



## Bacterial community dominance in a sewage-driven eutrophic coastal lagoon by next generation sequencing: initial findings

Dominância da comunidade bacteriana em uma lagoa costeira eutrófica induzida por esgoto utilizando sequenciamento de nova geração: observações iniciais

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**Abstract:** This study investigates the presence of bacterial dominance in one of the most studied sewage-driven eutrophic coastal lagoons, the Imboassica Lagoon in Macaé (RJ), Brazil, utilizing high-throughput sequencing of 16S rDNA. Water samples were collected from three sites within the lagoon. Total microbial DNA was extracted, and the V3-V4 region of the 16S rRNA gene was amplified and sequenced on the Illumina MiSeq platform. A total of 744,879 partial 16S rRNA sequences were clustered, revealing the absence of a single bacterial dominance in the sewage-driven eutrophic coastal lagoon. The prominent phyla detected in the lagoon were *Cyanobacteria* (27.8%), *Proteobacteria* (23.7%), and *Actinobacteria* (14.6%). *Proteobacteria* emerged as the most abundant phylum in the sewage-impacted lagoon site, whereas *Cyanobacteria* dominated the other two sampling sites. Among families, *Synechococcaceae* predominated with genus *Synechococcus* exhibited the highest prevalence. Families of potentially toxic *Cyanobacteria* represented less than 1% of the total families. The sewage-impacted lagoon section displayed greater bacterial diversity and richness. The dominance of bacterial communities associated with raw sewage, such as members of the *Enterobacteriaceae* family, was not confirmed, constituting only 0.75% of the families in the most affected site. This study presents the initial analysis of the bacterial community in the Imboassica Lagoon and suggests that dominance in the lagoon responds to the eutrophication and sewage discharge.

**Keywords:** bacterioplankton community; *Cyanobacteria*; *Proteobacteria*; 16S rRNA sequencing.

**Resumo:** Este estudo investiga a dominância bacteriana em uma das mais estudadas lagoas costeiras eutróficas contaminadas por esgoto, a Lagoa Imboassica em Macaé (RJ), Brasil, utilizando sequenciamento massivo do gene codificador do RNAr 16S. Foram coletadas amostras de água de três locais na lagoa. O DNA microbiano total foi extraído e a região V3-V4 do gene RNAr 16S foi amplificada e sequenciada na plataforma Illumina MiSeq. Um total de 744.879 sequências parciais do gene RNAr 16S foram agrupadas, revelando a ausência de uma dominância bacteriana única na lagoa costeira eutrófica influenciada por esgoto. Os filos predominantes detectados na lagoa foram *Cyanobacteria* (27,8%),

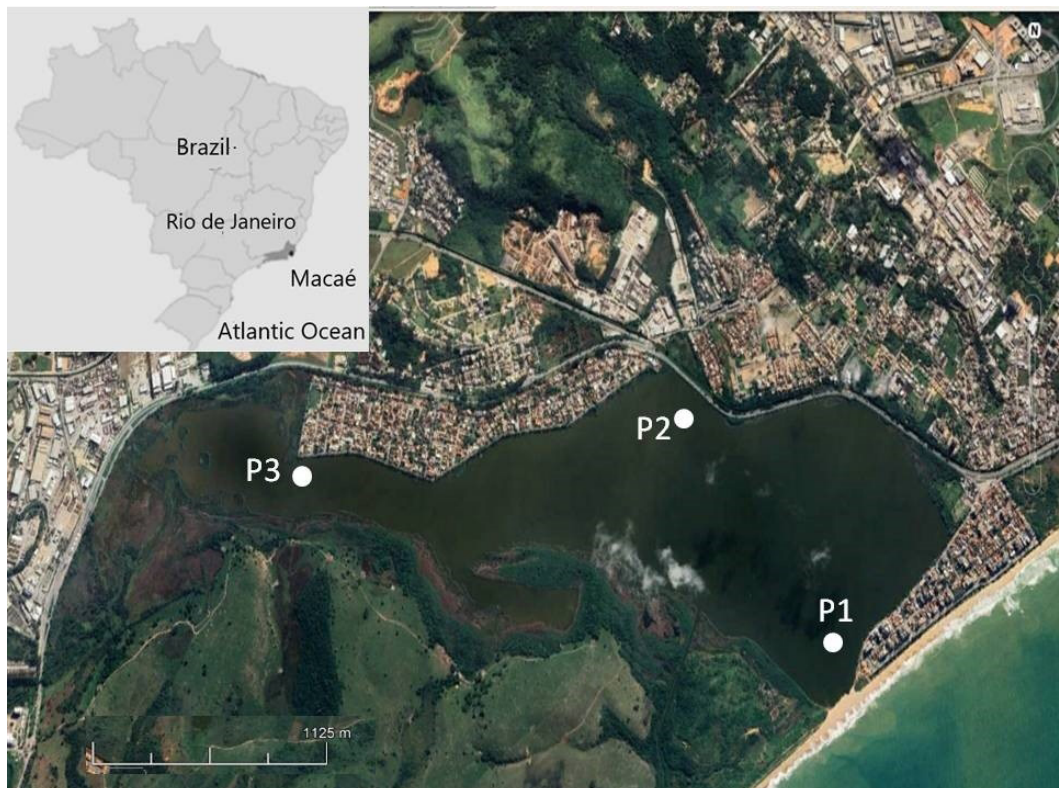


*Proteobacteria* (23,7%) e *Actinobacteria* (14,6%). *Proteobacteria* emergiu como o filo mais abundante no local da lagoa impactado pelo esgoto, enquanto *Cyanobacteria* dominou os outros dois locais de amostragem. A família de maior prevalência encontrada foi a *Synechococcaceae*, sendo representada pelo gênero *Synechococcus*. Famílias do filo *Cyanobacteria* consideradas potencialmente tóxicas representaram menos de 1% do total de famílias. A região da lagoa impactada pelo esgoto exibiu maior diversidade e riqueza bacteriana. A dominância de comunidades bacterianas associadas ao esgoto bruto, como membros da família *Enterobacteriaceae*, não foi confirmada, a qual representou somente 0,75% das famílias no local mais afetado. Este estudo apresenta a análise inicial da comunidade bacteriana na Lagoa Imboassica e sugere que a dominância responde a eutrofização e descarga de esgoto.

**Palavras-chave:** comunidade do bacterioplâncton; *Cyanobacteria*; *Proteobacteria*; sequenciamento RNAr 16S.

Coastal lagoons are prone to continuous discharge of untreated sewage, resulting in heightened nutrient concentrations that, in turn, trigger eutrophication and algal bloom, creating an environmental imbalance that negatively impacts the microbial community (Campaneli & Molisani, 2019; Wang et al., 2022). The knowledge of diversity and structure of bacterial communities is favored by the 16S rRNA gene sequencing technique which has been applied to provide a more comprehensive and wide bacterial profile in environmental samples (Xie et al., 2022). The bacterial dominance hypothesis in the sewage-driven eutrophic lagoons was tested in the coastal Imboassica lagoon where water quality

and limnological dynamic has been assessed since the 1980. These studies have documented the transition from clear and oligotrophic waters to turbid and eutrophic conditions caused by raw sewage discharge in the Imboassica lagoon, culminating in the current highly eutrophic state with intermittent algal blooms (Farias et al., 2019). Thus, this study investigates if there is bacterial dominance in the sewage-driven eutrophic coastal lagoon by using the 16S rRNA gene sequencing technique. In May 2015, surface water samples were collected from Imboassica Lagoon, located in the Macaé municipality (RJ), Brazil. Triplicate samples were acquired using sterile 1-liter flasks from three designated sampling sites (Figure 1).



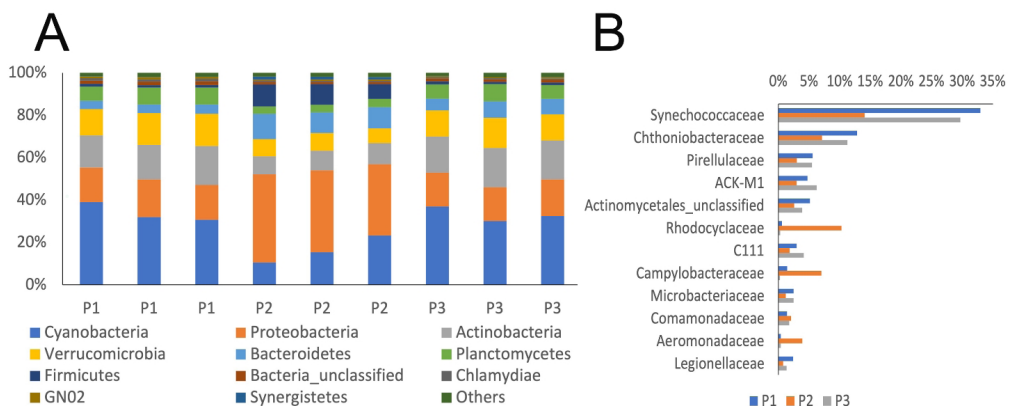
**Figure 1.** Location of the Imboassica lagoon and sampling sites (modified from Google Earth).

These sites corresponded to areas monitored by the long-term ecological monitoring project (PELD) and represented distinct influences in the lagoon (marine P1, 22°24'55"S and 41°49'03.58"S; sewage discharge P2, 22°24'24.5"S and 41°49'31.9"S; Imboassica River inflow P3, 22°24'35.63"S and 41°49'52.59"S).

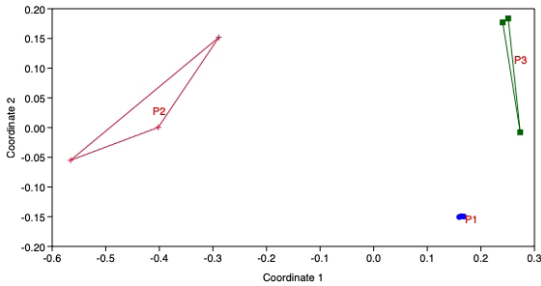
Subsequently, the samples were subjected to filtration (using AP20 filters with pore sizes of 0.45 µm and 0.22 µm), and DNA was extracted from the collected cells utilizing the Metagenomic DNA isolation kit for water (Epicentre®). Amplicon libraries were constructed and quantified using the Nextera XT index (Illumina) and KAPA library quantification kits (Kapa Biosystems), respectively. The targeted region for amplification was the V3-V4 region of the 16S rRNA gene (Klindworth et al., 2013). After normalization and pooling, the samples were sequenced on the Illumina MiSeq platform. To analyze the data, the paired-end reads (2 x 250 bp) were joined using Mothur v. 1.44.0 (Schloss et al., 2009). The sequences were deposited in the NCBI and can be downloaded from the Bioproject under PRJNA949180. Low-quality reads were filtered out using the following criteria: maxambig = 0, maxlength = 470, minlength = 365, homopolymer <8. Taxonomic classification was performed using the Greengenes database (v13\_8) with a confidence threshold of 80%. Sequences classified as singletons, not within the Bacteria domain, or identified as Chloroplast or Mitochondria were removed from the dataset. The normalized sequences were used to generate a distance matrix and were clustered into operational taxonomic units (OTUs) with a sequence similarity cutoff of 97%. The statistical significance of alpha-diversity at each sampling

point was assessed using ANOVA and Tukey's post hoc test, using R software v. 4.2.2. The normality of residuals was investigated with a Shapiro–Wilk test and the homogeneity of variance was determined with Levene's test. To visualize the OTU relative abundances, an ordination plot was generated using non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity coefficients. The statistical significance of sample grouping was evaluated using PERMANOVA, employing Past software v. 3.24 (Hammer et al., 2001).

After the exclusion of low-quality sequences, a resulting set of 744,879 partial 16S rRNA sequences was clustered into 7,069 operational taxonomic units (OTUs). The predominant phyla observed in the lagoon were *Cyanobacteria* (27.8%), *Proteobacteria* (23.7%), and *Actinobacteria* (14.6%) (Figure 2A). To assess the structure of bacterial communities, a non-metric multidimensional scaling (NMDS) was employed, revealing a clustering pattern of samples based on their spatial sampling points. This was further corroborated by PERMANOVA analysis ( $F=18.96$ ;  $p=0.0035$ ) (Figure 3). The sampling points less impacted by sewage (P1 and P3) exhibited similar communities, primarily dominated by the phylum *Cyanobacteria*, followed by *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Planctomycetes* (Figure 2A). Conversely, the sampling point most impacted by sewage (P2) displayed a dominance of the phylum *Proteobacteria*, succeeded by *Cyanobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* ( $p<0.0001$ ). Generally, the family abundance was predominantly represented by *Synechococcaceae*, followed by *Chthoniobacteraceae* at sampling points P1 and P3, while *Rhodocyclaceae*, *Chthoniobacteraceae*, and *Campylobacteraceae* were more prevalent at sampling point P2 (Figure 2B).



**Figure 2.** Relative bacterial community abundance of three sampling sites in the Imboassica lagoon (A: phyla; B: Most abundant families >2%). Marine (P1), sewage discharge (P2), and Imboassica river inflow (P3).



**Figure 3.** Non metric multidimensional scaling ordination based on Bray-curtis similarity of data from OTU abundance in the samples from the three sampling points: Marine (P1), sewage discharge (P2), and Imboassica river inflow (P3).

Diversity was assessed using the Shannon index, while richness was determined by the number of OTUs. The Shannon diversity values were 5.18, 4.43, and 4.14, and the bacterial richness was 3429, 2392, and 2085, respectively, for sites P2, P3, and P1, with the greatest richness and diversity found in the sewage-impacted site (P2) ( $p < 0.01$ ). The Shannon and richness values observed in the Imboassica lagoon were consistent with those typically found in eutrophic lentic environments (Shen et al., 2019). Similar results in  $\beta$ -diversity were described by Nascimento et al. (2018) in sewage sludges, showing *Proteobacteria* > *Bacteroidetes* > *Firmicutes* as the most abundant phyla. However, previous studies often associated eutrophication and sewage discharge with a decrease in bacterial diversity (Xie et al., 2022; Shen et al., 2019; Wang et al., 2022) which is an apparent contradiction when observed the higher diversity in the sewage-impacted site of the Imboassica lagoon. This could be explained by the influx of microorganisms via raw domestic effluent mixed with bacterial communities from natural and urban runoff, as well as treated sewage from a waste treatment plant that drains into this lagoon part. The study of Xie et al. (2022) discovered an increase in the richness of the bacterial community along a river section that receives raw sewage but it is mixed with treated sewage, as well as other water inputs characterizing a river self-purification that contributes to the increase in microbial diversity.

Past research has demonstrated that a lake's trophic status plays a pivotal role in shaping the taxonomic structure and diversity of planktonic microbial communities (Shen et al., 2019; Li et al., 2022). Considering the significance of the *Cyanobacteria* phylum in Imboassica Lagoon, its average dominance (27.8%) was lower than

values reported for other eutrophic lagoons, where *Cyanobacteria* can make up to 46% of the total phyla (Ji et al., 2018). The predominant family in Imboassica Lagoon was *Synechococcaceae*, accounting for an average of 26% of the total taxonomic group, primarily represented by the genus *Synechococcus*. Other potentially toxic families like *Pseudanabaenaceae*, *Cyanobacteriaceae*, and *Microcystaceae* constituted less than 1% of the total families detected. The *Cyanophyceae* class played a secondary role in Imboassica Lagoon prior to extensive sewage discharge and eutrophic conditions (Melo et al., 2007). After the lagoon's eutrophication, Bergamin (2020) identified 30 cyanobacterial taxa by microscopic analysis, including 17 toxin-producing species, such as *Microcystis* sp. and *Synechocystis* sp., which were also identified in our study. Beyond *Cyanobacteria*, *Proteobacteria* can also dominate lentic environments (Shen et al., 2019; Wang et al., 2022), as observed in Imboassica Lagoon. *Proteobacteria* is one of the most diverse and abundant phyla of microorganisms, contributing to essential ecological functions like organic matter degradation (Meyer et al., 2016). The presence of *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* has been linked to sewage discharge (Nascimento et al., 2018; Shi et al., 2021; Xie et al., 2022). Within this phylum, various pathogens such as *Vibrio*, *Helicobacter*, *Campylobacter*, *Escherichia*, and *Salmonella* are also found (Xie et al., 2022). However, bacterial communities belonging to this phylum, particularly those related to raw sewage, such as members of *Enterobacteriaceae*, constituted only 0.28% of total sequences, with a higher occurrence at the P2 sampling site (0.75%), where sewage discharge is well-documented (Farias et al., 2019). The presence of *Proteobacteria* in other sampling sites may also indicate the diffuse raw sewage influence from faulty septic tanks and dump in the watershed's soil which can contribute to the increase the bacterial biodiversity across the lagoon.

An antagonistic interaction between *Cyanobacteria* and *Enterobacteriaceae* has been described. For instance, microcystin produced by some cyanobacterial species can harm the survival of *E. coli* (Halac et al., 2019). The most abundant family within *Proteobacteria* found at the P2 sampling site was *Rhodocyclusaceae* (Figure 2B), encompassing about 18 genera with diverse physiological roles. Previous studies have reported this family in sewage treatment plants, both polluted and unpolluted waters, soil, and

plant roots (Xie et al., 2022; Nascimento et al., 2018), suggesting an influence not only from natural processes but also from sewage discharge in Imboassica Lagoon. A spatial shift in the predominant bacterioplankton community was observed, correlating with sewage discharge and the eutrophication of the lagoon. Notably, *Cyanobacteria*, *Proteobacteria*, and *Actinobacteria* were the dominant phyla in this context. Subsequent studies could explore the impacts of ongoing sewage treatment and measures aimed at reducing eutrophication and its effects on the bacterial communities within the lagoon.

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