



# Flood Season Microbiota from the Amazon Basin Lakes: Analysis with Metagenome Sequencing

Célio Dias Santos Júnior,<sup>a</sup> Danyelle Toyama,<sup>a</sup> Tereza Cristina Souza de Oliveira,<sup>b</sup> Fernando Pellon de Miranda,<sup>c</sup> Flávio Henrique-Silva<sup>a</sup>

<sup>a</sup>Laboratório de Biologia Molecular, Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil

<sup>b</sup>Departamento de Química, Universidade Federal do Amazonas, Manaus, Amazonas, Brazil

<sup>c</sup>Centro de Pesquisas e Desenvolvimento Leopoldo Américo Miguez de Mello, Petróleo Brasileiro S.A. (Petrobras), Rio de Janeiro, Rio de Janeiro, Brazil

**ABSTRACT** Despite an apparent geographic separation of the Amazon water bodies, they are an interconnected system. During floods, the microbiota of rivers, lakes, and soil combines. This study used metagenomics sequencing to survey the microbiota of the Amazon Basin lakes during flood season, showing important patterns in microbial communities.

The Amazon Basin is a complex fluvial system reworking almost 3,200 megatons (Mt)/year of floodplain sediment (1), with an annual discharge representing 16% of the freshwater released into the world's oceans (2). Amazon Basin rivers and lakes constitute two separate systems. However, the flood pulse concept (3, 4) shows a connection between the Amazon River and its floodplain, depending on its water level, which can vary more than 14 m between seasons (5). Previous findings (6, 7) revealed *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Crenarchaeota*, as well as some marine clades (e.g., *Ilumatobacter*), to be the predominant taxa in the Amazon Basin.

Although there are previous studies on microbial communities in the Amazon lakes, they used samples collected only during the dry season. Therefore, we performed metagenomics high-throughput sequencing to understand the ecology of microbial communities from the lakes throughout the flood season.

Samples from the three lakes were collected in the morning (9:00 a.m. to 12:00 p.m.) in March 2015 (flood season) from Lake Baixio (03°17'4.69"S, 60°03'24.419"W, at a depth of 1 m below the surface), Manacapuru Great Lake (03°19'1.38"S, 60°47'53"W, at a depth of 2 m below the surface), and Lake Preto (03°21'21.76"S, 60°37'36.76"W, at a depth of 1 m below the surface). The sampling procedures and total DNA extraction were conducted as described previously (7). Metadata included the following: for Lake Baixio, temperature of 29.3°C, pH 6.53, turbidity of 24.63 nephelometric turbidity units (NTU), dissolved oxygen (DO) concentration of 2.77 mg/liter, and biological oxygen demand (BOD) of 2.82 mg/liter; for Lake Preto, temperature of 27.8°C, pH 6.2, turbidity of 16.63 NTU, DO concentration of 1.65 mg/liter, and BOD of 1.76 mg/liter; and for Manacapuru Great Lake, temperature of 28.8°C, pH 6.2, turbidity of 19.43 NTU, DO concentration of 5.6 mg/liter, and BOD of 6.1 mg/liter. Environmental DNA libraries were prepared using a Nextera XT DNA kit (Illumina, San Diego, CA, USA) and sequenced with the HiSeq 2500 platform (Illumina). Sequencing yielded 144 million, 132 million, and 180 million paired-end reads of 2 × 100 bp for Lake Baixio, Lake Preto, and Manacapuru Great Lake, respectively. These reads were quality filtered (Q > 20) and assessed for ambiguities using the next-generation sequencing quality control (NGS QC) Toolkit\_v2.3.3 (Roche, Indianapolis, IN, USA) (8), utilizing default settings. All high-quality sequences for each metagenome were given functional

**Citation** Santos Júnior CD, Toyama D, de Oliveira TCS, Pellon de Miranda F, Henrique-Silva F. 2019. Flood season microbiota from the Amazon Basin lakes: analysis with metagenome sequencing. *Microbiol Resour Announc* 8:e00229-19. <https://doi.org/10.1128/MRA.00229-19>.

**Editor** Frank J. Stewart, Georgia Institute of Technology

**Copyright** © 2019 Santos Júnior et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Flávio Henrique-Silva, [dfhs@ufscar.br](mailto:dfhs@ufscar.br).

**Received** 27 February 2019

**Accepted** 8 April 2019

**Published** 25 April 2019

and taxonomic assignments using the MG-RAST server (Argonne National Laboratory [operated by the UChicago Argonne LLC, Chicago, IL, USA]) (9) at default settings.

The taxonomic composition (percentage of assigned reads) of these metagenomes was 94.0 to 95.2% bacteria, 0.2 to 0.7% archaea, and 0.2 to 0.3% viruses. The phyla observed most often were *Proteobacteria* (abundance, 46.0 to 70.8%), *Actinobacteria* (14.6 to 24.4%), *Cyanobacteria* (2.1 to 13.2%), *Bacteroidetes* (1.2 to 2.0%), and *Firmicutes* (2.1 to 3.4%). Alpha diversity was estimated to be from 281 to 393 species. The functional annotations of the subsystems revealed a predominance of open reading frames (ORFs) related to carbohydrate processing (abundance, 12.7 to 13.5%), protein metabolism (8.6 to 9.0%), and cofactors, vitamins, prosthetic groups, and pigments (7.2 to 7.5%). We observed a predominant ubiquitous genus in all samples, *Polynucleobacter* (from 1.4 to 9.4%), reinforcing its presence in this ecosystem (6, 7, 10, 11). In summary, this metagenome project reveals initial insight about the Amazon lakes' microbial communities during the flood season.

**Data availability.** The sequences obtained in this project have been deposited in the European Nucleotide Archive (ENA) under BioProject number [PRJEB25171](https://www.ebi.ac.uk/ena/browser/view/PRJEB25171).

## ACKNOWLEDGMENTS

This work was supported by Petróleo Brasileiro S.A. (Petrobras) as part of a research agreement (number 0050.0081178.13.9) with the Federal University of São Carlos, São Paulo, Brazil, within the context of the Geochemistry Thematic Network. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, under finance code 001. F.H.-S. is the recipient of a Research Productivity Scholarship from the National Council for Research and Development (CNPq grant 311746/2017-9). C.D.S.J. and D.T. were recipients of a doctoral scholarship from the CNPq.

## REFERENCES

- Mertes LAK, Dunne T, Martinelli LA. 1996. Channel-floodplain geomorphology along the Solimões-Amazon River, Brazil. *Geol Soc Am Bull* 108: 1089–1107. [https://doi.org/10.1130/0016-7606\(1996\)108<1089:CFGATS>2.3.CO;2](https://doi.org/10.1130/0016-7606(1996)108<1089:CFGATS>2.3.CO;2).
- Oltman RE. 1968. Reconnaissance investigations of the discharge and water quality of the Amazon River, p 16. United States Geological Survey, Washington, DC.
- Junk WJ. 1983. Ecology of swamps in the middle Amazon, p 269–294. In Gore AJP (ed), *Ecosystems of the world. Mires, swamp, bog, fen and moor. 3. Regional studies*. Elsevier, Amsterdam, The Netherlands.
- Junk WJ. 1997. General aspects of floodplain ecology with special reference to Amazonian floodplains, p 3–17. In Junk WJ (ed), *The Central Amazon floodplain—ecology of a pulsing system*. Springer-Verlag, Berlin, Germany.
- Beisl CH, Miranda FP, Pedroso EC, Landau L. 2003. Generation of oil sensitivity index information in Western Amazonia, Brazil, using dual season SAR image mosaics of the Global Rain Forest Mapping Project, p 823–830. In *Anais do XI SBSR*, 5 to 10 April 2003. INPE, Belo Horizonte, Brazil.
- Satinsky BM, Fortunato CS, Doherty M, Smith CB, Sharma S, Ward ND, Krusche AV, Yager PL, Richey JE, Moran MA, Crump BC. 2015. Metagenomic and metatranscriptomic inventories of the lower Amazon River, May 2011. *Microbiome* 3:39. <https://doi.org/10.1186/s40168-015-0099-0>.
- Ghai R, Rodríguez-Valera F, McMahon KD, Toyama D, Rinke R, de Oliveira TCS, Garcia JW, Miranda FP, Henrique-Silva F. 2011. Metagenomics of the water column in the pristine upper course of the Amazon River. *PLoS One* 6:e23785. <https://doi.org/10.1371/journal.pone.0023785>.
- Patel RK, Jain M. 2012. NGS QC toolkit: a toolkit for quality control of next generation sequencing data. *PLoS One* 7:e30619. <https://doi.org/10.1371/journal.pone.0030619>.
- Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards RA. 2008. The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9:386. <https://doi.org/10.1186/1471-2105-9-386>.
- Santos-Júnior CD, Kishi LT, Toyama D, Soares-Costa A, Oliveira TCS, de Miranda FP, Henrique-Silva F. 2017. Metagenome sequencing of prokaryotic microbiota collected from rivers in the Upper Amazon Basin. *Genome Announc* 5:e01450-16. <https://doi.org/10.1128/genomeA.01450-16>.
- Toyama D, Kishi LT, Santos-Júnior CD, Soares-Costa A, de Oliveira TCS, de Miranda FP, Henrique-Silva F. 2016. Metagenomics analysis of microorganisms in freshwater lakes of the Amazon Basin. *Genome Announc* 4:e01440-16. <https://doi.org/10.1128/genomeA.01440-16>.