Characterization of Bacterial Communities From The Surface And Adjacent Bottom Layers Water of Billings Reservoir

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Abstract

Here, we describe the microbial diversity and physicochemical properties in freshwater samples from the surface and bottom layer of Billings reservoir in São Paulo state, Brazil. Twenty-two matched samples were characterized using the 16S rRNA gene Illumina MiSeq platform. Taxonomical composition revealed an abundance of *Cyanobacteria* phyla, followed by *Proteobacteria*, with 1903 and 2689 known bacterial genera in the surface and deep-water layers, respectively. *Chroobacteria*, *Actinobacteria*, *Betaproteobacteria*, *Alphaproteobacteria*, *Sphingobacteriia*, and *Acidimicrobiia* were the most dominant classes. Shannon diversity index ranging from 2.3 - 5.39 and 4.04 - 6.86 in the surface and bottom layer, respectively. Among the diverse pathogenic genera identified, *Flavobacterium* was the most predominant genus. Temperature and phosphorus concentration were among the most influential factors in shaping the microbial communities of both layers. Predictive functional analysis suggests that the reservoir is enriched in motility genes involved in the flagellar assembly. The overall results present new information on the significantly altered diversity composition of the bacterial community detected in Billings freshwater reservoir.

Introduction

The Billings reservoir in the State of São Paulo, Brazil, is the largest freshwater storage aquatic body in the São Paulo Metropolitan Region, which covers 127 km², have a total volume of 1228.7 × 10⁶ m³ and a maximum depth of 18 meters [1, 2]. The reservoir has multiple-use, including hydropower generation, water supply for 4.5 million people, and industries, irrigation, fishery, and flood control. The basin has a narrow central body which measures over 20 km and connected and fed by eight branches, namely Rio Grande, Rio Pequeno, Capivari, Pedra Branca, Taquacetuba, Bororé, Cocaia, and Alvarenga rivers [3–5]. The reservoir has undergone considerable changes in its properties since the 1940s. At that time, part of the polluted water from the Tiete River (São Paulo city) was allowed to flow into the reservoir, believing that it would raise the water level to generate electric power [6]. This process, besides the pressure of uncontrolled urban growth, significantly contributed to considerable anthropogenic eutrophication and algal bloom of the reservoir [7].

Reliable access to clean and affordable water is one of the most basic humanitarian goals and is a major global challenge for the 21st century [8, 9]. Water pollution is caused by the discharge of harmful domestic and industrial wastes into surface water bodies like rivers, dams, reservoirs, lakes, and canals due to the lack of or inefficient wastewater treatment plants [10–12].

Reservoirs are critical artificial aquatic bodies of many drinking water supply systems; they can maintain an equilibrium of storing or releasing water, play a central role in the biogeochemical cycling, energy flows, and the recycling of nutrients. The functioning of these cycling is mainly maintained by the inhabiting microorganisms. Despite their potential importance, the structure and functioning of the microbial communities in these ecosystems have received relatively less attention compared to other freshwater bodies, such as natural lakes and rivers [13]. There are very few studies on bacterial community structures and compositions of the surface waters in Brazil [14, 15]. Thus, this study aimed to (i) to explore the microbial communities in surface and bottom layer water along the Billings reservoir using the 16 S rRNA gene-based Illumina MiSeq sequencing (ii) evaluate the presence of potential pathogens in these water samples and (iii) explore the predicted functional profiles of the obtained microbial communities in the basin to determine their role in the ecosystem.

Material And Methods

Study Sites and Sample Collection

The study area covered the entire 127 km² of the Billings reservoir, which is located west of the city of São Paulo at 23° 47'S, 46° 40'W, W, an altitude of 746 m a.s.l. Surface and bottom layer water samples (250 ml each) were collected in July 2019 from 30 locations (about 17 km apart; **Fig. 1**) in triplicate using a Van Dorn sampler as previously described [11]. Surface samples were collected from the side of a boat just below the water surface (10 cm) and bottom- water was collected of approximately 1m above from the sediment. Depending on the locations of collected samples, water depth of the study area from the reservoir ranged from 1.16 m to 13.6 m. A total of 180 samples were collected and transported to the laboratory on ice the same day they collected. For the 16S rRNA gene analysis, duplicate samples from each point were combined in 500 ml sterile bottle and thoroughly mixed. Then, a sub-sample of 40 ml was transferred into sterile 50-ml tube (BD Falcon), centrifuged for 10 min at 5000 g at 4°C, and supernatant was discarded leaving approximately 600 µL of residual liquid. The pellets were frozen at – 80°C until DNA extraction. Water temperature (Temp), pH, dissolved oxygen (DO), and pH were evaluated on-site from each sample using a handheld multi-parameter water quality sonde (YSI Inc./Xylem Inc.) Other variables including turbidity, nitrate (NO₃⁻), sulfate (SO₄²⁻), phosphorus (P), and ammonia (NH₃) were determined from another sub-sample (250 ml) according to the Brazilian standard issued by Environment National Council (CONAMA resolution 357/2005). Table 1–2 provide a list of sample sites and their physicochemical data in both layers.

DNA isolation and library preparation

Total genomic DNA was extracted from 200 µl resuspended pellet using the PowerSoil DNA kit (MO BIO Laboratories™: Carlsbad, CA, USA) as per the manufacturer's instructions. To minimize the potential bias during DNA extraction, each sample was extracted in duplicate and then pooled to quantify their DNA yield with a *Qubit*® fluorometer (Invitrogen, USA). The extracted DNA from each sample was subjected to PCR amplification of the V3-V4 variable region of the 16S rRNA gene using the previously published primers Bakt_341F/Bakt_805R [16] and conditions previously described by our group [17, 18]. Library preparation and massively parallel sequencing (MPS) were performed according to the manufacturer's protocol for paired-end sequencing using MiSeq Reagent Kit v3 as previously reported [11, 17, 18].

Detection of toxin-producing cyanobacterial genes

Three regions of the microcystin synthetase (*mcy*) gene cluster was selected to search for potential microcystins (MCs) producers in Billings samples. Representative samples (n = 15) with cyanobacteria in > 40% of their bacterial communities were selected for the investigation. A different set of specific published primers designed to detect *mcyA* (*mcyA*-Cd_1F; 5'-AAA ATT AAA AGC CGT ATC AAA-3' and *mcyA*-Cd_1R; 5'-AAA AGT GTT TTA TTA GCG GCT CAT-3') [19], *mcyD* (*mcyD*F; 5'-GAT CCG ATT GAA TTA GAA AG-3' and *mcyD*R; 5'-GTA TTC CCC AAG ATT GCC-3'), and *mcyE* gene (*mcyE*-F2; 5'-GAA ATT TGT GTA GAA GGT GC-3' and *mcyE*-R4; 5'-AAT TCT AAA GCC CAA AGA CG-3') [20] were used. The amplification of these fragments were conducted using 50–100 ng DNA template, 2 mM MgCl₂, 0.1 mM dNTPs, 0.5 µM of each primer, and 2.5 U high-fidelity *Taq* platinum DNA polymerase (Invitrogen, Carlsbad, CA) in a MgSO₄ reaction buffer. After an initial denaturation of 5 min at 94°C, 35 cycles of 30s at 94°C, 30s at 55°C, 60s at 72°C and a final extension at 72°C for 5 min were performed. Each PCR included a known cyanobacterial DNA positive control, and an interspersed no DNA template negative control. The amplified product was electrophoresed through 1% (wt/vol) agarose gels containing 0.5 × Tris Borate EDTA, followed by ethidium bromide staining.

Bioinformatics and Statistical Analysis

Base-calling and data quality were initially assessed on the MiSeq instrument using RTA v1.18.54, and MiSeq Reporter v2.6.2.3 software (Illumina Inc., CA). The sequences were analyzed by a pipeline of the 16S Microbiome Taxonomic Profiling (MTP) of the EzBioCloud (<https://www.ezbiocloud.net/>) application. The MTP index is a data unit that represents a single microbiome sample and contains information such as run quality control, taxonomic hierarchy and composition, α and β -diversities. The cleaned valid reads were clustered into operational taxonomic units (OTUs) at 97% sequence similarity and taxonomy was assigned using the EzBioCloud Database update 2019.04.09 with default parameters. A rarefaction curve was created to estimate the indices of bacterial diversity using the OTUs. The ACE, Chao1, and Jackknife α -diversity indices were used to calculate the bacterial richness and the Shannon, Simpson function, and NPSHannon indices to estimate the bacterial evenness in each group using the Wilcoxon rank-sum test. Beta diversity was calculated using the Bray-Curtis dissimilarity distance for revealing differences between surface and bottom layer bacteriome on the based of their OTUs data. The predicted profiles were categorized into clusters of Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology and pathways. The differences in the surface and bottom layer characteristics were investigated between the groups using the t-test. To determine whether the reservoir bacterial compositions are affected by depth status, we performed a principal component analysis (PCA) at genus level using the Bray-Curtis dissimilarity distance implemented in the EzBioCloud tool. Pairwise permutational multivariate analysis of variance (PERMANOVA) test was computed to determine whether there are differences in the bacterial communities between both layers. The enrichment in the assigned taxonomic and functional profiles of the two groups were defined by the linear discriminant analysis (LDA) of the effect size (LEfSe) algorithm. The correlations between bacterial community diversity (richness and evenness) and water properties were assessed using the bootstrap based confidence limits (100 iterations) in PCA implemented in the Past 3.25 package [21]. For all analyses, a *p*-value threshold of 0.05 was considered significant.

For the detection of bacterial pathogens, we considered any bacteria potentially pathogenic if at least one species with a minimum abundance of 10 strains of any genus was categorized as biosafety level 2 or 3 by the American Biological Safety Association (<https://my.absa.org>). The analyzes were performed based on uncultivated microorganisms.

Results

Physicochemical characteristics of water samples

The physicochemical properties of the surface and bottom layer water samples from Billings reservoir at 30 different sites are demonstrated **Tables 1 & 2**. Samples were collected in July 2019 where the temperature was above 20°C and the season was slightly dry. Temperature from both layers ranged from 18.8°C to 22.1°C and DO from 3.5 to 9.5. Both parameters were significantly higher (*p* < 0.5) in

surface water when compared to the bottom layer (Average temperature: 21.1°C versus 20.7°C, Averaged DO: 9.5 mg/L versus 8.4 mg/L), while the phosphorus concentrations showed the opposite patterns. The first PCA axis depicted in **Figs. 2 & 3** explained 43.2% and 46.2% and the second axis explained 28.5 and 36% of the variations of bacterial communities from the surface and bottom layer water, respectively. The PCA component 1 in samples from the surface water showed that NO_3^- , temperature, NH_3 , and SO_4^{2-} are positively correlated with the bacterial diversity whereas the pH, DO, P, turbidity, and depth are negatively correlated (Fig. 2). On the other hand, the PCA component 1 in samples from the bottom layer water demonstrated that the bacterial diversities are positively correlated with NO_3^- and P (Fig. 3).

Bacterial community structure

Of the 30 locations, 22 paired water samples from the surface and bottom layer were successfully amplified, sequenced, and submitted for the 16S rRNA analysis. Up to 100,000 MPS reads from each sample were uploaded, quality controlled, and profiled by the EzBioCloud tool. This resulted in 1,468,033 (Min: 15,766 in B7; Max: 87,913 in B21) and 1,861,126 (Min: 35,189 in B22F; Max: 96,434 in B12F) valid reads in surface and bottom layer water, respectively. The distribution of sequence lengths produced agreed with the amplicon length (Average 427 bp) of the 16S rRNA. The good's coverage estimator of the OTUs in the surface and bottom layer samples ranged from 99.4 to 99.9% and 99 to 99.8%, respectively (**Tables 3 & 4**). Rarefaction curves reached stable values, indicating that the diversity of bacterial populations in both groups were sufficiently covered by the generated sequences (data not shown). The number of OTUs per sample ranged from 305 to 2150 and from 1063 to 5919 in the surface and bottom layer samples, respectively. The median Shannon's diversity index showed no significant difference between the two layers ($p > 0.3$). Both in surface and bottom layer water, the Shannon index of B04 was the highest at 5.39 and 6.19, respectively, indicating abundant community diversity. Our results also showed that the bacteriome of the bottom layer had significantly higher phylogenetic diversity indices than those of the surface layer ($p < 0.005$) (Fig. 4). We found no distinct clusters corresponding to the two groups, implying that the bacterial communities from the surface and bottom layer of the reservoir are not significantly different ($p = 0.131$).

Identification of Billings bacteriome in the surface and bottom layer samples.

We found seven phyla (*Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Planctomycetes*, and *Chlorobi*) were highly abundant in matched samples from both layers. The phylum *Proteobacteria* and *Bacteroidetes* were less abundant in the surface water samples than in the matched bottom layer samples (Fig. 5). Across all the samples, *Chroobacteria*, *Actinobacteria*, *Betaproteobacteria*, *Alphaproteobacteria*, *Sphingobacteriia*, and *Acidimicrobiia* represented the most dominant classes (**Tables 1 and 2 supplementary**). At the order level, 21.9% and 16.9% of OTUs were assigned to *Oscillatoriales* in surface and bottom layer, respectively. The order *Planctophila* accounts for > 12% of all detected OTUs from Billings cyanobacterial sources followed by the Burkholderiales that accounts for > 24%. At genus level, 20.2% and 14.5% of OTUs were assigned to the *Planktothrix* genus within the *Microcoleaceae* family in surface and bottom layer, respectively, and > 8% OTUs were assigned to *Nanopelagicus* genus within the family *Nanopelagicaceae* in both layers. Within the *Cyanobacteria*, *Chroobacteria* are particularly presented as the most abundant group in the reservoir with median relative abundance of 18.96 % and 18.08% of all OTUs in the surface and bottom layer samples, respectively. Differentially abundant taxa between the two water samples were identified using the LEfSe algorithm (minimum LDA score: 2.0). This analysis revealed 126 taxa, including 11 class, 24 families, 29 genera, 24 order, 6 phyla, and 32 species. Of these, two families, four genera, one order, and nine species were significantly abundant and discriminative between the groups (FDR-adjusted p-value < 0.02, **Table 3 supplementary**). The bacterial genera *Stenotrophomonas*, *Achromobacter*, *Comamonas*, *Pseudomonas*, *Acinetobacter*, and *Schlesneria* were highly abundant in the bottom layer compared to the surface samples (FDR-adjusted p-value < 0.05), while the *Nanopelagicus* species was substantially depleted.

Identification of metabolic-functional pathways between surface and bottom layer.

A LEfSe analysis was conducted to identify the most pertinent functional pathways responsible for shaping the Billings bacteriome between both layers. We used the software package PICRUST2 implemented in the EzBiocloud online tool to infer the content of bacterial genes from the data of the 16S rRNA and aggregated relative abundance of functional genes into metabolic pathways. A total of 83 KEGG orthology (KO) terms were predicted from all OTUs detected in matched samples. Of these, seven differentially abundant (FDR-adjusted $P < 0.05$) KO terms between surface and bottom layer water were identified (**Table 4 supplementary**). Most of the differentially abundant predicted KO terms, including protein metabolism, signaling and cellular processes, and cell motility were highest in bottom layer samples. The pathway of the flagellar assembly was also enriched in the bottom water layer group, which suggests that the growth environment for the bacterial communities in the bottom layer water was much better than that of the surface. PICRUST module analyses demonstrated

that microbiota in the bottom layer exhibits increased biosynthesis of tetrahydrofolate (M00841) and C21-Steroid hormone (M00109) while the surface microbiota showed an increase use of Mce transport system (M00670).

Search for pre-defined bacterial groups, pathogens, and cyanotoxin genes

The screening for pre-defined bacterial groups in the surface and bottom layer water of Billings reservoir revealed important taxa associated with the human gut that included the phylum *Proteobacteria* (surface; median abundance value 24.7%, bottom layer; median abundance value 27.7%).

The search for bacterial pathogen in both layers identified several bacterial genera known to be human, animal and/or plant pathogens including *Flavobacterium*, *legionella*, *Staphylococcus*, *Pseudomonas*, *Aeromonas*, *Acidovorax*, and *Acinetobacter*. The *Flavobacterium* (surface; median abundance value 0.65%, bottom layer; median abundance value 0.94%) and *legionella* (surface; median abundance value 0.21%, bottom layer; median abundance value 0.19%) were the predominant genera.

The three selected genes DNA that involved in the biosynthesis of *mcy* (*mcyA*, *mcyE*, and *mcyD*) were successfully amplified in all analyzed samples, confirming the genetic potential of the strains in the reservoir to produce microcystin (data not shown).

Discussion

In the current study, the relatively high Shannon diversity indices of 2.38–5.39 and 4.04–6.86 and the detection of 305–2207 and 1063–5919 OTUs in surface and bottom layer water, respectively, showing a greater level of overall biodiversity. Perhaps the greatest value of biodiversity is attributed to intensive anthropogenic disturbance in the reservoir that possibly led to increased nutrient discharges, which resulted in higher nitrogen and phosphorous concentrations, as evidenced in this study. It has been documented by Dias et al [22] that the influence of the surrounding human activities, surrogated by the human footprint index, on the functional diversity and the trait composition of fish assemblages in Billings reservoir was significantly high. It is possible that other variables that were not included in our study could be contributed to the bacterial community variability in the Billings reservoir. The observed bacterial richness and diversity indices in this study were comparable to those in other reservoirs [23]. The taxonomical composition revealed that the *Proteobacteria* phyla were most common, followed by *Cyanobacteria* and *Actinobacteria*. These results are consistent with other Brazilian studies that describe the microbial communities in the Amazon basin [24–26] and Tocantins River [27] but somewhat different from those previously described for reservoirs in China. For instance, Qu J *et al* [23] used the 16S rDNA Illumina approach and showed that *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes* were the dominant bacterial phyla in the Miyun Reservoir, which is considered the largest man-made reservoir in North China. These differences of dominant phyla may be due to a variety of environmental factors including air, soil, and water pollution, rainfall-induced nutrient fluctuations, and changes in local conditions. The difference may also be attributed to the use of distinct DNA extraction methods and/or primers selection [28]. Although the bacterial richness indices varied little between the surface and bottom layer samples in this study, some specific bacterial groups showed a clear difference. For instance, members of the β -*Proteobacteria* and γ -*proteobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Sphingobacteriales* were significantly abundant in the bottom layer than those in the surface water. Since the water flow within a certain layer could contribute significantly to the displacement of bacterial populations, it is reasonable to assume that the latter could constitute a contribution to the observed bacterial community differences between both layers. It is also conceivable that the phosphorus availability in the bottom layer compared to the surface water could positively influence the growth rates of these bacterial groups [29].

In addition to the seven dominant phyla in Billings reservoir, there were approximately 3.5% and 4.4% of unclassified OTU's labeled as ETC in the surface and bottom layer water, respectively, indicating that as-yet-unidentified bacterial populations with unknown metabolic functions are an important part of the reservoir bacteriome, these findings warrant further metagenomics analysis.

Members of *Cyanobacteria* were detected as the most dominant genus and that all *Cyanobacteria* bloom tested here were toxic. These results lend further support to previous studies that demonstrated Brazilian semiarid reservoirs harbor cyanobacterial communities [15, 30–34]. The presence of these bacteria in high abundance in the reservoir can be linked to uploading nutrients like ammonia and to the increase in water temperature (20.7°C) at the time of sampling. OTUs of these bacteria are known to out-compete other planktonic microbes for nutrients in eutrophic systems [35]. A previous study on the Neuse River, North Carolina, conducted by Paerl recommended that a reduction of 30–40% of NO₃ had the optimum power of minimizing *M. aeruginosa* as a dominant phytoplankter [36]. Concerning temperature, there is a consensus among researchers that water temperature below 20°C is generally considered unfavorable for the development of the common water bloom forming-genera like *Dolichospermum* and *Microcystis*. In contrast to this, our finding showed wide spread of *Microcystis* and *Planktothrix* in the reservoir. There are various effects of increasing numbers of *Cyanobacteria* in freshwater systems including oxygen depletion, fish mortalities and toxicity that poses a wide range of health hazards [37]. Because the

Cyanobacteria detected in this study are capable of producing toxin, their presence in the reservoir poses biggest threats to animal and human health.

In this study, *Proteobacteria* represented a second huge portion of the bacterial population in the reservoir, which is similar to that in other studies [38, 39]. Despite the dominance of *Proteobacteria* in both layers, a significant difference in the composition of these members was observed at detailed in taxonomic analysis. *Betaproteobacteria* were the most detected *Proteobacterial* group in this work, which agrees with other studies [40, 41]. OTUs belonging to the *Betaproteobacteria* class have been associated with anthropogenic activities [42]. The *Alpha* and *Gammaproteobacteria* found in both layers probably indicate an increase of organic and inorganic inputs and phytoplankton production [29, 40].

The *Actinobacteria* phylum was the third most abundant OTUs in the reservoir. OTUs of this phylum are among the most abundant groups in freshwater habitats [40, 43]. Abundances of these microbes inversely correlated with those of *Cyanobacteria* that cause prolonged and irretrievable ecological disturbances to freshwater ecosystems and serve as sentinels of impending ecological damage [44, 45]. OTUs of *Actinobacteria* were less abundant when compared to *Proteobacteria*, which comprised 23.7% and 28.5% of the total bacterial abundance in the surface and bottom layer water of Billings reservoir, respectively. *Actinobacteria*, which are well-recognized soil bacteria [46], are frequently detected in oligotrophic freshwater habitats [47], and are often associated with oligotrophic ecosystems [48]. They have long been known to produce pigments that protect them against UV radiation, which easily penetrates deep into a freshwater habitat [40].

The alpha diversity analysis conducted between the surface and bottom layer revealed high phylogenetic diversity indices of the bacteriome inhabiting the bottom layer. This result may indicate that bacterial communities in the bottom layer have experienced high diversification rates or immigration of multiple lineages that radiated successfully. One factor that may promote higher bacterial diversification in the bottom layer is that this habitat is possibly less extreme than that of the surface layer and permit easier radiations [49]. An examination of Billings's water did not reveal significant differences in microbial community beta diversity between the two layers, suggesting that a core bacteriome exists between both layers of the reservoir.

Application of the LEfSe method demonstrated the presence of *Stenotrophomonas species* as the most significant specific biomarkers in the bottom layer. These species play an important ecological role in the nitrogen and Sulphur cycles and several *Stenotrophomonas species* can engage in beneficial interactions with plants, promoting growth and protecting plants from attack [50]. Since the reservoir contains a huge number of *Cyanobacteria*, the death of many of these bacteria could lead to the release of high content of sulfur-containing amino acids from their cells [51] resulting in sulfur-rich water and thus explain the detection of *Stenotrophomonas species*. However, these conclusions must be interpreted with cautions given the serious limitations of deducing the biofunctions of the reservoir bacteria from 16S rRNA gene sequencing and potential risk of bias.

Many countries in the developing world are experiencing intense contamination of their freshwater resources by bacterial pathogens, which have been caused to various waterborne disease outbreaks [52]. In the current study, we found that pathogenic bacteria were ubiquitous across all the sampled waters in Billings reservoir. Among these pathogens, *Flavobacterium* and *Legionella* genera were the most prevalent in both layers. Although most *Flavobacterium* are harmless, but some are opportunistic or true pathogens and cause disease in animals, plants and humans [53–55]. The majority of *legionella species* are considered pathogenic and have been reported as one of the leading bacterial etiological pathogen of waterborne outbreak in the United State between 2007–2009 [56].

In addition to taxonomic compositions, we identified a pathway containing genes encoding for flagellar assembly that were present at the greater relative abundance in the Billings 16S metagenome. The abundance of flagellar assembly within the bottom layer indicates that the reservoir contains abundant bacterial communities, which utilize flagellum for its locomotion. It is possible that the adhesion process of these bacteria was influenced by environmental factors like pH and higher levels of metals introduced to the reservoir because of the rapid expansion of industry and intense domestic activities.

The main limitation of this study is that we restricted the analysis to samples at a single point in time. While the spatiotemporal data is not yet complete, analysis of the data already in hand is well underway. In addition, we used bacterial DNA genomics for this investigation, which would have revealed the presence of bacterial populations regardless of whether they are dead or alive, culturable cells, or non-culturable cells. Another limitation, inherent to 16S rRNA diversity surveillance by Illumine MiSeq platform, is the fact that the taxonomic and ecological resolution of the used marker gene is simply too low to enable accurate identification of pathogenic bacteria and strains with flagella genes to species level as demonstrated in functional analysis. These results should be amenable to robust metagenomic data, RNA sequencing, and cultivable fractions to expand our understanding of the bacterial strains associated with reservoir, their genes and metabolic pathways. That being said, our findings reveal the extent of microbial diversity in the reservoir, the health hazards on households drinking or use directly of water by residents of the reservoir communities.

Conclusions

In conclusion, this study provides important information about the numerous bacteria inhabiting the Billings reservoir and the combination of environmental that shaped the structure. These results may help pave the way for future studies devoted to control and improve the water quality in the Billings reservoir, which is facing rapid urban development and urbanization.

Declarations

Data Availability

All sequence data described here are available in the online Zenodo repository: <https://doi.org/10.5281/zenodo.4751698>

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Contributions

M.A.M: Data curation, visualization. investigation, methodology. A.N: Investigation, data curation, visualization. R.P: Formal analysis, visualization. J.R.V: Writing - review & editing, visualization. A.J.S.D: Writing - review & editing, visualization. PBC: Formal analysis, visualization, writing - review & editing. S.S.S: Conceptualization, supervision, project administration, funding acquisition.

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Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics Approval

Not applicable

Consent to Participate

Consent for Publication

All authors read and approved the final manuscript.

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Tables

Table 1 Physiochemical characteristics of water samples from the bottom layer of Billings reservoir.

Sample ID	DO* mg/l	Temp	pH	Average Water Depth (Km)	Turbidity (NTU)	Sulfate mg/L	Phosphorus mg/L	Ammonia mg/L	Nitrate mg/L
B1F	3.7	20.6	7.4	9.5	4.13	0.4	0.12	2.5	1.1
B2F	6.4	20.8	8.3	5.5	3.03	0.5	0.2	2.3	1.2
B3F	5.1	19.8	7.2	3.2	3.86	0.4	0.23	2.8	1.5
B4F	4	18.8	7	2.1	1.16	0.1	0.14	2.4	1.2
B5F	7.1	21.1	8.2	6.9	2.99	0.6	0.08	2.3	0.05
B6F	7.1	21.7	8.3	12.8	4.38	0.4	0.1	3.2	0.09
B7F	5.6	21.2	8.1	12.4	6.28	0.5	0.09	2.8	1.2
B8F	4.6	21.1	7.9	10.1	8.08	0.2	0.08	2.2	1.1
B9F	4.5	20.9	7.2	9	9.81	0.4	0.043	2.4	0.9
B10F	4.8	21.2	7.6	7.6	13.55	0.5	0.1	3.7	0.8
B11F	3.5	21.2	7.6	5.9	10.3	0.2	0.04	3.1	1.1
B12F	3.5	20.9	7.4	8.1	6.72	0.3	0.04	2.8	0.8
B13F	4.9	20.8	7.7	10.4	10.27	0.5	0.03	3.1	1.2
B14F	8.3	21	8.3	3.9	31.9	0.3	0.02	2.5	0.9
B15F	4.3	20.8	7.5	13.6	6.09	0.5	0.06	2.7	1.2
B16F	8.4	20.6	8.5	10.1	11.5	0.4	1.2	2.3	0.09
B17F	6.3	20.5	7.8	6.9	16.5	0.4	1.1	2.6	1.1
B18F	6	20.3	7.6	8.9	13.1	0.4	0.08	2.7	0.09
B19F	5.9	20.5	7.6	6.1	9.93	0.8	1.1	2.6	1
B20F	5.5	20.4	7.5	4.5	9.87	0.6	2.1	2.4	2.7
B21F	6.6	20.5	7.7	7	8.36	0.3	0.9	2.8	1.1
B22F	7.8	20.5	8.4	5.4	10.2	0.6	1.2	2.7	1.6
B23F	6.9	20.9	8.1	5.8	8.16	0.5	1.3	2.3	1.8
B24F	7.1	20.7	8	5.5	6.79	0.4	0.9	3	2.1
B25F	6.6	20.9	7.9	6.9	6.15	0.5	1.2	3.5	1.9
B26F	7.6	21.3	8	6.8	7.78	0.5	1.9	2.9	2.3
B27F	8.3	20.5	7.8	3.2	3.4	0.6	2.1	3.1	1.9
B28F	7.6	20.5	7.9	7.1	7.19	0.4	1.9	2.9	1.6
B29F	8.2	20.6	8.6	8.7	7.72	0.5	2.1	2.4	1.1
B30F	7.7	20.5	8	10.2	7.6	0.5	0.9	2.3	1.3

*Dissolved
Oxygen

Table 2 Physiochemical characteristics of water samples from the surface of Billings reservoir.

Sample ID	DO* mg/l	Temp	pH	Average Water Depth (Km)	Turbidity (NTU)	Sulfate mg/L	Phosphorus mg/L	Ammonia mg/L	Nitrate mg/L
B1S	4	20.5	7.3	9.5	3.05	0.4	0.08	2.4	0.9
B2S	7.1	21.2	8.3	5.5	3.08	0.5	0.21	2.8	1.1
B3S	5.7	21.2	7.5	3.2	2.5	0.4	0.06	2.8	2.2
B4S	5.5	22.1	7	2.1	1.86	0.6	0.02	2.7	7.4
B5S	7.4	21.5	8.1	6.9	2.51	0.4	0.17	2.7	0.9
B6S	8.3	21.6	8.7	12.8	4.28	0.3	0.32	2.2	1.2
B7S	7.5	21.2	8.2	12.4	6.91	0.3	0.06	2.6	0.9
B8S	7.3	21.1	7.9	10.1	15	0.4	0.27	2.5	1.2
B9S	7.2	21.2	7.6	9	11.5	0.7	0.3	2.5	1
B10S	7.9	21.5	7.9	7.6	18.53	0.3	0.84	2.9	2.2
B11S	5.8	21.5	7.6	5.9	12	0.6	0.12	3	1.8
B12S	4.3	21.2	7.5	8.1	14.1	0.5	0.02	2.6	1.4
B13S	6.7	20.8	7.8	10.4	10	0.5	0.14	2.7	1.9
B14S	9.5	20.9	8.5	3.9	12	0.54	0.12	3.3	1.8
B15S	4.7	20.9	7.5	13.6	6.22	0.56	0.03	2.7	1.7
B16S	9.2	21	8.6	10.1	12.9	0.4	0.11	2.8	1.3
B17S	7.9	20.6	7.7	6.9	11.8	0.6	0.09	2.7	2.3
B18S	6.5	20.5	7.5	8.9	18	0.4	0.15	2.7	2.65
B19S	6.3	20.5	7.6	6.1	7.82	0.5	0.09	2.1	1.3
B20S	6.5	20.9	7.3	4.5	10.5	0.3	0.11	2.6	2.9
B21S	7.7	20.9	7.6	7	12	0.5	0.16	2.8	2.2
B22S	9.4	20.6	8.3	5.4	11	0.5	0.19	2.1	1.1
B23S	9	21	7.3	5.8	6.5	0.4	0.15	2.4	1.8
B24S	7.9	21.3	8	5.5	8.07	0.5	0.04	2.9	1.02
B25S	5.8	21.8	7.6	6.9	5.05	0.5	0.05	3.4	1.98
B26S	7.6	21.6	8	6.8	6.52	0.4	0.11	3.2	0.9
B27S	9.2	20.8	7.4	3.2	5.31	0.3	0.08	2.4	0.8
B28S	9.3	20.6	7.9	7.1	4.79	0.4	0.11	2.8	0.9
B29S	9.3	21.6	8.9	8.7	5.28	0.5	0.08	2.9	1.2
B30S	8.1	20.9	7.8	10.2	8.3	0.4	0.19	2.3	2.8

*Dissolved
Oxygen

Table 3.Valid reads sequenced for each matched sample from the surface layer and alpha diversity indices at the 97% OTU level of 16S rRNA gene fragments.

MTP name*	Valid reads	OTUs	ACE	CHAO	Jackknife	NPSannon	Shannon	Simpson	Phylogenetic Diversity	Good's coverage of library(%)
B4S	83.790	2116	2185	2144	2281	5.4	5.39	0.01	2231	99.8
B5S	84.101	1550	1619	1570	1688	5.3	5.29	0.01	1655	99.7
B7S	92.440	798	852	820	896	5.1	5.08	0.02	1175	99.4
B8S	91.036	1990	2127	2044	2236	4.9	4.80	0.07	2304	99.6
B9S	76.820	433	457	446	473	2.4	2.38	0.40	808	100.0
B10S	60.821	305	358	337	365	2.8	2.83	0.18	685	99.9
B11S	93.392	1943	2112	2014	2229	4.4	4.30	0.10	2320	99.6
B12S	96.434	1777	1920	1839	2021	3.7	3.61	0.22	2264	99.7
B13S	95.249	1531	1674	1603	1761	4.0	3.97	0.15	1874	99.7
B14S	91.409	1673	1811	1726	1902	4.8	4.78	0.04	2020	99.7
B15S	92.202	1730	1848	1779	1941	4.0	3.99	0.17	2058	99.7
B16S	95.132	1635	1789	1699	1879	4.6	4.52	0.06	2024	99.6
B18S	92.605	1926	2054	1967	2156	5.2	5.17	0.02	2258	99.7
B19S	92.066	2003	2104	2032	2203	5.0	4.96	0.04	2308	99.7
B20S	86.364	2085	2233	2132	2347	5.2	5.14	0.02	2377	99.6
B21S	88.101	1660	1758	1704	1841	5.0	5.01	0.02	2235	99.8
B22S	35.189	1735	1902	1813	2009	4.9	4.85	0.03	2102	99.6
B23S	77.192	1825	1927	1855	2015	4.8	4.74	0.04	2120	99.7
B24S	90.412	1353	1404	1371	1464	4.8	4.74	0.03	1844	99.8
B25S	90.585	2072	2177	2103	2282	5.1	5.02	0.02	2213	99.7
B29S	66.790	2150	2283	2195	2407	5.2	5.17	0.02	2307	99.6
B30S	88.996	1526	1586	1548	1654	5.0	4.98	0.02	1932	99.8

* microbiome taxonomic profile

Table 4. Valid reads sequenced for each matched sample from the bottom layer and alpha diversity indices at the 97% OTU level of 16S rRNA gene fragments.

MTP name*	Valid reads	OTUs	ACE	CHAO	Jackknife	NPS Shannon	Shannon	Simpson	Phylogenetic Diversity	Good's coverage of library(%)
B4F	87.428	5142	5581	5323	5940	6.3	6.2	0.01	5300	99.1
B5F	51.832	1630	1789	1718	1877	5.3	5.2	0.02	2250	99.7
B7F	15.766	1398	1477	1433	1544	5.1	5.0	0.02	1859	99.8
B8F	65.835	1700	1813	1759	1902	4.7	4.7	0.07	2247	99.8
B9F	84.508	1529	1628	1580	1709	4.3	4.2	0.12	2149	99.8
B10F	45.319	1287	1381	1340	1452	4.1	4.0	0.15	1968	99.7
B11F	64.536	2112	2280	2197	2394	5.3	5.2	0.03	2802	99.7
B12F	81.047	2035	2188	2122	2306	4.8	4.7	0.06	2874	99.7
B13F	72.290	1717	1843	1780	1933	4.9	4.8	0.04	2323	99.8
B14F	70.107	1672	1854	1768	1942	4.7	4.7	0.05	2297	99.7
B15F	72.382	1789	1885	1832	1971	5.0	4.9	0.05	2301	99.8
B16F	68.314	1402	1506	1455	1577	4.6	4.6	0.04	2053	99.8
B18F	65.941	1920	2046	1970	2144	5.2	5.2	0.02	2532	99.8
B19F	67.777	1792	1922	1853	2014	5.1	5.1	0.02	2440	99.8
B20F	62.497	5919	6302	6084	6649	7.0	6.9	0.01	6001	99.2
B21F	87.913	1917	2097	2001	2205	5.2	5.2	0.02	2588	99.7
B22F	68.781	1063	1155	1112	1214	4.9	4.9	0.02	1731	99.6
B23F	74.190	1530	1616	1569	1696	4.8	4.8	0.03	2244	99.8
B24F	57.108	1842	2023	1926	2122	4.8	4.8	0.03	2530	99.7
B25F	67.702	1659	1815	1744	1901	5.0	5.0	0.02	2345	99.7
B29F	69.843	1356	1447	1403	1520	4.8	4.8	0.03	1990	99.8
B30F	66.917	1673	1845	1761	1934	5.1	5.1	0.02	2354	99.7

*
microbiome
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Figures

Figure 1

Map showing sampling site locations in the Billings reservoir in São Paulo. Locations are indicated by the green Global Positioning System (GPS) symbol. Map was obtained using the Google Earth mapping engine (<https://www.google.com/earth/>).

Figure 2

Bootstrap observation plots derived from principal component analysis (PCA) of the surface water physicochemical properties and a bacterial diversities represented by chao1 and Shannon index based on 100 resampling of the data. The two principal components (PC1 and PC2) explained 71.7 % of total variation in environmental data. Temperature (Temp), dissolved oxygen (DO), phosphorus (P), ammonia (NH₃⁺), nitrate (NO₃⁻). Black symbols in plot indicates sample IDs. Green lines represent environmental parameters, and black circles represent sampling points.

Figure 3

Bootstrap observation plots derived from principal component analysis (PCA) of physicochemical properties and a bacterial diversities represented by chao1 and Shannon index based on 100 resampling of the data in the bottom layer water of the reservoir. The two principal components (PC1 and PC2) explained 82.2 % of total variation in environmental data. Temperature (Temp), dissolved oxygen (DO), phosphorus (P), ammonia (NH₃⁺), nitrate (NO₃⁻). Black symbols in plot indicates sample IDs. Green lines represent environmental parameters, and black circles represent sampling points.

Figure 4

Community diversity represented by phylogenetic diversity index of bacteriome between water samples from the surface and bottom layer.

Figure 5

The relative abundance of A) Proteobacteria B) Bacteroidetes, and C) Phylum level taxonomical abundance of bacteriome between water samples from the surface and bottom layer.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.zip](#)