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Identification of cyanobacteria in Lagoa Salgada by metagenomic approach, Rio de Janeiro, Brazil

Thiago Corrêa de Almeida[a*], Rodrigo Jardim Monteiro da Fonseca[b], Priscila Gonçalves Moura[a], Elvira Carvajal[c], Nelson Peixoto Kotowski-Filho[b], Adriana Sotero-Martins[d]

[a] *Programa de Pós-Graduação em Saúde Pública e Meio Ambiente, Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, Rio de Janeiro, 21041-210, Rio de Janeiro, Brasil. * almeida.thiago.bio@gmail.com*

[b] *Instituto Oswaldo Cruz, Fundação Oswaldo Cruz Rio de Janeiro, 21040-360, Rio de Janeiro, Brasil.*

[c] *Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 20550-900, Rio de Janeiro, Brasil.*

[d] *Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, Rio de Janeiro, 21041-210, Rio de Janeiro, Brasil.*

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Abstract

Lagoa Salgada is a coastal hypersaline water body located in the north of Rio de Janeiro State and recognized by the presence of stromatolites. Cyanobacteria are the main primary producers of these structures in coastal hypersaline lagoons. The aim of the study was to identify the cyanobacteria present in Lagoa Salgada through metagenomics. The genetic material obtained from a water sample collected in June 2019 was submitted to sequencing by the shotgun method. The metagenomic data were analyzed using the MetaWrap version 1.3 pipeline to identify cyanobacteria and biological processes. The genus *Synechococcus* showed greater abundance, corresponding to 64.3% of the identified cyanobacteria, followed by *Synechocystis* (23.6%), *Geminocystis* (2%), and *Calothrix* (1.8%). The most abundant species were *Synechococcus* sp. RS9909 (46.7%), *Synechocystis* sp. PCC 6714 (16.4%), *Synechococcus* sp. WH 8101 (9.2%), *Synechocystis* sp. CACIAM 05 (4.3%). Thirty-three biological processes associated with genes present in the sample were identified. The Lagoa Salgada has a wide diversity of cyanobacteria in its aquatic ecosystem that is still little explored, justifying the need for protection and preservation of this lagoon environment.

Keywords: Biological processes; coastal ecosystem; hypersaline environment; stromatolites; metagenomics.

Identificação de cianobactérias na Lagoa Salgada por abordagem metagenômica, Rio de Janeiro, Brasil

Resumo

A Lagoa Salgada é um corpo aquático hipersalino costeiro localizado no norte do Estado do Rio de Janeiro e reconhecido pela presença de estromatólitos. As cianobactérias são os principais produtores primários dessas estruturas em lagoas hipersalinas costeiras. O objetivo do estudo foi identificar as cianobactérias presentes na Lagoa Salgada por meio da metagenômica. O material genético obtido de uma amostra de água coletada no mês de junho de 2019 foi submetido ao sequenciamento pelo método *shotgun*. Os dados metagenômicos foram analisados utilizando o pipeline MetaWrap versão 1.3 para identificação das cianobactérias e dos processos biológicos. O gênero *Synechococcus* apresentou maior abundância correspondendo por 64,3% das cianobactérias identificadas, seguidos por *Synechocystis* (23,6%), *Geminocystis* (2%) e *Calothrix* (1,8%). As espécies mais abundantes foram *Synechococcus* sp. RS9909 (46,7%), *Synechocystis* sp. PCC 6714 (16,4%), *Synechococcus* sp. WH 8101 (9,2%), *Synechocystis* sp. CACIAM 05 (4,3%). Foram identificados 33 processos biológicos associados aos genes presentes na amostra. A Lagoa Salgada apresenta uma ampla diversidade de cianobactérias em seu ecossistema aquático ainda pouco explorado, fundamentando a necessidade de proteção e preservação deste ambiente lagunar.

Palavras-chave: Processos biológicos; ecossistema costeiro; ambiente hipersalino; estromatólitos; metagenômica.

Introduction

Coastal lagoons are shallow aquatic ecosystems under marine influence but isolated from the sea by a physical barrier or connected to it by one or more inlets, where exchanges of water and organisms are restricted (Pérez-Ruzafa, Marcos, & Pérez-Ruzafa, 2011). They are important for their biological, geological, physical, and chemical characteristics (Cataudella, Crosetti, & Massa, 2015), besides being exploited by mankind for the development of various activities, such as transportation, food supply, mining, and recreation (Pérez-Ruzafa, Marcos, & Pérez-Ruzafa, 2011).

Lagoa Salgada is characterized as a hypersaline coastal aquatic body located in the northern region of the state of Rio de Janeiro. This ecosystem has great potential for geopaleontological studies due to the presence of microorganisms responsible for the formation of stromatolites, especially cyanobacteria.

Cyanobacteria are the main primary producers in inland hypersaline lakes, coastal hypersaline lagoons, and other environments with higher salt concentrations than seawater, with several unicellular and filamentous non-heterocytic species identified in such environments (Oren, 2015).

In hypersaline aquatic environments, the metagenomic approach, which is characterized by sequencing and analyzing the genomic DNA of non-culturable organisms, combined with the analysis of entire environmental genomes (Lapidus & Korobeynikov, 2021), has allowed the generation of detailed information on microbial diversity and on the metabolic activities of the microorganisms existing in such environments (Ventosa, Haba, Sánchez-Porro, & Papke, 2015).

This study aimed to identify the cyanobacteria present in the aquatic environment of Lagoa Salgada through metagenomics.

Materials and Methods

Study area

Lagoa Salgada is a hypersaline aquatic lagoon located on the northern coast of the state of Rio de Janeiro (41°00'30"W and 21°54'10"S), near Cabo de São Tomé, between the municipalities of Campos dos Goytacazes and São João da Barra. Its surface extension is approximately 16 km^2, presenting an artificial connection with the sea through the Açu River (Silva e Silva, Alves, Magina, & Gomes, 2013). With a water body measuring approximately 4.5 km in length and 1.2 km in width in the central area, its depth can reach up to one meter during the period of greatest rainfall (August to September) (Ramos, Araújo, & Oliveira, 2019).

Regarding physical-chemical parameters, the lagoon has a predominantly alkaline pH, with values ranging from 7.7 to 11.5 depending on the seasonal period (Silva, Mansur, & Borghi, 2018); salinity of 64.7‰; and water temperature ranging from 27 to 32 °C between March and April (Silva e Silva *et al*., 2013).

Currently, the Lagoa Salgada region presents a scenario of development of agricultural activity and significant urban expansion. Such activities have been promoting changes in the physical and hydrochemical environment of the lagoon, where the following changes stand out: the removal of stromatolytic structures by the local population; process of silting of the margins; use of pesticides in crops surrounding the lagoon; and increased pollution (Srivastava, 2002). The presence of the Açu Superport Industrial Complex in the region is another factor that contributes to increase the human pressure on the environment.

Sample collections

A sample of 20 liters of surface water was collected during July, 2019, in the area of Lagoa Salgada (21°54'04.5"S 41°01'35.0"W), located between the municipalities of São João da Barra and Campos dos Goytacazes. The water volume was obtained using a polypropylene gallon (20 liters) duly sterilized (Extran 5% detergent rinsed tenfold for its total removal; then rinsed with acetone a.p., rinsed fivefold with deionized water; rinsed with ethanol a.p.; and rinsed threefold with deionized water). The gallon was transported on ice to the laboratory, being stored in a refrigerator at 5 °C for filtration and DNA extraction.

Filtration and DNA extraction

The collected water was submitted to a vacuum filtration process (Suryha 5CFM) using Millipore membranes (0.8 μm, 0.45 μm, and 0.22 μm). The membranes containing the filtered material were subjected to DNA extraction using the DNeasy Power Water kit (Quiagen). The total DNA from each membrane was extracted following the manufacturer's protocol and instructions.

Sequencing of the extracted DNA

Metagenomic libraries were prepared with Nextera XT DNA and Nextera XT Kits (Illumina, Inc., San Diego, CA, USA), following the manufacturer's instructions. The samples were sequenced using the shotgun method on the Illumina Hiseq 2500 sequencer (Illumina, Inc., San Diego, CA, USA), with sequencing being performed at the Sequencing Platform of the Technology Center for the Chemical and Textile Industries - SENAI CETIQT.

Metagenomic data analysis

For metagenomic data analysis, the methodology described in the MetaWrap pipeline, version 1.3, was used (Uritskiy, DiRuggiero, & Taylor, 2018).

First, in the metaWRAP-Read_qc module, the raw sequences of the sequencer (reads) were evaluated in relation to sequencing quality using the Phred index above 20 as the cutoff score. Then, the sequences passed through the assembly module, with k-mer lengths of 21. The raw and assembled sequences (contigs) were taxonomically profiled in the Kraken module, version 2, and the results were generated by the Krona plot, which presented the interactive taxonomic representations of the microbial community. After the assembly was carried out, the sequences were forwarded to the binning module, being consolidated into a single complete set. The evaluation of integrity and contamination potential was performed. The Reassemble_bins module was used to reassemble the reads, improving the quality of the generated set.

The reassembled sequences were analyzed using the Annotate_bins module to annotate the gene functions present in the sample.

The results of the analyzes were visualized in the software KronaTools, version 2.7, and REVIGO Gene

Ontology treemap. The abundance level was obtained using descriptive statistics (percentage).

Results and Discussion

After analyzing the results, it was observed that the phylum *Cyanobacteria* presented a total of 141,964,138 sequences, with the order Synechococcales being the majority among its taxonomic level.

Equally evident, the genus *Synechococcus* was the one presenting the highest abundance, corresponding to 64% of the number of identified cyanobacterial sequences. This prevalence of the genus *Synechococcus* may be related to its ability to be distributed and to adapt to different types of environments, such as tropical oceans, coastal and freshwater marine ecosystems, microbial mats in hot springs, and nutrient-rich polar waters (Sohm *et al.*, 2015). In hypersaline lake environments, Clementino *et al.* (2008) detected the genus *Synechococcus* as one of the dominant groups comprising the bacterial community of Lagoa de Araruama (Araruama, RJ). In Lagoa Salgada, the family Synechococcaceae was identified as the one with the highest frequency of species related to microbial composition in the development of stromatolites (Silva and Silva *et al*., 2013). The genus *Synechocystis* was the second most abundant group, representing 23% of the diversity of cyanobacteria found in the sample. This result is superior to that obtained by Fourçans *et al*. (2004) in a shallow hypersaline lake in the Salins-de-Giraud saltern, (Camargue, France), which has similar characteristics to the Lagoa Salgada. In the study, the authors identified that 26.4% of the cyanobacterial population found corresponded to the unicellular type, with the genus *Synechocystis* being responsible for 6.6% of the diversity of the group.

The genera *Geminocystis* and *Calothrix* showed an abundance of 2% and 1.8%, respectively, being significantly lower when compared to the genera *Synechococcus* and *Synechocystis*. The genus *Geminocystis* was identified by Ramos *et al*. (2017) in three hypersaline lagoons of the Araruama lagoon complex (Lagoa de Araruama, Lagoa Pitanguinha, and Lagoa Pernambuco), which are ecosystems that present similar characteristics to Lagoa Salgada. The identification of the genus *Calothrix* was expected, since this group has a wide global distribution, with records in cyanobacterial mats and tropical reefs. Silva and Silva *et al.* (2013) identified species of *Calothirx* in the water and in microbial mats of Lagoa Salgada, corroborating the result obtained.

The other genera identified are listed in Table 1. The percentage of unclassified cyanobacteria was 3%.

Table 1. Classification of cyanobacterial genera identified in the sample according to the number of sequences and abundance.

Genus	Number of Sequences	Abundance (%)
Synechococcus	90.752.615	64.3
Synechocystis	32.648.842	23.6
Geminocystis	1.997.889	2.0
Calothrix	1.920.799	1.8
Nostoc	1.282.132	0.9
Microcystis	643.281	0.5
Candidatus Atelocyanobacterium	358.763	0.3
Oxynema	296.740	0.2
Halothece	276.548	0.2
Planktothrix	257.574	0.2
Gloeothece	220.701	0.2
Crocosphaera	209.298	0.1
Cyanothece	191.671	0.1
Oscillatoria	183.630	0.1
Fischerella	142.574	0.1
Geitlerinema	134.518	0.09
Microcoleus	121.500	0.09
Scytonema	110.399	0.08
Chondrocystis	104.364	0.07
Raphidiopsis	99.049	0.07
Gloeocapsa	82.261	0.06
Nodularia	75.241	0.05
Anabaena	61.882	0.04
Sphaerospermopsis	61.313	0.04

The species *Synechococcus* sp. RS9909 (46.7%) presented the highest abundance in the group of cyanobacteria, followed by *Synechocystis* sp. PCC 6714 (16.4%), *Synechococcus* sp. WH 8101 (9.2%), *Synechocystis* sp. CACIAM 05 (4.3%), *Synechococcus* sp. BMK-MC-1(1.2%), *Geminocystis* sp. NIES-3708 (0.9%), and *Calothrix* sp. PCC 6303 (0.8%). The values referring to the number of sequences of the main species are shown in Table 2.

Table 2. Classification of the main species of cyanobacteria identified according to the number of sequences.

Order	Genus	Main species	No. of sequences
Synechococcales	*Synechococcus*	*Synechococcus* sp. RS9909	65.756.909
		Synechococcus sp. WH 8101	13.318.747
		Synechococcus sp. BMK-MC-1	1.530.004
	Synechocystis	*Synechocystis* sp. PCC 6714	23.379.164
		Synechocystis sp. CACIAM 05	4.985.028
Chroococcales	*Geminocystis*	*Geminocystis* sp. NIES-3708	1.230.249
Nostocales	*Calothrix*	*Calothrix* sp. PCC 6303	1.100.406

When analyzing the functionality of the genes in the sample, 33 biological processes were identified, 14 of which were related to molecular functions. The main biological processes are shown in table 3.

Among the various biological processes identified, cyanobacteria stand out in their participation in the process

of photosynthesis, regulation of nitrogen use, carbon fixation, and in the process of sulfate reduction.

Cyanobacteria are directly related to the process of formation of microbial mats in coastal hypersaline lagoons. Stal (2012) stated that dense communities of microorganisms are present in such formations, which are likely to have an abundance of microbial metabolism. Glunk *et al*. (2010) highlighted that cyanobacteria, together with aerobic and anaerobic heterotrophic bacteria, efficiently participate in carbon, nitrogen, sulfur, and oxygen cycles in shallow benthic mats. Silva and Silva et. al (2013) stated that the microbial mats in Lagoa Salgada present a green superficial green stratum of variable thickness where organic matter is produced through the metabolic processes of photosynthesis. According to Stal (2012), most of the organic matter produced by photosynthetic CO_2 fixation is released into the sediment, being degraded by chemotrophic microorganisms, among which sulfate-reducing bacteria are particularly prominent. The author also highlighted that the combined activities of cyanobacteria and sulfate-reducing bacteria result in high sulfide and oxygen gradients.

Table 3. Classification of the main biological processes identified in the sample, associated with the respective numbers of molecular functions.

Biological processes	Number of molecular functions
Metabolic processes with folic acid	33
Dephosphorylation	31
Sodium-dependent phosphate transport	21
Metabolic process of oligosaccharide	12
Binding of protein-phycocyanobilin	9
Carbon fixation *	0
Regulation of nitrogen use *	8
Outer membrane of Gram-negative bacteria	5
Photosynthesis *	0
Antibiotic biosynthesis	4
Amino sugar metabolic process	5
Cellular lipid metabolic process	4
Electron transport chain	1
Glycerol ether metabolic process	5
Defense response to the virus	5
Sulfate reduction *	6
Methylation	1
ATP metabolic process	1
Sulfate reduction process	0

* Main biological processes associated with the cyanobacterial group.

The metabolic process of carbon fixation identified in the sample is directly related to the formation of stromatolites existing in Lagoa Salgada. Dupraz *et al*. (2009) reported that the process of their formation occurs through the carbon cycle, in which bacteria transform inorganic carbon into bioavailable organic carbon, resulting in the release of inorganic carbon, which in turn, under alkaline conditions, binds to cations and precipitates mainly as calcium carbonate. This precipitate, together with the sediment grains, can be trapped within bacterial biofilms, forming the lithified layers. The author concluded by pointing out that changes in pH and solubility index can promote mineralization or dissolution of carbonate minerals through microbial cycling of sensitive redox compounds, such as phosphate, nitrogen, sulfur, and other nutrients within the biofilm.

Cyanobacteria also play an important role in the process of regulating the use of nitrogen identified in the sample. According to Baumgarten and Pozza (2021), such microorganisms are considered the main providers of nitrogen for the trophic chains of several aquatic ecosystems through the process of fixing molecular nitrogen gas in the presence of the nitrogenase enzyme. Nitrogenase is inhibited by the supply of ammonium, in a process of self-regulation with the aim of saving energy by organisms that fix molecular nitrogen. All cyanobacterial mats have the ability to fix atmospheric dinitrogen, thus obtaining part of their nitrogen demand, which is limited by physicochemical gradients (Stal, 2012).

Conclusion

Lagoa Salgada has a significant diversity of cyanobacteria in its ecosystem. Species belonging to the genera *Synechococcus* and *Synechocystis* presented greater abundance in relation to the others identified. The biological processes associated with cyanobacteria are directly related to the formation of microbial mats present in the lagoon. The metagenomic approach was significantly effective, allowing the identification of groups of cyanobacteria still unknown in the lagoon environment. The microbiological diversity present in Lagoa Salgada justifies the need for integral protection of the lagoon environment, contributing to the preservation of its biological richness.

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