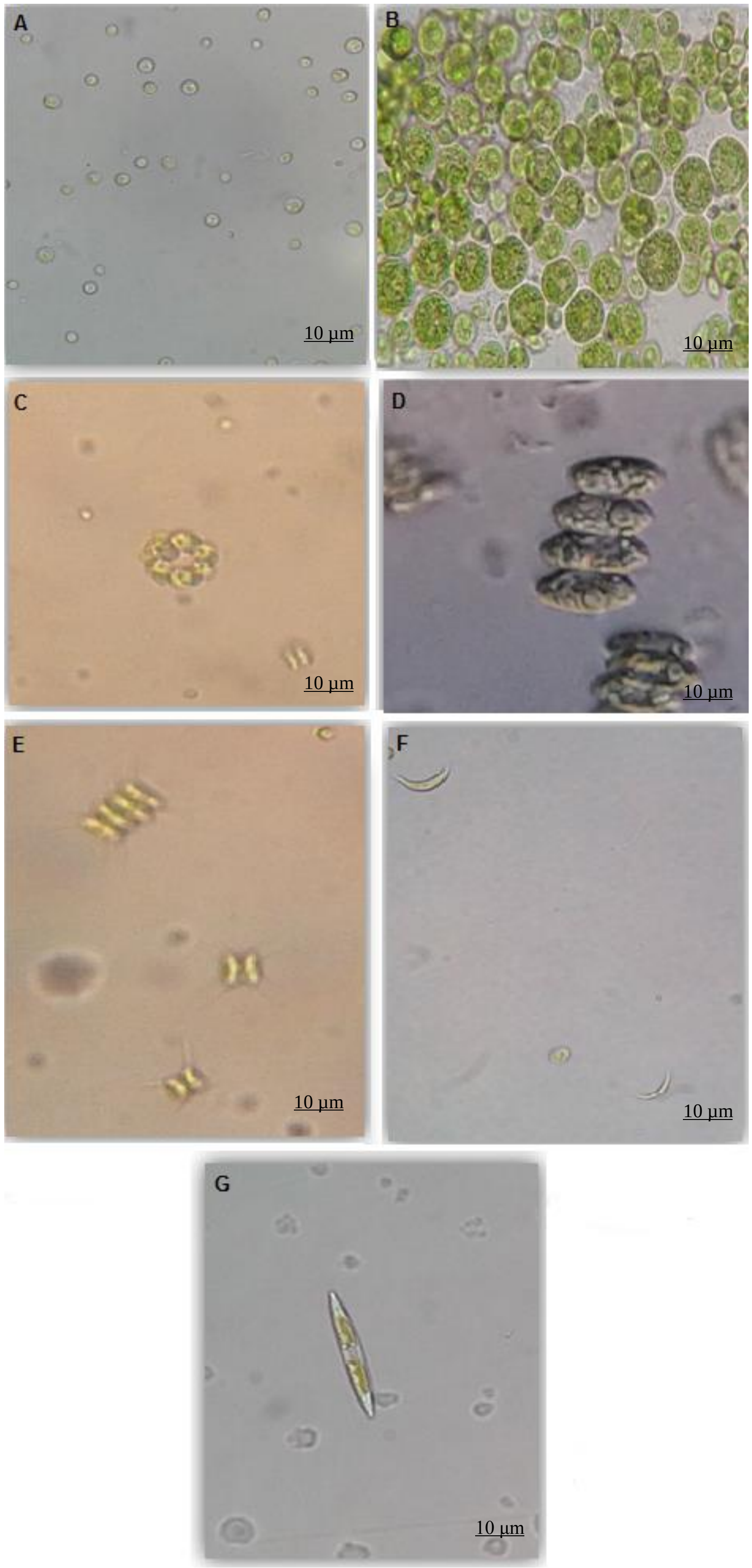

Figure 1. Bioprospected microalgae, where: 2a *Chlorella* sp; 2b *Chlorococcum* sp; 2c *Coelastrum* sp; 2d *Desmodesmus brasiliensis*; 2e *Desmodesmus communis*; 2f *Monoraphidium caribeum*; 2g *Nitzschia* sp.

(5) *Desmodesmus communis* (Figure 1e) from family Scenedesmaceae was identified by the cells with four or more cells aligned linearly grouped inside the coenoby. The elliptic cells presented rounded poles, obtuse/truncated, differentiated by the presence of spines at their extremities, usually four spines. Quite variable characteristics in this species are the dimensions of cells and spines. It was formerly known as *Scenedesmus quadricauda* (Turpin) Brébisson sensu Chodat (1913, 1926), being renamed by Hegewald (1977) for *S. communis* Hegewald and later for *D. communis* Hegewald (Hegewald, 2000). He included most of the taxonomic varieties of *S. quadricauda* in this new classification, remaining this way up till now without any other change.

(6) *Monoraphidium caribeum* (Figure 1f), from family Selenastraceae was identified by the solitary cells, arched in a semicircle and slightly tapered at the extremities. Parietal chloroplast was absent of pyrenoids. This species is found mainly in plankton and periphyton of eutrophic aquatic environments (Ramos et al., 2012).

(7) *Nitzschia* sp. (Figure 1g) from family Bacillariaceae was identified by the cylindrical shape with rounded apices, with two plastids arranged symmetrically one on each side of the transapical medial plane.

All the species presented here were able to develop in the culture medium, but not all the species found in the ponds were adaptable to the in vitro culture conditions. The difficult was evident as only few strains were successfully isolated. The microalgae species isolated here took a long time to adapt themselves to the given conditions of cultivation in the laboratory, since the culture medium does not meet the specific conditions of their natural habitat. The waters of the region of Bonito present a high concentration of calcium carbonate. They are subject to receiving fragments of rocks existing in the surface of that sediment in the water, and a small amount that enters in suspension is soon deposited by the precipitation of the carbonate. This characteristic turns the water of the rivers very clear, favoring the biological activity and, consequently, the precipitation of carbonate (Boggiani, 1999). The development of a medium that faithfully reproduce the same conditions would allow the isolation of a higher microbial biodiversity.

Studies involving the isolation and identification of microalgae in the region of Mato Grosso do Sul are not frequently reported. It is challenged by knowledge of the local biodiversity within the scope of promising species of microalgae that can be further studied. Mendes et al. (2012) report the need for the creation of new collections of microalgae from the Brazilian scarcely studied biomes.

The bioprospection of local microalgae is the first step for more complex studies and obtaining viable algal raw material. In addition, it is a preponderant factor for the knowledge of local biodiversity and for the sustainable development. The bioprospected species are now part of a collection of microalgae, which may be accessed for other researchers.

Conclusion

Seven microalgae taxa were isolated from samples. Adaptation of the species was a limiting factor to obtain a greater number of strains, since not all the microalgae species found in the lakes have this capacity, requiring a long period for this adaptation and often resulting in loss of the species. The development of appropriate media would facilitate the isolation of a higher microbial biodiversity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Barcellos FC, Oliveira JC, Carvalho PGM (2009). Environmental investment in dirty and Natural resources and energy (In Portuguese). Iberoamericana Econ. Ecol. Mag. 12:33-50.

Bischoff HW, Bold HC (1963). Phycological studies. IV. Some soil algae from enchanted rock and related algal species, University of Texas Publications. 6318:1-95.

Boggiani PC (1999). Why Bonito city is beautiful? (In Portuguese). In: Scremin-Dias, Edna; Pott, Vali Joana. et al. In the submerged gardens of Bodoquena. Campo Grande, MS: UFMS Ed. pp. 11-23.

Boggiani PC, Scremin-Dias E, Pott VJ, Hora RC, Souza PR (1999). Bodoquena Geology. p.10-23. In: In the submerged gardens of Bodoquena: Guide for aquatic plants identification from Bonito city and region (In Portuguese). UFMS Editor. 160p.

Chodat R (1913). Seaweed monograph in pure culture. Materials for Cryptogamic Flora Switzerland (In French). 4:1-266.

Chodat R (1926). *Scenedesmus*: Study of genetics, experimental systematics and hydrobiology. Zeitschrift für Hydrologie (In French). 3:71-258.

Comas AG (1996). Cuban freshwater chlorococcals (In Spanish). Berlin, Stuttgart, Gebrüder Borntraeger Verlagsbuchhandlung. P 192.

Schmitz R, Dal Magro C, Colla LM (2012). Environmental applications of microalgae. CIATEC – UPF Mag. 4(1):48-60.

Fanés I, Sánchez P, Comas A (2009). Contribution to the taxonomic study of the family Botryococcaceae (Trebouxiophyceae, Chlorophyta) in southern Spain. Cryptog. Algolog. 30(1):17-30.

Gadd GM (2009). Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J. Chem. Technol. Biotechnol. 84:13-28.

Godinho LR, Gonzáles AAC, Bicudo CEM (2010). Cryptogams of the “Parque Estadual das Fontes do Ipiranga”, São Paulo, SP. Algae, 30:

Chlorophyceae (Family Scenedesmaceae). Hoehnea. São Paulo. Hoehnea 37(3):513-553.

Hegewald E (1977). *Scenedesmus communis* Hegewald, a new species and its relation to *Scenedesmus quadricauda* (Turp.) Bréb. Algolog. Stud. 18:142-155.

Hegewald E (2000). New combinations in the genus *Desmodesmus* (Chlorophyceae, Scenedesmaceae). Algolog. Stud. 96:1-18.

Komarék J, Marvan P (1992). Morphological Diff. in Natural Populations of the Genus *Botryococcus* (Chlorophyceae). Archiv fuer Protistenkunde 141:65-100.

Lourenço SO (2006). Culture of marine microalgae - Principles and applications (In Portuguese). Rima Ed, São Carlos, SP. 588p.

Mendes MCQ, Gonzalez AAC, Menezes M, Nunes JMC, Pereira S, Nascimento IA (2012) Collection of microalgae from natural freshwater environments of Bahia, Brazil, as a potential source for biofuel production: A taxonomic approach. Acta Bot. Brasil. 26(3):691-696.

Menezes M, Bicudo CE (2006). Genus of freshwater algae in Brazil, key for identification and descriptions (In Portuguese). 2º Ed. Rima. São Carlos. 502p.

Menezes M, Bicudo CE (2008). Flagellate green algae from four water bodies in the state of Rio de Janeiro, Southeast Brazil. Hoehnea 35(3):435-468.

Mezzomo N, Saggiorato AG, Siebert R, Tatsch PO, Lago MC, Hemkemeier M, Costa JAV, Bertolin TE, Colla LM (2010). Cultivation of microalgae *Spirulina platensis* (*Arthrospira platensis*) from biological treatment of swine wastewater. Food Sci. Technol. 30(1):173-178.

Morais MG, Costa JAV (2007). Biofixation of carbon dioxide by *Spirulina sp*. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. J. Biotechnol. 129:439-445.

Ramos GJP, Bicudo CEM, Goes Neto A, Moura CWN (2012). *Monoraphidium* and *Ankistrodesmus* (Chlorophyceae, Chlorophyta) from Pantanal dos Marimbus, Chapada Diamantina, Bahia State, Brazil. Hoehnea 39(3):421-434.

Round FE, Crawford RM, Mann DG (1990).The Diatoms, Biology & Morphology of the Genera. Cambridge, Cambridge University Press. 747p.

Sabino J (2002). Bodoquena´s Plateau: Nature in a state of Grace (In Portuguese). The Paths Earth Mag. 125:58-65.

Xu R, Mi Y (2011) Simplifying the process of microalgal biodiesel production through in situ transesterification technology. J. Am. Oil Chem. Soc. 88(1):91-99.

Xu H, Miao X, Wu Q (2006). High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. J. Biotechnol. 126(4):499-506.