

## MIT Open Access Articles

*Draft Genome Sequence of a Tropical Freshwater Cyanobacterium, Limnothrix sp. Strain P13C2*

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

**Citation:** Tan, Boon Fei, Shu Harn Te, Karina Yew-Hoong Gin, and Janelle R. Thompson. "Draft Genome Sequence of a Tropical Freshwater Cyanobacterium, Limnothrix Sp. Strain P13C2." *Genome Announcements* 4, no. 5 (October 20, 2016): e01117–16.

**As Published:** <http://dx.doi.org/10.1128/genomeA.01117-16>

**Publisher:** American Society for Microbiology (ASM)


**Persistent URL:** <http://hdl.handle.net/1721.1/107220>

**Version:** Final published version: final published article, as it appeared in a journal, conference proceedings, or other formally published context

**Terms of use:** Creative Commons Attribution 4.0 International License



# Draft Genome Sequence of a Tropical Freshwater Cyanobacterium, *Limnothrix* sp. Strain P13C2

Boon Fei Tan,<sup>a</sup>  Shu Harn Te,<sup>b</sup> Karina Yew-Hoong Gin,<sup>b,c</sup> Janelle R. Thompson<sup>a</sup>

Centre for Environmental Sensing and Modelling, Singapore-MIT Alliance for Research and Technology, Singapore<sup>a</sup>; NUS Environmental Research Institute, National University of Singapore, Singapore<sup>b</sup>; Department of Civil and Environmental Engineering, National University of Singapore, Singapore<sup>c</sup>

**A nonaxenic unialgal culture of *Limnothrix* sp. strain P13C2 was obtained through multiple subculturing of an inoculum obtained from a tropical freshwater lake. Here, we report the genome of P13C2 of 4.6 Mbp, extracted from the metagenome of this coculture.**

Received 26 August 2016 Accepted 26 August 2016 Published 20 October 2016

**Citation** Tan BF, Te SH, Gin KY-H, Thompson JR. 2016. Draft genome sequence of a tropical freshwater cyanobacterium, *Limnothrix* sp. strain P13C2. *Genome Announc* 4(5): e01117-16. doi:10.1128/genomeA.01117-16.

**Copyright** © 2016 Tan 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 Janelle R. Thompson, [janelle@mit.edu](mailto:janelle@mit.edu).

*Limnothrix* is a ubiquitous cyanobacterium commonly found in many freshwater and marine environments (1, 2). Research conducted on this genus is limited because the organism is rarely the most abundant species during bloom events—they have been found only occasionally to codominate an algal bloom with other cyanobacterial species (3). *Limnothrix* spp. were not known to be toxin producers in the past until recent studies demonstrated that they cause toxicological effects in aquatic organisms, with symptoms similar to neurotoxin exposure (4, 5). Phylogenetic analysis based on 16S rRNA gene sequences showed that members that have been previously classified as *Limnothrix* are polyphyletic, forming two clades with similarity lower than 90% (6); although the difference at the genomic scale for these strains is unknown.

A filamentous cyanobacterium identified as *Limnothrix* sp. strain P13C2 based on 16S rRNA gene and morphology was isolated and cultivated using methods described previously (7). The nonaxenic unialgal culture of strain P13C2 used in this study was cultivated at room temperature for 2 weeks, after which total DNA was isolated and sequenced using the Illumina HiSeq 2000 platform as previously described (7). Adaptor and bar code sequences were removed from all reads using BBDuk of the BBTTool packages (<https://sourceforge.net/projects/bbmap/>), and the reads were *de novo* assembled into scaffolds using CLC Genomics Workbench V8 with default settings. All quality-controlled unassembled reads were subjected to BLASTX searches against the NCBI NR database using DIAMOND (8) and taxonomic assignment using MEGAN 6 (9), revealing that the metagenome was represented with >50% of reads being assigned to *Cyanobacteria*. The genome of P13C2 was extracted from the minimetagenome using MetaBAT (10), and genome completeness and contamination were determined using checkM (11). The genome was subsequently annotated using the RAST platform (12).

The genome of P13C2 is >99% complete and contained zero sequence contaminant, determined using checkM (11). The genome of 4.6 Mbp is contained in 32 scaffolds ( $N_{50}$  192 kbp) with 55% GC content. A single copy the 16S rRNA gene is 99% to 100%

identical to those in several *Limnothrix* spp. (e.g., *Limnothrix planktonica* CHAB763; JQ004026.2). Comparison to genomes of other *Limnothrix* spp. was not conducted as these genomes were unavailable in the JGI IMG (13) and NCBI genome databases at the time of this analysis. Certain *Limnothrix* spp. strains have been previously found to produce toxin (4, 5), although the gene encoding hepatotoxin (e.g., microcystin, saxitoxin), as well as off-flavor compounds (e.g., geosmin and 2-MIB), were not detected in the P13C2 genome. The genome encodes various pathways for central carbohydrate metabolism, the citric acid cycle, pentose-phosphate pathway, Entner-Doudoroff pathway, and glycolysis, thus suggesting that P13C2 may be physiologically versatile. The genome lacks the gene encoding nitrogenase, suggesting that P13C2 is incapable of nitrogen fixation. The organism likely obtains its nitrogen source through import of nitrogenous compounds using cyanate ABC transporter, nitrate ABC transporter, and ammonium transporter, whereby genes encoding these permeases were detected in the draft genome. In light of current knowledge gaps, additional genomic information on *Limnothrix* sp. is essential for taxonomic revision and evaluation of their potential public health risk.

**Accession number(s).** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [MBRF00000000](https://www.ncbi.nlm.nih.gov/nuccore/MBRF00000000). The version described in this paper is version MBRF01000000.

## ACKNOWLEDGMENTS

We thank the Public Utilities Board of Singapore (PUB) for their collaboration in this project.

This research is supported by the Singapore National Research Foundation under its Environmental & Water Technologies Strategies Research Programme and administered by PUB. B.F.T. and J.R.T. were supported by the National Research Foundation Singapore through the Singapore MIT Alliance for Research and Technology's (SMART) Center for Environmental Sensing and Modeling (CENSAM) research program.

## FUNDING INFORMATION

This work, including the efforts of Shu Harn Te and Karina Yew Hoong Gin, was funded by National Research Foundation Singapore (NRF) (1102-IRIS-14-02). This work, including the efforts of Boon Fei Tan and Janelle R. Thompson, was funded by Singapore-MIT Alliance for Research and Technology Centre (SMART).

## REFERENCES

1. Acinas SG, Haverkamp TH, Huisman J, Stal LJ. 2009. Phenotypic and genetic diversification of *Pseudanabaena* spp. (cyanobacteria). *ISME J* 3:31–46. <http://dx.doi.org/10.1038/ismej.2008.78>.
2. Rucker J, Wiedner C, Zippel P. 1997. Factors controlling the dominance of *Planktothrix agardhii* and *Limnothrix redekei* in eutrophic shallow lakes, p. 107–115. In *Shallow Lakes 1995*. Springer, Netherlands, Dordrecht.
3. Zwart G, Kamst-van Agterveld MP, van der Werff-Staverman I, Hagen F, Hoogveld HL, Gons HJ. 2005. Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake. *Environ Microbiol* 7:365–377. <http://dx.doi.org/10.1111/j.1462-2920.2005.00715.x>.
4. Daniels O, Fabbro L, Makiela S. 2014. The effects of the toxic cyanobacterium *Limnothrix* (strain AC0243) on *Bufo marinus* larvae. *Toxins (Basel)* 6:1021–1035. <http://dx.doi.org/10.3390/toxins6031021>.
5. Humpage A, Falconer I, Bernard C, Froscio S, Fabbro L. 2012. Toxicity of the cyanobacterium *Limnothrix* AC0243 to male BALB/c mice. *Water Res* 46:1576–1578. <http://dx.doi.org/10.1016/j.watres.2011.11.019>.
6. Zhu M, Yu G, Li X, Tan W, Li R. 2012. Taxonomic and phylogenetic evaluation of *Limnothrix* strains (*Oscillatoriales*, *Cyanobacteria*) by adding *Limnothrix planktonica* strains isolated from central China. *Hydrobiologia* 698:367–374. <http://dx.doi.org/10.1007/s10750-012-1127-8>.
7. Mohamed Nor NH, Tan BF, Te SH, Thompson JR, Gin KY. 2016. Draft genome sequence of *Cylindrospermopsis* sp. Strain CR12 extracted from the minimetagenome of a nonaxenic unialgal culture from a tropical freshwater lake. *Genome Announc* 4(1):e01726-15. <http://dx.doi.org/10.1128/genomeA.01726-15>.
8. Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. *Nat Methods* 12:59–60. <http://dx.doi.org/10.1038/nmeth.3176>.
9. Huson DH, Auch AF, Qi J, Schuster SC. 2007. MegaN analysis of metagenomic data. *Genome Res* 17:377–386. <http://dx.doi.org/10.1101/gr.5969107>.
10. Kang DD, Froula J, Egan R, Wang Z. 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. *PeerJ* 3:e1165. <http://dx.doi.org/10.7717/peerj.1165>.
11. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes *Genome Res* 25:1043–1055. <http://dx.doi.org/10.1101/gr.186072.114>.
12. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res* 42:D206–D214. <http://dx.doi.org/10.1093/nar/gkt1226>.
13. Markowitz VM, Chen IM, Palaniappan K, Chu K, Szeto E, Pillay M, Ratner A, Huang J, Woyke T, Huntemann M, Anderson I, Billis K, Varghese N, Mavromatis K, Pati A, Ivanova NN, Kyrpides NC. 2014. IMG 4 version of the integrated microbial genomes comparative analysis system. *Nucleic Acids Res* 42:D560–D567. <http://dx.doi.org/10.1093/nar/gkt963>.