



High-Quality Draft Genome Sequence of *Pantanalinema* sp. GBBB05, a Cyanobacterium From Cerrado Biome

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INTRODUCTION

Cyanobacteria comprise one of the oldest and most diverse phyla in the Bacteria domain and are recognized for their importance in the biosphere evolution. Members of this phylum can be found in a wide variety of environments reflecting their photosynthetic ability, adaptability to various environmental conditions, and diversified metabolism. Such characteristics make cyanobacteria one of the preferred targets for research on bioactive compounds and new enzymes (Schirrmeister et al., 2011; Dittmann et al., 2015).

Pantanalinema was described as a new genus of the Leptolyngbyaceae cyanobacterial family by a polyphasic approach, which included morphological characteristics, 16S rRNA gene phylogeny, 16S-23S ITS rRNA secondary structures, and physiological characteristics such as adaptability to pH variations (Vaz et al., 2015).

This genus has been described only in Brazilian biomes such as the Pantanal and the Amazon, the first isolates being found in a lake. These *Pantanalinema* isolates were characterized by their ability to grow over a wide pH range (pH 4 to 11) as well as to modify the culture medium pH around neutrality (pH 6 to 7.4). Due to these characteristics, it is thought that this genus can occupy a variety of ecological niches, such as alkaline or slightly acidic water bodies (Vaz et al., 2015; Genuário et al., 2017). Taxonomic classification of *Pantanalinema* isolates requires the use of molecular markers as this genus is morphologically very similar to the recently described genus *Amazoninema*, which, in turn, has comparable morphology to other genera of the Leptolyngbyaceae family (Genuário et al., 2018).

In this work, we report the genome sequence of a new *Pantanalinema* strain, named GBBB05, which was isolated from the Brazilian Cerrado biome. This is the first genome assembly for the *Pantanalinema* genus, which, along with the analyses provided here, is expected to enhance our understanding of this genus's metabolic potential.

Value of Data

Pantanalinema is a recently described new genus of cyanobacteria. Here we describe the first genome of a strain in this genus. The high-quality draft genome assembled from an environmental culture shows the feasibility of this method, especially for cyanobacteria samples that are underrepresented in metagenomic samples and difficult to obtain from axenic cultures. The reported genome will allow further understanding of this species' biology.

RESULTS

Total DNA isolated from a non-axenic unialgal culture of strain GBBB05 was sequenced using the MiSeq-Illumina platform. The sequencing generated 8,728,802 reads, and after quality control, *de novo* assembly and binning of 5,745,014 quality-filtered PE reads allowed the recovery of eight bacterial genomes with completeness varying from 50 to 99.5% (**Supplementary Table 1**). With the initial taxonomic placement using GTDB-Tk (Chaumeil et al., 2020) and the module “classify_bins” from Metawrap (Uritskiy et al., 2018) (**Supplementary Table 2**), one of the recovered genomes (GBBB05) is from cyanobacteria (Leptolyngbyaceae family) while the other seven are from heterotrophic bacteria.

The cyanobacterial genome of the GBBB05 strain was assembled into 94 contigs and has an estimated size of 7,181,771 bp and a GC content of 48.43% (**Table 1**). The genome showed high completeness (99.05%) and low contamination (0.4%). Selected features of the GBBB05 genome are presented in **Table 1** and **Supplementary Table 1**.

Based on the morphology shown in the culture, the GBBB05 strain was assigned to either *Pantanalinema* or *Amazoninema* genera for which there are no genome sequences currently available. *In silico* DDH values with reference genomes from the Leptolyngbyaceae family ranged from 18.4 to 22.8% (**Supplementary Table 5**). Nevertheless, phylogenomic analysis grouped GBBB05 with one representative genome of the genus *Leptolyngbya* with 100% bootstrap support (**Figure 1A**). Phylogenetic reconstruction using Bayesian inference of partial 16S rRNA sequences from Leptolyngbyaceae shows that GBBB05 belongs to the *Pantanalinema* genus (**Figure 1C**).

Pan-genome analysis revealed that the *Pantanalinema* sp. GBBB05 shares a set of 1,624 core genes (~28% CDSs) with the reference strains *Leptolyngbya* PCC 6306, *Leptolyngbya* JSC 12, and *Geitlerinema* PCC 7407 (**Figure 1B** and **Supplementary Table 6**).

The functional prediction performed by the RAST annotation found about 25 functional categories (**Figure 1D**). Among all the categories, “Carbohydrates” with 176, “Amino Acids and Derivatives” with 169, “Cofactors, Vitamins, Prosthetic Groups, Pigments” with 143, and “Protein metabolism” with 135 genes were the biggest group. In the category “Secondary metabolites,” five genes related to plant alkaloids and plant hormones (auxins) were identified. Six genes related to metal (cadmium, cobalt, mercury, and zinc) resistance and two to fluoroquinolones were found.

TABLE 1 | Genome features of *Pantanalinema* GBBB05.

Features	Chromosome
Strain	<i>Pantanalinema</i> GBBB05
Number of contigs	94
L50 value	17
N50 value (bp)	142,797
Completeness	99.05%
Contamination	0.4%
Sequencing coverage	18x
GC content	48.43%
Estimated chromosome size (bp)	7,181,771
Protein-coding genes (CDS) ¹	5,976
rRNAs ¹	6
5S rRNAs	2
16S rRNAs	2
23S rRNA	2
tRNAs	106
ncRNAs	4
Pseudo genes	152
CRISPR ^{2*}	16 sequences
CAS ^{2*}	1 sequence
Phage ^{3*}	1

Genome statistics were obtained through CheckM. ¹Prokaryotic Genome Annotation Pipeline (PGAP); ²CRISPRCasFinder; ³PHAST; *Complete results from analysis with CRISPRCasFinder and PHAST are shown in **Supplementary Tables 2 and 3**.

Analyses using antiSMASH resulted in the prediction of 17 biosynthetic gene clusters (BGCs), which included the following: 5 clusters for terpene production; 4 bacteriocin clusters; 1 cluster for betalactone biosynthesis; 1 cluster for resorcinol production; 1 mixed module of polyketide synthase (PKS) type 1 and non-ribosomal peptide synthetase (NRPS) related to nostophycin; and 6 clusters containing NRPS biosynthetic pathways (**Supplementary Table 7**).

Analysis in the NaPDoS server, using the default setting, predicted 11 CDSs related to metabolite production in domain C pathways and 4 CDSs related to metabolite production in KS domain pathways (**Supplementary Tables 8, 9**).

The PRISM4 analysis identified seven gene clusters: five non-ribosomal peptide, one mixed module of polyketide synthase (PKS) type 1 and non-ribosomal peptide synthetase (NRPS), and one prochlorosin cluster (**Supplementary Table 10**).

MATERIALS AND METHODS

Growth Conditions and Genomic DNA Isolation

The strain GBBB05 was isolated from a waterfall in the outside border of the Chapada das Mesas National Park, Carolina County, Maranhão State, Brazil (S07°02.6575 / W047°30.4508). Aerobic cultivation was performed in the BG-11 medium (Stanier and Cohen-Bazire, 1977) under the illumination of 3–15 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 4 weeks at 28°C. Non-axenic unialgal culture was obtained by the spread plate

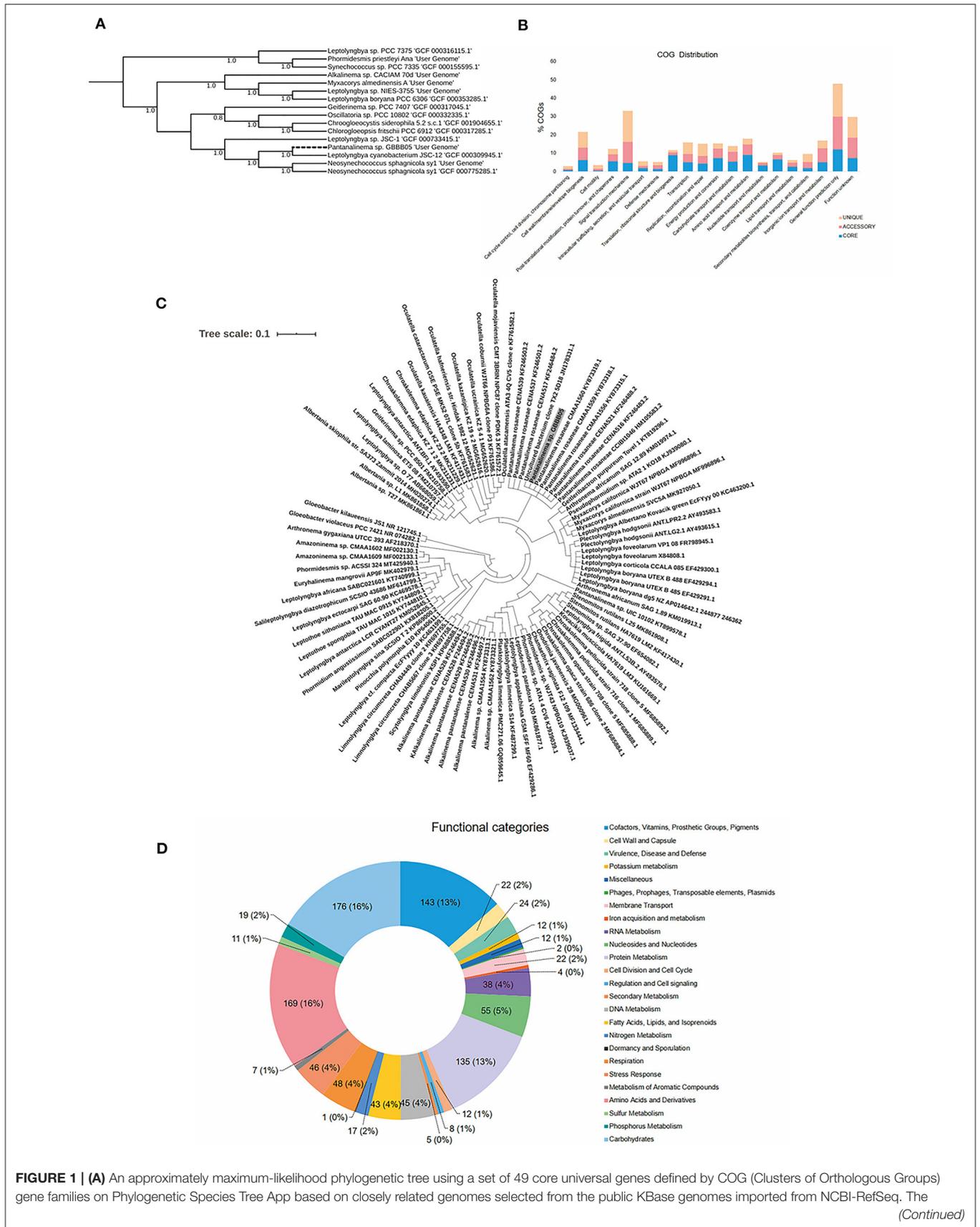


FIGURE 1 | (A) An approximately maximum-likelihood phylogenetic tree using a set of 49 core universal genes defined by COG (Clusters of Orthologous Groups) gene families on Phylogenetic Species Tree App based on closely related genomes selected from the public KBase genomes imported from NCBI-RefSeq. The (Continued)

FIGURE 1 | Jukes-Cantor model of nucleotide evolution was used with 1,000 resamples. *Pantanalinema* GBBB05 is highlighted with a black edge. The numbers beside each node indicate the percentage bootstrap values. Default parameters are used by Species Tree App. **(B)** COG distribution of core, accessory, and unique genes in all four analyzed genomes generated by Bacterial Pan Genome Analysis Tool (BPGA). **(C)** *Pantanalinema* GBBB05 Bayesian tree reconstructed using MrBayes based on partial 16S rRNA sequence (alignment of 1,179 bp) with *Gloeobacter* as the outgroup. Sequences of other Leptolyngbyaceae strains obtained from GenBank are represented with their respective accession numbers. *Pantanalinema* GBBB05 strain is highlighted in gray background. **(D)** Functional categories identified in *Pantanalinema* GBBB05 genome by RAST server. Total number of genes related to each category and its respective percentage in brackets.

method and serial dilutions, and each step was monitored with an optical microscope. Total DNA was extracted from 25 ml of stationary phase non-axenic unialgal culture using the PowerPlant kit (MoBio, California, USA) according to the manufacturer's instructions and stored at -20°C . DNA purity and concentration were evaluated by the absorbance at 260 and 280 nm on a NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA concentration was further quantified with a Quant-iT Picogreen dsDNA assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

Genome Sequencing, Assembly, and Taxonomic Classification

The sequencing library was prepared with 30 ng of total DNA using the Illumina Nextera DNA library preparation kit (Illumina, Inc.) and sequenced with MiSeq-Illumina platform (Illumina, Inc., San Diego, USA) using the MiSeq Reagent kit v2 (500-cycle format). Paired-end (PE) reads were quality-filtered and trimmed (PHRED quality score ≥ 30) with Read_qc module from Metawrap (Uritskiy et al., 2018). *De novo* assembly was performed with the Metawrap Assembly module using metaSPAdes v. 3.13 (Nurk et al., 2017). Assembled contigs were subjected to three different binning rounds using CONCOCT (Alneberg et al., 2014), MaxBin2 (Wu et al., 2016), and MetaBAT2 (Kang et al., 2019). The results were compared using the Bin_refinement module, and the highest-quality bins were selected. The genome statistics were obtained through CheckM (Parks et al., 2015). Digital DNA-DNA hybridization (DDH) values were calculated using the Genome-To-Genome Distance Calculator (GGDC) server (Meier-Kolthoff et al., 2013, 2014). GTDB-TK (Chaumeil et al., 2020) and Classify_bins from metaWRAP (Uritskiy et al., 2018) were used to do the genomic classification (Supplementary Table 1). All parameters were kept at default values.

Phylogenetic Analysis

A phylogenomic tree of strain GBBB05 closely related genomes was constructed using the Species Tree App present in KBase (Arkin et al., 2018). Further phylogenetic analysis was performed using the GBBB05 16S rRNA partial sequence retrieved from its genome assembly plus 89 partial 16S rRNA sequences from various strains of the Leptolyngbyaceae family and *Gloeobacter* genus, retrieved from the NCBI. The nucleotide substitution model used was SYM+G, determined by PAUP 4.0b10¹ and MrModeltest 2 (Nylander, 2004). The tree was constructed using MrBayes 3.2.6 (Ronquist et al., 2012), running with 10⁷ generations, sampling every 100th iteration. TRACER 1.6

(Rambaut et al., 2018) was used to check the performance of tree construction with all parameters at default settings.

Genome Annotation and Functional Analyses

The genome assembly was submitted to the Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016) at the National Center for Biotechnology Information (NCBI). The CRISPRCasFinder server (Couvin et al., 2018) was used to predict CRISPR sequences and CAS genes, and the PFAST web server tool (Zhou et al., 2011) was used to identify the phage-related sequences (Table 1). Pan-genome analysis was performed using the Bacterial Pan Genome Analysis Tool (BPGA) (Chaudhari et al., 2016), with default parameters. To predict gene clusters related to secondary metabolite production, the online servers AntiSMASH 5.0 (Blin et al., 2019) and PRISM4 (Skinnider et al., 2017) were used with all their options enabled. For the detection of C domains and KS domains, the NaPDos online server pipeline was used with its default configuration (Ziemert et al., 2012). Prediction of functional categories was performed using RAST server annotation at default settings (Aziz et al., 2008; Overbeek et al., 2014).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: NCBI [accession: PRJNA560270].

AUTHOR CONTRIBUTIONS

HD, AS, JS, KS, DF, and LD conceived and supervised this study. RS, MO, and PM collected and established the enriched cultures of *Pantanalinema*. LF, AB, IR, PS, RR, CM, and AL performed sequencing and bioinformatic analysis. LF, AB, HD, AS, JS, EG, DF, and LD wrote and revised the manuscript. All authors read and approved the final manuscript.

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¹ Available at: <http://phylosolutions.com/paup-test/>.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2021.639852/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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