



# Metagenome Sequencing of Prokaryotic Microbiota Collected from Rivers in the Upper Amazon Basin

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**ABSTRACT** Tropical freshwater environments, like rivers, are important reservoirs of microbial life. This study employed metagenomic sequencing to survey prokaryotic microbiota in the Solimões, Purus, and Urucu Rivers of the Amazon Basin in Brazil. We report a rich and diverse microbial community.

The Amazon Basin spans eight countries and occupies approximately 40% of the South American continent (1). This basin holds 17% of the planet's freshwater (2). Moreover, the species richness of its plants and animals is currently estimated to be five times that of North America (3), and more species are being discovered every year (4). However, little is known about its microbiota. Previous studies (1, 5) described the initial microbial landscape of freshwater Amazon rivers being rich in *Actinobacteria*, *Alpha-proteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Crenarchaeota*. Some marine clades, e.g., *Ilumatobacter*, were also reported (5).

The runoff from rivers in this region account for nearly 15% of the world's runoff from rivers. There are two well-established seasons (dry and flood) as described in references 6–8. These complex ecosystems work actively in carbon budget processes (9–11).

Because of the presence of noncultivable microorganisms in environmental samples, it is impossible to isolate many of these organisms and difficult to recreate environmental microbiotas in the laboratory. Thus, the most appropriate approach to gain details regarding the community structure is metagenomics. Despite its importance, data on the microbial diversity of the Upper Amazon Basin are very limited. Therefore, this study aimed to form a landscape of the most frequent species in this environment.

We sequenced total DNA extracted from freshwater samples collected from different rivers in the Upper Amazon Basin. These samples were collected in September 2008 (dry season) from the Solimões (03°17'03.86"S, 60°01'23.04"W), Purus (03°41'07.4"S, 61°28'13.91"W), and Urucu (04°08'08.03"S, 63°29'54.69"W) Rivers. Sampling and total DNA extractions were performed as previously described (1). Total DNA was sequenced on an Illumina HiSeq 2500 platform (Illumina, Inc., San Diego, CA); an average of 300,095,181 paired reads of 100 bp per sampling site were obtained. Reads were quality filtered for ambiguities and homopolymers using the next-generation sequencing (NGS) QC Toolkit (12); bases with Phred quality scores <20 were removed.

Taxonomic assignment was made using the MG-RAST server (13) and revealed that 95.4 to 95.8%, 0.3 to 2.2%, and 1.0 to 1.8% of the assigned reads were bacteria, archaea, and viruses, respectively. Major bacterial phyla observed were *Proteobacteria* (abun-

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dance percentages of 34.9 to 54.5%), *Actinobacteria* (14.9 to 24.3%), *Cyanobacteria* (1.9 to 24.3%), *Bacteroidetes* (2.0 to 6.6%), *Planctomycetes* (1.8 to 8.2%), and *Firmicutes* (2.5 to 3.2%). Archaea were mainly assigned to *Euryarchaeota* (0.2 to 0.5%) and *Thaumarchaeota* (0 to 1.6%). Alpha diversity was estimated to be between 346 and 620 species. Functional annotations revealed that a considerable portion of reads were associated with respiration (3.4 to 3.7%), photosynthesis (0.2 to 0.8%), and phages, prophages, transposable elements, and plasmids (1.9 to 2.2%). The present metagenomic project holds important insights on the metabolic potential of microbial communities from tropical freshwater rivers.

**Accession number(s).** The sequences obtained in this project have been deposited in the NCBI Short Read Archive under the following accession numbers: [SRR1514963](https://www.ncbi.nlm.nih.gov/sra/SRR1514963) (Solimões River), [SRR1515032](https://www.ncbi.nlm.nih.gov/sra/SRR1515032) (Purus River), and [SRR1518285](https://www.ncbi.nlm.nih.gov/sra/SRR1518285) (Urucu River).

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## REFERENCES

- Ghai R, Rodriguez-Valera F, McMahon KD, Toyama D, Rinke R, de Oliveira TCS, Wagner Garcia J, de 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>.
- Maretti CC, Riveros SJC, Hofstede R, Oliveira D, Charity S, Granizo T, Alvarez C, Valdujo P, Thompson C. 2014. State of the Amazon: ecological representation in protected areas and indigenous territories. WWF Living Amazon (Global) Initiative, Brasília, Brazil.
- Kricher J. 1999. *A Neotropical companion: an introduction to the animals, plants, and ecosystems of the new world tropics*, 2nd ed. Princeton University Press, Princeton, NJ.
- Albert JS, Reis RE. 2011. *Historical biogeography of Neotropical freshwater fishes*. University of California Press, Berkeley, CA.
- 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>.
- Camargo M, Giarrizzo T, Jesus AJS. 2015. Effect of seasonal flooding cycle on litterfall production in alluvial rainforest on the middle Xingu River (Amazon Basin, Brazil). *Brazil J Biol* 75:S250–S256. <https://doi.org/10.1590/1519-6984.00514BM>.
- Christoffersen BO, Restrepo-Coupe N, Arain MA, Baker IT, Cestaro BP, Ciais P, Fisher JB, Galbraith D, Guan XD, Gulden L, van den Hurk B, Ichii K, Imbuzeiro H, Jain A, Levine N, Miguez-Macho G, Poulter B, Roberti DR, Sakaguchi K, Sahoo A, Schaefer K, Shi MJ, Verbeeck H, Yang ZL, Araújo AC, Kruijt B, Manzi AO, da Rocha HR, von Randow C, Muza MN, Borak J, Costa MH, Gonçalves de Gonçalves LGG, Zeng XB, Saleska SR. 2014. Mechanisms of water supply and vegetation demand govern the seasonality and magnitude of evapotranspiration in Amazonia and Cerrado. *Agric Forest Meteorol* 191:33–50. <https://doi.org/10.1016/j.agrformet.2014.02.008>.
- Rongxing G. 2006. *Territorial disputes and resource management: a global handbook*. Nova Science Publishers, Hauppauge, NY.
- Ward ND, Keil RG, Medeiros PM, Brito DC, Cunha AC, Dittmar T, Yager PL, Krusche AV, Richey JE. 2013. Degradation of terrestrially derived macromolecules in the Amazon River. *Nat Geosci* 6:530–533. <https://doi.org/10.1038/ngeo1817>.
- Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middelburg JJ, Melack J. 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. *Ecosystems* 10:172–185. <https://doi.org/10.1007/s10021-006-9013-8>.
- Downing JA, Cole JJ, Middelburg JJ, Striegl RG, Duarte CM, Kortelainen P, Prairie YT, Laube KA. 2008. Sediment organic carbon burial in agriculturally eutrophic impoundments over the last century. *Global Biogeochem Cycles* 22:10. <https://doi.org/10.1029/2006GB002854>.
- 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>.