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Original Article

Morphology, molecular phylogeny and biomass evaluation of *Desmodesmus abundans* (Scenedesmaceae-Chlorophyceae) from Brazil

Morfologia, filogenia molecular e avaliação da biomassa de *Desmodesmus abundans* (Scenedesmaceae-Chlorophyceae) do Brasil

G. Burgel[a,b], P. G. Ribas[a,b], P. C. Ferreira[c], M. F. Passos[a,b], B. Santos[a,b], D. C. Savi[d], T. A. V. Ludwig[c], J. V. C. Vargas[b], L. V. Galli-Terasawa[a] and V. M. Kava[a,b]*

[a]Universidade Federal do Paraná, Departamento de Genética, Programa de Pós-Graduação em Genética, Laboratório de Genética de Microrganismos (LabGeM), Curitiba, PR, Brasil

[b]Universidade Federal do Paraná, Núcleo de Pesquisa e Desenvolvimento de Energia Autossustentável – NPDEAS, Curitiba, PR, Brasil

[c]Universidade Federal do Paraná, Departamento de Botânica, Laboratório de Ficologia, Curitiba, PR, Brasil

[d]Centro Universitário – Católica de Santa Catarina, Departamento de Biomedicina, Joinville, SC, Brasil

Abstract

The biotechnological potential of microalgae has been the target of a range of research aimed at using its potential to produce macromolecules with high added value. Particular focus has been given to biofuels' production, such as biohydrogen, biodiesel, and bioethanol from lipids and carbohydrates extracted from microalgal biomass. Bioprospecting and accurate identification of microalgae from the environment are important in the search for strains with better performance. Methodologies that combine morphology and molecular techniques allow more precise knowledge of species. Thereby, this work aimed to identify the new strain LGMM0013 collected at Iraí Reservoir, located in Paraná state, Brazil, and to evaluate the production of biomass, carbohydrates, and lipids from this new microalgal strain. Based on morphology and phylogenetic tree from internal transcribed spacer (ITS), strain LGMM0013 was identified as *Desmodesmus abundans*. *D. abundans* accumulated 1500 mg L^{-1} of dried biomass after 22 days of cultivation in autotrophic conditions, 50% higher than *Tetradesmus obliquus* (LGMM0001) (Scenedesmaceae-Chlorophyceae), usually grown in photobioreactors located at NPDEAS at the Federal University of Paraná (UFPR) to produce biomass. Analysis of the *D. abundans* biomass from showed an accumulation of 673.39 mg L^{-1} of carbohydrates, 130% higher than *T. obliquus* (LGMM0001). Lipid production was 259.7 mg L^{-1}, equivalent to that of *T. obliquus*. Nitrogen deprivation increased the production of biomass and carbohydrates in *D. abundans* LGMM0013, indicating this new strain greater biomass production capacity.

Keywords: bioprospection, carbohydrates, ITS sequences, lipids, nitrogen deprivation.

Resumo

O potencial biotecnológico das microalgas tem sido alvo de uma série de pesquisas que visam utilizar seu potencial para produzir macromoléculas de alto valor agregado. Uma especial atenção tem sido dada à produção de biocombustíveis, como biohidrogênio, biodiesel e bioetanol, a partir de lipídios e carboidratos de microalgas. A bioprospecção e a identificação precisa de microalgas são importantes na busca de linhagens com melhor desempenho em termos de produção de biomassa e lipídios. Metodologias que combinam morfologia e técnicas moleculares permitem uma identificação mais precisa das espécies. Assim, este trabalho teve como objetivo identificar a nova cepa LGMM0013 coletada na Represa do Iraí, localizado no estado do Paraná, Brasil e avaliar a produção de biomassa, carboidratos e lipídios. Com base na morfologia e na árvore filogenética obtida com sequências ITS ("Internal Transcribed Spacer"), a cepa LGMM0013 foi identificada como *Desmodesmus abundans*. *D. abundans* acumulou 1500 mg L^{-1} de biomassa seca após 22 dias de crescimento em condições autotróficas, 50% superior a *Tetradesmus obliquus* (LGMM0001) (Scenedesmaceae-Chlorophyceae), uma cepa cultivada frequentemente em fotobiorreatores para produção de biomassa no NPDEAS, na Universidade Federal do Paraná (UFPR) para a produção de biomassa. Na análise da biomassa de *D. abundans* LGMM0013 constatou-se acúmulo de 673,39 mg L^{-1} de carboidratos, 130% maior que *T. obliquus* (LGMM0001). A produção de lipídios foi de 259,7 mg L^{-1}, equivalente à de *T. obliquus*. A privação de nitrogênio aumentou a produção de biomassa e carboidratos em *D. abundans* LGMM0013, indicando uma maior capacidade de produção de biomassa desta cepa.

Palavras-chave: bioprospecção, carboidratos, sequências ITS, lipídios, privação de nitrogênio.

*e-mail: vankava@ufpr.br
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1. Introduction

Microalgae have been the focus of numerous biotechnological researches due to their potential for producing molecules with high added value (Pang et al., 2019). The use of biomass from microalgae to produce biofuels is one of the biggest challenges in the area. Obtaining sufficient biomass for industrial-scale application and the extraction of the macromolecules of interest suffers from the process's high costs (González-Balderas et al., 2020). The search for strains with high productive potential, easy to handle and survive in a controlled environment could be the solution to this problem (Hadi et al., 2016). The production of macromolecules by microalgae may be affected by cultivation factors, such as nutrient deprivation, pH, temperature, and light incidence (Wase et al., 2018). Find the best cultivation condition is a strategy to overcome the limitations.

Microalgae cultivation has gained prominence for enhancing the production of molecules of interest while promoting wastewater decontamination. Nitrogen and phosphorous could be removed from wastewater (Wang et al., 2022), and even wastewater from swine (Caprio et al., 2019; Miyawaki et al., 2021) or cattle (Scherer et al., 2017) manure could be used for microalgae cultivation. By combining different cultivation conditions with the idea of biorefineries, a wide variety of biomass products would be obtained, eventually allowing the process to be sustainable, by becoming energetically and economically viable ('t Lam et al., 2018). Aiming to achieve such goals, the NPDEAS (http://npdeas.blogspot.com/) research group has been developing microalgae cultivation based projects, especially through an indigenous strain of *Tetradesmus obliquus* (LGMM0001), which has been shown to bear different environmental conditions, even when grown in 10,000 liter photobioreactors. Biomass (Silva et al., 2013; Selesu et al., 2016; Miyawaki et al., 2021), lipids (Hesse et al., 2017; Escorsim et al., 2018), and biohydrogen (Vargas et al., 2014; Corrêa et al., 2017; Dias et al., 2019) are some of the bioproducts evaluated for *T. obliquus* (syn. *Acutodesmus obliquus, Scenedesmus obliquus*) in the search for economically viable energy production. Such studies have shown that bioproducts diversification and production maximization could eventually lead to feasible and economically competitive plants.

Some Scenedesmaceae genera like *Desmodesmus, Tetradesmus,* and *Scenedesmus* have demonstrated good biomass productivity, and interest molecules accumulation, such as lipids and carbohydrates (Hess et al., 2018; Akgül et al., 2021; Najeeb et al., 2024). Changes in cultivation conditions, such as nutrient deprivation and addition of heavy metals, can affect the biomass and macromolecules' production by *Desmodesmus* (Abinandan et al., 2019).

An essential step beyond the bioprospecting of new microalgae isolated from the natural habitat is the taxonomic identification approaches that combine morphological and molecular analysis (Leliaert et al., 2012). Morphologically, the genus *Desmodesmus* is commonly identified by the analysis of its unique cells, formation and alignment of the coenobia, in addition to the presence of a layer of sporolenin in the cell wall, which allows the formation of spines and other ornamentations, very characteristic of the genus (An et al., 1999; Hegewald, 2000).

The high degree of phenotypic variability found in different organisms leads to inconsistencies and misidentification based only on morphological characteristics. This challenge has been overcome through taxonomic reviews and the identification of new strains based on molecular analyzes (Lortou and Gkelis, 2019), such as the sequencing of the internal transcribed spacer 2 (ITS-2) rDNA region, that allowed the separation of the genus *Desmodesmus*, previously identified as *Scenedesmus* (An et al., 1999).

In this sense, the main objectives of this study were: i) to bioprospecting the isolate *D. abundans* LGMM0013 and quantify its biomass, carbohydrates and lipids production; ii) to identify the isolate *D. abundans* LGMM0013 taxonomically by morphological and molecular analysis; iii) to know its physiological response to changes in cultivation condition.

2. Material and Methods

2.1. Isolation

Samples were collected from the Iraí Reservoir (25°25.31'S, 49°06.22'W), located in the metropolitan region of Curitiba city, Brazil. Samples were plated directly in an autotrophic CHU medium (Chu, 1942) and were incubated at 21 ºC with a photoperiod of 12:12 light/dark and light intensity of 7.4 µmol m^{-2} s^{-1}. Isolated colonies were transferred to liquid cultures kept in a cultivation room at 21 ºC with continuous light and aeration of 0.4% CO_2 (v/v), with 0.2 µm filters coupled to air entrance. The first pre-inoculums were prepared using stationary phase cultures (30 days) grown in 50 mL flasks (21 ºC, photoperiod 12/12 h) with 3 mL of CHU. The fastest growing and most promising strain was selected to proceed for further investigations.

The samples were fixed with the Transeau solution in a proportion of 1:1 (Bicudo and Menezes, 2006) and deposited in the Herbarium of the Botany Department of the UFPR (UPCB: 93121).

2.2. Comparison of growth, biomass, carbohydrates, and lipids production of D. abundans LGMM0013 AND T. obliquus LGMM0001

The growth, biomass, carbohydrates, and lipids production of the new isolate *D. abundans* LGMM0013 and *T. obliquus* (LGMM0001), the main strain used by NPDEAS´ researchers, at Federal University of Paraná (UFPR), were compared. The methodology used in all experiments was strictly the same for the two strains LGMM0013 and *Tetradesmus obliquus* LGMM0001.

2.2.1. Growth conditions

The culture medium used was liquid CHU [$NaNO_3$ 2.5 mg L^{-1}, $CaCl_2.2H_2O$ 0.25 mg L^{-1}, $MgSO_4.7H_2O$ 0.75 mg L^{-1}, K_2HPO_4 0.75 mg L^{-1}, KH_2PO_4 1.75 mg L^{-1}, NaCl 0.25 mg L^{-1}, EDTA–Na_2 0.05 mg L^{-1}, KOH 0.031 mg L^{-1},

$FeSO_4.7H_2O$ 0.00498 mg L^{-1}, H_3BO_3 0.01142 mg L^{-1}, $ZnSO_4.7H_2O$ 0.0000882 mg L^{-1}, $MnCl_2.4H_2O$ 0.0000144 mg L^{-1}, $CuSO_4.5H_2O$ 0.0000157 mg L^{-1}, $Co(NO_3)2.6H_2O$ 0.0000049 mg L^{-1}, $NaMoO_4.2H_2O$ 0.0000119 mg L^{-1}, Chu (1942)] for autotrophic conditions. Cultures were incubated in 2 L Erlenmeyer flasks with 1.5 L of CHU medium, to provide autotrophic conditions, with aeration 0.4% CO_2 (v/v). The flasks were kept in a cultivation chamber at 25ºC with continuous light of 7.4 µmol m^{-2} s^{-1}. To the mixotrophic conditions, 1000 mg L^{-1} or 1500 mg L^{-1} of $NaHCO_3$ was added to CHU.

2.2.2. Growth kinetics

Growth kinetics of *D. abundans* LGMM0013 and *T. obliquus* (LGMM0001) were evaluated considering the dry biomass (see item 2.2.3). A pre-inoculum grown for 14 days was used and inoculated in a 2 L Erlenmeyer with an initial concentration of 0.01 g L^{-1} of dry biomass. Growth was assessed by dry biomass every 48 hours over 22 days. At the end of the experiment, carbohydrates (see item 2.2.5) and total lipids quantification (see item 2.2.6) were extracted from biomass.

2.2.3. Biomass recovery and quantification

Biomass was recovered according to the methodology described by Selesu et al. (2016). Dry biomass quantification was obtained by filtering an aliquot of the culture through glass fiber microfilters (GF1 0.7 µm) using the gravimetric method. Filters were previously dried in an oven at 60 ºC for 24 hours and then weighed on an analytical scale model SHIMADZU AUW220D (Schimadzu Corporation, Kyoto, Japan). After filtering the culture, the filters were dried and weighed again. The data were treated by this equation (Equation 1):

$$DB = (M_2 - M_1)/V \quad (1)$$

Where, DB = dry biomass (mg L^{-1}); M_1 = mass of the empty microfilter (mg); M_2 = microfilter mass with microalgae biomass (mg); V = filtered aliquot (L).

2.2.4. Carbohydrates quantification

The methodology used for the quantification of carbohydrates was described by Dubois et al. (1956) using phenol and sulfuric acid. In a tube, 20 mg of freeze-dried biomass was added with 1 mL of 80% H_2SO_4 and then rested for 20 hours. For the first 4 hours, the bottles were kept on ice and for the next hours at room temperature.

After resting, the sample was diluted in 9 mL of distilled water. A 1.5 mL aliquot was removed and centrifuged at 8,000 x *g* for 10 minutes. A volume of 50 µL of the supernatant was removed and placed in a test tube, then 500 µL of 5% phenol solution and 2.5 mL of concentrated H_2SO_4 were added. Samples were read on a spectrophotometer with 490 nm of absorbance.

2.2.5. Lipids quantification

Lipids were extracted using the Bligh and Dyer method with modifications (Bligh and Dyer, 1959; Hess et al., 2018). A sample of 50 mg of freeze-dried biomass was added in 3 mL $CHCl_3/CH_3OH$ solution (1:2, v:v), mixed by ultrasound (USC 700, 55 Hz) in 3 cycles of 15 minutes and incubated overnight at 4 ºC in dark conditions. Samples were treated by ultrasound again and harvested at 2000 x g for 10 minutes. The supernatant was recovered, $CHCl_3/CH_3OH$ solution was added to the remaining precipitate, and the process was repeated. The supernatant's oil content was separated by adding 2 mL of distilled water and 1 mL of $CHCl_3$. The organic phase was recovered, and chloroform was evaporated in an exhaustion chamber. Lipid mass was weighed on an analytical scale model SHIMADZU AUW220D.

Total lipid content was calculated by the following equation (Equation 2):

$$L\% = (m_f - m_0) \times 100/m_b \quad (2)$$

Where L% = total lipids, m_f = mass of flask containing lipids, m_0 = mass of the empty flask, m_b = microalgae biomass used for lipid quantification.

2.3. Growth under different culture medium composition

The biomass, carbohydrates, and lipids production of the isolate *D. abundans* LGMM0013 were evaluated after 16 days of cultivation, varying nitrogen and carbon source concentration. Three concentrations of nitrogen (2.5, 1.25 or 0.625 mg L^{-1} of $NaNO_3$) in an autotrophic condition, or with 1000 mg L^{-1} or 1500 mg L^{-1} sodium bicarbonate ($NaHCO_3$) as a carbon source to mixotrophic condition, in liquid CHU medium, were measured (as shown in Table 1), resulting in eight different culture medium composition. A pre-inoculum grown for 16 days was used and inoculated with an initial concentration of 50 mg L^{-1} of dry biomass at the same cultivation conditions described previously. After 16 days of culture, the dry biomass was measured, and from the total biomass recovered, the production of carbohydrates and lipids were estimated from the different media as previously described.

2.4. Statistical analysis

Tests were performed in triplicates. The significance of results was verified by the ANOVA test, accepting a p-value < 0.05.

2.5. Morphological and molecular analysis

Morphological and molecular identification were carried out with samples taken from the same culture. Growth was in 200 mL of liquid TAP medium (Gorman and Levine, 1965), for seven days at 21° C, without aeration in a culture chamber.

Morphological identification was conducted by light (LM) and Scanning Electron Microscopy (SEM). *Desmodesmus* cells were visualized and photographed under the light microscope Olympus BX40 with an attached Olympus DP71 capture camera. Samples of the *D. abundans* LGMM0013 culture were prepared according to Costa et al. (2018) and analyzed with a JEOL JSM6360 scanning electron microscope at the Center of Electron Microscopy from Federal University of Paraná.

The morphological identification was achieved by analyzing coenobia morphology, cell number, marginal

cell morphology, median cell morphology, length (µm), width (µm), and presence or absence of thorns and tubes. Characteristics were compared with relevant literature, such as Chodat (1926), Komarek and Fott (1983), and González (1996).

The molecular identification of LGMM0013 using the internal transcribed sequence (ITS) of ribosomal DNA was performed. The genomic DNA was extracted using NucleoSpin® Plant II kit (Macherey-Nagel), according to fabricant instructions. ITS region (ITS 1, 5.8S, and ITS2) was amplified using the primers V9 (5'-TGCGTTGATTACGTCCCTGC-3') (Hoog and van den Ende, 1998) and LS266 (5'-GCATTCCCAAACAACTCGACTC-3') (Masclaux et al., 1995). Amplification was performed as suggested for Platinum Taq DNA Polymerase (Thermo Fisher Scientific) with an annealing temperature of 55 ºC. Sequencing was performed after EXO-SAP purification in ACTGene Biotechnology (RS/Brazil).

The sequence qualities were checked by BioEdit 7.1 (Hall, 1999) and edited using MEGA 10.1 software (Kumar and Gopal, 2015).

For phylogenetic analyzes, only the most reliable sequences from ITS-1 and ITS-2 regions available at Genbank for the genus *Desmodesmus* were selected, prioritizing sequences mainly used in identification articles (An et al., 1999; Hegewald, 2000; Vanormelingen et al., 2007; Fawley et al., 2011; Fawley et al., 2013; Kaplan-Levy et al., 2016; Hegewald and Braband, 2017). Among them, sequences from identified strains collections such as Culture Collection of Algae and Protozoa (CCAP), Culture Collection of Algae (SAG), and Culture Collection of Algae at the University of Texas at Austin (UTEX) were used. Alignments were inferred with MUSCLE default parameters (Edgar, 2004). Bayesian inference tree was generated in CIPRES (Miller et al., 2010), evolutionary model of Kimura 2 parameters with distribution in range five categories. A maximum-likelihood tree was created using GARLI 2.01 software through the CIPRES platform (Miller et al., 2010). The resulting trees were plotted and edited in Figtree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. Performance of D. abundans LGMM0013 versus T. obliquus LGMM0001

Cultivation started with 10 mg L^{-1} of dry biomass from both species of microalgae. From the 8th day, strain *D. abundans* LGMM0013 began to have higher dry biomass than *T. obliquus* (LGMM0001).The exponential phase started on the 10th day for both isolates (see Figure 1). Dry biomass on the 22nd day was 1500 mg L^{-1} for *D. abundans* LGMM0013, whereas for *T. obliquus* LGMM0001 it was 980 mg L^{-1}. Therefore, *D. abundans* LGMM0013 produces a higher amount of dry biomass compared to *T. obliquus* LGMM0001.

Values of carbohydrates and lipids were obtained by freeze-dried biomass from the 22nd day (see Figure 2). Accumulation of carbohydrates in *D. abundans*

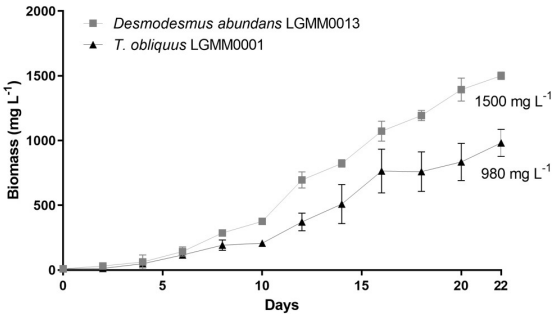

Figure 1. Growth comparison in autotrophic conditions between *D. abundans* LGMM0013 and *T. obliquus* LGMM0001 by dry biomass production. Bars indicate standard deviation.

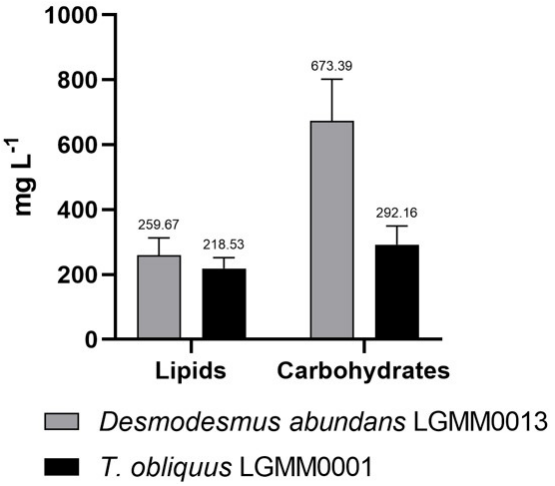

Figure 2. Lipids and carbohydrate production of *D. abundans* LGMM0013 and *T. obliquus* LGMM0001 under autotrophic conditions after 22 days. Bars indicate standard deviation.

LGMM0013 was 673.39 mg L^{-1}, 130% higher than *T. obliquus* (LGMM0001) that was 292.16 mg L^{1}.

For lipids, *D. abundans* LGMM0013 shared similar results to *T. obliquus* (LGMM0001) (see Figure 2). *D. abundans* LGMM0013 manages to match lipid content (259.7 mg L^{-1}) due to its high biomass production (see Figure 2).

3.2. Growth under different culture medium composition

Treatments with the addition of $NaHCO_3$ as a carbon source showed high accumulation of lipids along with the treatment 1 (control). Treatment 8 using a combination of nitrogen deprivation and addition of $NaHCO_3$ accumulated 207.48 mg L^{-1} of lipids in its biomass, closer to the treatment 1 that was 194.04 mg L^{-1}. Treatments with the addition of $NaHCO_3$ as a carbon source did not show a higher accumulation of biomass than autotrophic cultures, without a carbon source (see Table 1).

Highest accumulation of total carbohydrates occurred in two scenarios, using 50% nitrogen deprivation in the culture medium (treatment 2) and with the addition

of 1000 mg L^{-1} of $NaHCO_3$ in the medium without nitrogen deprivation (treatment 4), 688.44 mg L^{-1} and 641.79 mg L^{-1} respectively.

Treatment with a reduction of 50% of nitrogen source and no addition of $NaHCO_3$ (treatment 2) had the most significant biomass recovery by the 16th day with 1034 mg L^{-1}. The same treatment resulted in one of the highest percentages of carbohydrates in dry biomass (688.44 mg L^{-1}/ 66.58%), alongside treatment 4 (641.79 mg L^{-1}).

3.3. LGMM0013 identification and species description

Through optical and Scanning Electron Microscopy (SEM) (see Figure 3), LGMM0013 was characterized as *Desmodesmus abundans* (Kirchner) E.H.Hegewald. The *Desmodesmus abundans* description is given below (Hegewald, 2000).

Desmodesmus abundans (Kirchner) E.H.Hegewald Algological Studies, v. 96, p. 1, 2000.

Solitary cells elliptical. Coenobia with 2 to 4 cells arranged linearly. Cells elliptical, rounded poles, 5.2–6.2 µm long and 1.5–4 µm wide. Coenobia and solitary cells with one spine at each pole (see Figure 3A, 3C, 3D, 3F) and few short spines. Two celled colonies with 4 to 6 short spines irregularly distributed on the cell surface (see Figure 3B, 3E). Cell walls smooth with longitudinal ridges and one or two chimney-like rosettes per cell (see Figure 3D). Chloroplast parietal, with single pyrenoid.

The population studied here is morphologically similar to *D. abundans* morphotype IIIa presented by Hegewald and Schnepf (1991), differing by the shorter cell length of our specimens (5.2–6.2 µm), compared to *D. abundans*

Table 1. Growth conditions and dry biomass, lipids, and carbohydrates from *Desmodesmus abundans* LGMM0013 after 16 days of cultivation.

Growth conditions	$NaHCO_3$ in CHU medium (mg L^{-1})	$NaNO_3$ in CHU medium (mg L^{-1})	Dry Biomass (mg L^{-1})	Lipids (mg L^{-1})	Lipids (%)	Carbohydrates (mg L^{-1})	Carbohydrates (%)
1 (control)	0	2.5	880 ± 0.01	194.04* ± 8.06	22.05%	194.22 ± 38.94	22.07%
2	0	1.25	1034* ± 0.17	178.16 ± 8.44	17.23%	688.44* ± 87.73	66.58%
3	0	0.625	896 ± 0,01	167.64 ± 14.07	18.71%	258.59 ± 43.28	28.86%
4	1000	2.5	930 ± 0,01	153.36 ± 10.09	16.49%	641.79* ± 122.34	69.01%
5	1000	1.25	890 ± 0.01	168.21 ± 4.58	18.9%	453.54 ± 43.04	50.96%
6	1000	0.625	617 ± 0.02	144.50 ± 10.41	23.42%	371.62 ± 21.01	60.23%
7	1500	2.5	629 ± 0.01	112.28 ± 7.57	17.85%	414.89 ± 38.16	65.96%
8	1500	1.25	912 ± 0.03	207.48* ± 7.13	22.72%	445.42 ± 44.16	48.84%
9	1500	0.625	775 ± 0.03	175.85 ± 9.61	22.69%	485.00 ± 62.62	62.58%

*Significantly higher ($p < 0.05$).

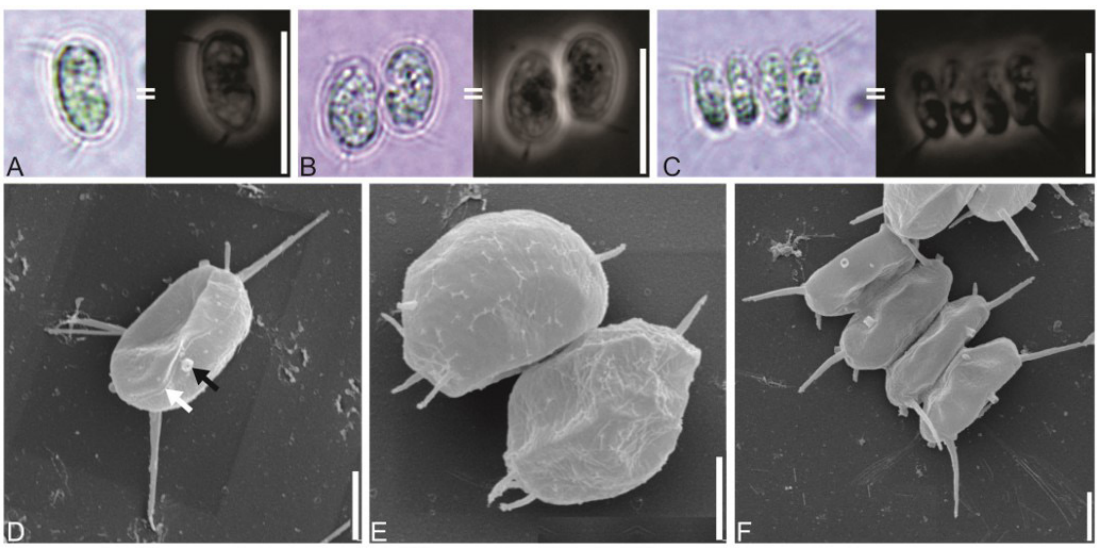

Figure 3. Morphology of *D. abundans* LGMM0013 by optical microscopy (A-C) and scanning electron microscopy (D-F). From A to C scale bar (black) equivalent to 10 µm. A: single cell. B: Coenobium with two cells. C: Coenobium with four cells. From D to F scale bar equivalent to 5 µm. D: Single-cell, presence of spines and tubes. E: Coenobium with two cells, presence of thorns. F: Coenobium with four cells, presence of spines and tubes. Black arrow: chimney-like rosettes; white arrow: longitudinal ridges.

morphotype IIIa (7–9.2 µm). However, according to Hegewald and Schnepf (1991), *D. abundans* morphotypes include cells with smaller length (4.5–38 µm), thus admitting our population in the species.

Desmodesmus abundans morphologically resembles *D. spinosus* and *D. subspicatus*. However, *D. abundans* differs by having a longitudinal ridge, one or two chimney-like rosettes per cell, and smaller measures of cell length and width. *D. spinosus* has a median spine on the outer cells of the coenobia, beyond the apical ones. In addition, on the internal cells lateral spines are arranged in series and may or may not have apical spines on the coenobia. *D. subspicatus*, a species very similar to *D. spinosus*, is distinguished by one or two simple rosettes decorating each cell (Hindak, 1990).

Morphological comparison between *Desmodesmus abundans*-like species was presented in Table 2.

The obtained ITS sequence of *D. abundans* LGMM0013 (GenBank accession number: ON191015) was used for phylogenetic analysis. Based on the phylogenetic tree, using sequences of ITS region of *Desmodesmus* species (see Figure 4), the *D. abundans* LGMM0013 strain is closely related to *D. abundans, D. spinosus* and *D. asymetricus* species, corroborating with the morphological data.

Table 2. Morphological comparison between similar species of *Desmodesmus*.

	***D. abundans* LGMM0013**	***D. abundans* morphotype IIIa**	***D. abundans***	***D. spinosus***	***D. subspicatus***
Coenobia	Linear	Linear	Linear	Linear	Linear, slight alternate
Nº. cells	1-2-4	1-8	2-4-8	2-4 (8)	(1) 2-4-8
Marginal cell shape	Elliptical	Elliptical	Rounded, cylindrical-fusiform, elliptical	Oblong, elliptical, cylindrical, long-ovoid	Oblong, elliptical, oval
Median cell shape	Elliptical	Elliptical	Oblong-cylindrical, cylindrical-fusiform, elliptical	Oblong, elliptical, cylindrical	Oblong, elliptical, oval
Poles	Rounded	Rounded	Obtusely-rounded, rounded	Rounded or conical	Rounded
Length (µm)	2,2-6,2	7-9,2	4,5-38	5-20	4,5-12
Width (µm)	1,5-4	1,8-4,8	2-15	2-8	2,4-7
Seta	Present	Present	Present	Present	Present
Seta - nº/cell	2	2	2	2	2
Spines - nº	2-6	Many	5-10	2-4	1-10
Spines - measure	Large	Large	Large	Short to Large	Short to Large
Spines	Disordely, across cell surface	Disordely, across cell surface	In series or disordely, ridges or across cell surface	In series and apical , across cell surface	In series and apical, poles and margin the marginal cells or across cell surface
Ribs	Present	Present	Present	Absent	Absent
Ridges	Absent	Absent	Present	Absent or present	Absent
Rosette	Present	Present	Present	Absent	Present
References	This work	Hegewald and Schnepf, 1991	Kirchner, 1878; Wolle, 1887; Hansgirg, 1888; Kutzing, 1849; Printz, 1914; Smith, 1916, Korshikov, 1953; Shihira and Krauss, 1965; Hegewald and Schnepf, 1991; Domingues and Torgan, 2012; Godinho et al., 2010; Oliveira, 2015; Shubert et al., 2014	González, 1996; Godinho et al., 2010; Rosini et al., 2013; Chodat, 1926; Komarek and Fott, 1983; Hindak, 1990; Kim, 2015; Hentschke and Torgan, 2010; Moresco and Bueno, 2007; Patil, 2013; Rosini et al., 2013	González, 1996; Hentschke and Torgan, 2010; Correa et al., 2019; Chodat, 1926; Komarek and Fott, 1983; Hindak, 1990; Hentschke and Torgan, 2010; Shubert et al., 2014; Tsarenko et al., 2005

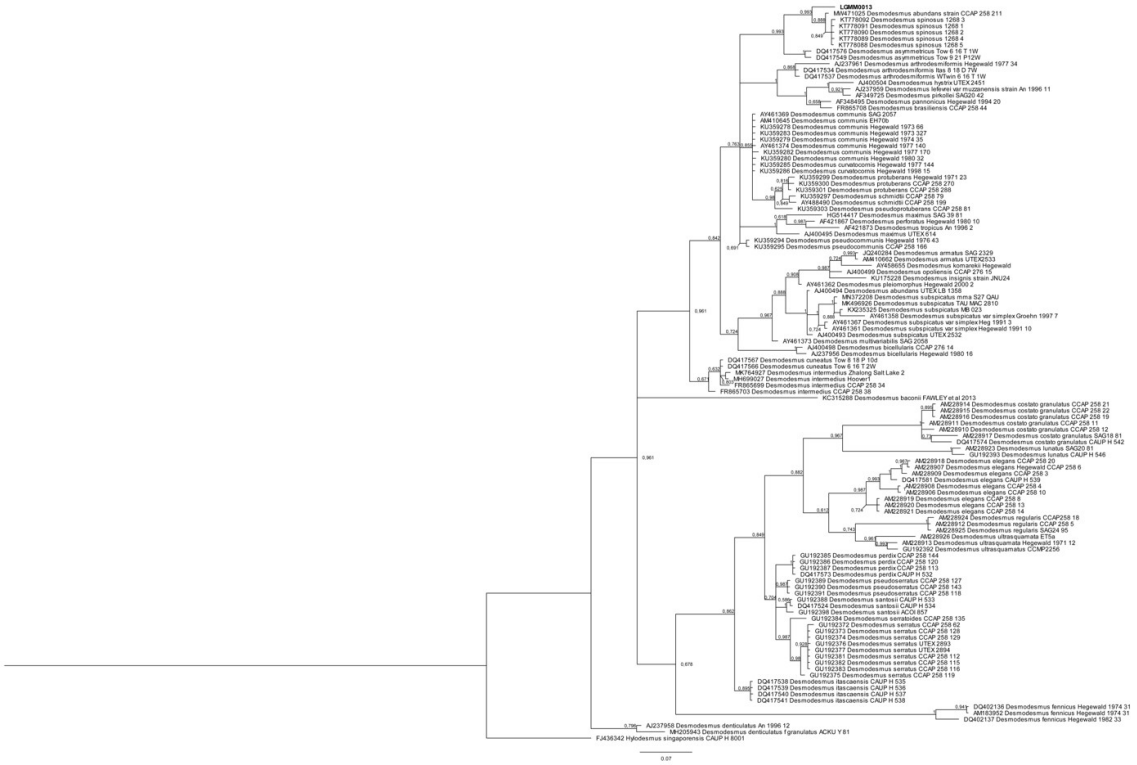

Figure 4. Phylogenetic analysis of *Desmodesmus* species by Bayesian inference using ITS sequences. Posteriori probability values are left from nodes.

4. Discussion

In this work, we isolated a new strain of the genus *Desmodesmus* from the Iraí Reservoir, Brazil. Autotrophic conditions allowed *D. abundans* LGMM0013 a recovery of biomass 50% larger than *T. obliquus* LGMM0001. The mixotrophic cultivation resulted in a biomass production up to 5 times greater than autotrophic cultivation since this condition support an overcome of possible limitations imposed by photosynthesis (Patel et al., 2021). Mixotrophic cultivation reduces the energy source's dependence, allowing improvement of growth and macromolecules production by microalgae, using both the photosynthesis and oxidative phosphorylation mechanisms (Pandey et al., 2018).

Another widely explored point is the induction of stressors to the cultivation of microalgae, a factor that enhances the production of macromolecules, especially lipids. One of the main forms of stress is caused by nitrogen limitation in growth conditions (Converti et al., 2009). This response may be related to the great adaptive capacity found in several species belonging to the Scenedesmacea (Ismagulova et al., 2018).

Here, we explore the adaptability and response of *D. abundans* LGMM0013 in culture using sodium bicarbonate as a carbon source along with changes in concentrations of nitrogen, inducing stress by nutrient limitation. *D. abundans* LGMM0013 showed higher production of carbohydrates (688.44 mg L^{-1}) and biomass (1034 mg L^{-1}) in treatment with a 50% reduction in nitrogen concentration. The 50% reduction in $NaNO_3$ was associated with accumulating carbohydrates in the cell (González-Fernández and Ballesteros, 2012). A similar result was observed with the addition of 1.5 g L^{-1} of sodium bicarbonate without altering the nitrogen concentration, a condition in which 641.79 mg L^{-1} of carbohydrates and 930 mg L^{-1} of biomass were recovered.

Our results revealed that *D. abundans* LGMM0013 responds positively to different growth conditions. In addition, it shows its ability to modulate this response. The accumulation of lipids was similar to that observed for *T. obliquus* LGMM0001 (see Figure 2), reaching 207.48 mg L^{-1} of this metabolite in the dry biomass, with 1.5 g L^{-1} of sodium bicarbonate coupled with reduction (50% less) of nitrogen. Sipaúba-Tavares et al. (2020) compared photoautotrophic and mixotrophic cultivation of *Messastrum gracile* (Chlorophyceae), and observed more than a twofold increase in the dry biomass lipid content (19%), under mixotrophic cultivation.

Rizza et al. (2017) explores the ability of *Desmodesmus* for carbohydrate production and its application in biohydrogen production, besides addressing the importance of selecting strains with high productivity of macromolecules to reduce the costs of applying microalgae in the biofuel industry. The same authors managed to convert 81.4% of sugars to bioethanol from biomass of a *Desmodesmus* strain, showing this genus can produce this biofuel. Microalgae with superior biomass production capacity and adaptation

to different environments can be an alternative to enable a process that, until now, faces the challenge of imposing high economic costs.

The isolation of new strains from the environment also involves the process of identification, which allows for a deeper understanding of their physiology (Li et al., 2015). For a long time, the identification of microalgae was based only on morphological analysis. However, due to microalgae morphological plasticity, the application of molecular identification techniques is essential (Zou et al., 2016). For the molecular identification of microalgae, ITS region sequencing is often used (An et al., 1999), and was used to elucidate many doubts regarding the identification at the level of species in genera such as *Desmodesmus* and *Tetradesmus*, for example (Hegewald, 2000; Hegewald and Wolf, 2003).

Morphological analysis showed that LGMM0013 was identified as *D. abundans* (as shown in Figure 3 and Table 2). The molecular analysis confirmed this result, where LGMM0013 is closer to *D. abundans*, *D. spinosus* and *D. asymmetricus* (see Figure 4). It is important to note that there are two *D. abundans* sequences in the phylogenetic tree, a CCAP 258/211 sequence that was close to the strain studied in this work and another UTEX LB 1358 that appeared on a distant branch. Both come from important microalgae collections, however the UTEX strain was identified only by molecular analysis and no morphological description was found by us (Lara-Gil et al., 2014). The CCAP strain (originally Hegewald 1977-222) was isolated and identified morphologically by Hegewald (Hegewald and Schnepf, 1991). The use of only one technique for the identification of microalgae is not enough and can lead to inaccurate results and this is still a common practice in microalgae identification. Therefore, it is important that future works include cross-analysis of molecular and morphological characteristics to obtain more accurate results.

Tests carried out in this study shown that *D. abundans* LGMM0013 has the potential for the production of molecules with applicability in the biotechnology industry of biofuels, denoting its potential to produce bioethanol. The results observed in this work provide a basis for future studies and substantial knowledge of *D. abundans* LGMM0013 physiology.

5. Conclusion

In this work, a new strain of the species *Desmodesmus abundans* LGMM0013 was isolated from the Iraí Reservoir, Brazil. Through morphological and DNA sequences analysis it was possible to identify this strain at the species level. Laboratory analyzes were performed to better understand the physiology of this strain and quantify molecules of biotechnological interest. The comparison of *D. abundans* LGMM0013 with the *T. obliquus* LGMM0001 allows denoting the high potential of *D. abundans* LGMM0013 for biomass production for different purposes, like biofuels. The main conclusions of this study are: *i*) it was confirmed through morphological and DNA sequences analysis that the microalgae LGMM0013 belongs to the species *Desmodesmus abundans*; *ii*) autotrophic conditions allowed *D. abundans* LGMM0013 a recovery of biomass 50% larger than *T. obliquus* LGMM0001; *iii*) *D. abundans* LGMM0013 showed an accumulation of carbohydrates 130% larger than *T. obliquus* LGMM0001; *iv*) nitrogen deprivation was correlated with an increase in the production of biomass and carbohydrates in *D. abundans* LGMM0013, denoting its potential to produce bioethanol, and *v*) preliminary tests carried out in this study shown that *D. abundans* LGMM0013 has the potential for the production of molecules with applicability in the biotechnology industry of biofuels.

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