

Draft genome sequence of the anatoxin-a producing cyanobacterium *Tychonema bourrellyi* B0820 isolated from the epilimnion of the deep Alpine Lake Garda

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ABSTRACT We report the draft genome sequence of strain B0820 of the cyanobacterium *Tychonema bourrellyi* isolated from the epilimnion of Lake Garda and assembled from a metagenome of a non-axenic culture. The strain analyzed was shown to produce anatoxin-a, a potent neurotoxin that can cause fatal intoxication in exposed organisms.

KEYWORDS cyanobacteria, *Tychonema*, draft genome, full shotgun, anatoxin-a, lake, non-axenic culture

Following the discovery (1) of anatoxin-a (ATX)-producing strains of *Tychonema bourrellyi* (2, 3) in the epilimnion of the southern Alpine lakes, other ATX-producing *Tychonema* species in freshwater mats were described in Germany, where they caused dog deaths (4, 5).

Tychonema strain B0820 was collected from the epilimnion of Lake Garda (45.69 N, 10.72 E) in August 2020. Under the microscope, a single filament was isolated, washed repeatedly with sterilized Z8 medium, and cultured in the same medium at 20°C with 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ illumination (6). The 16S rRNA (OR088434) and ATX (OR124837) sequences [Sanger sequencing (6)] showed 100% identity with previously reported *T. bourrellyi* genes. Using standard LC-MS methods (7), strain B0820 was shown to produce anatoxin-a.

For metagenomic analysis, to reduce the bacteria in the non-axenic culture, filaments were diluted in 1 L of sterilized Z8 medium, filtered through sterilized 80 μm mesh, resuspended in 100 mL Z8 medium, and filtered through 5 μm cellulose nitrate membranes (Whatman). DNA was extracted using PowerWater Kit (Qiagen). The library was prepared using Kapa HyperPlus Kit (Roche) and processed by 150-bp paired-end sequencing on an Illumina NovaSeq 6000, which generated 98,364,812 paired-end reads. Unless otherwise stated, the software was run with default parameters. Reads were checked with FastQC 0.11.9 (8). Removal of residual adapters and trimming (trimq = 20 maq = 20 maxns = 0 minlen = 35, ftm = 5, tpe, tbo) were performed using bbdutk 38.95 (<https://jgi.doe.gov>). After correction with metaSPAdes 3.15.3 (9) (--only-error-correction), the paired reads were assembled with megahit 1.2.9 (10) (--presets meta-sensitive), yielding 6,411 contigs larger than 1,000 bp, with a total size of 160 Mbp and N50 of 201,376 bp. The draft genomes were binned using CONCOCT 1.1.0 (11), MetaBAT 2 2.12.1 (12), and MaxBin 2 2.2.7 (13), and results combined using DAS Tool 1.1.6 (14). The *T. bourrellyi* bin was checked and confirmed with anvio 7.1 (15). The genome was 5.37 Mbp assembled into 223 contigs, with N50 39,298 bp, GC content 44.6%, and coverage 336x. Using the phylum Cyanobacteria and the order Oscillatoriales as marker lineages, CheckM 1.2.2 (16) estimated a completeness of 98.9% and 99.3%, respectively, with almost no evidence of contamination (0.07% and 0.08%) and no strain heterogeneity. The taxonomy of the draft genome was confirmed by GTDB-Tk 2.1.1 (17) and a

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phylogenomic analysis using PhyloPhlAn 3.0.3 (18), which indicated coincidence (ANI = 99.6) with a strain previously isolated from Lake Garda (19).

The genome annotation was completed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP 6.5) (20), which identified 5,125 coding sequences and 52 tRNAs. After blastn (nr/nt) analysis, the 16S rRNA and *rbcX* genes from the draft genome were 99.9%–100% and 100% identical, respectively, to previously reported *T. bourrellyi* genes. A polyketide synthase protein gene cluster associated with ATX biosynthesis was identified by blastn (99%–100% query coverage) with 93.1% and 93.4% identity to the anatoxin-a synthetase gene clusters of *Anabaena* sp. 37 (JF803645) and *Cuspidothrix issatschenkoii* (LT984882), respectively; the same sequence showed 99.8%–100% identity to *anaF* sequences previously determined in *T. bourrellyi* isolates (query coverage 3%–7%).

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AUTHOR CONTRIBUTIONS

Nico Salmaso, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review and editing | Adriano Boscaini, Conceptualization, Data curation, Investigation, Methodology, Resources, Validation, Writing – review and editing | Leonardo Cerasino, Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Writing – review and editing | Massimo Pindo, Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – review and editing | Federica Pinto, Conceptualization, Investigation, Methodology, Writing – review and

editing | Nicola Segata, Conceptualization, Investigation, Methodology, Writing – review and editing | Claudio Donati, Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Software, Validation, Writing – review and editing

DATA AVAILABILITY

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession [JAVCBQ000000000](https://doi.org/10.1093/bioinformatics/btv638). The version described in this paper is version [JAVCBQ000000000.1](https://doi.org/10.1093/bioinformatics/btv638). The sequences are publicly available under BioProject accession number [PRJNA979152](https://doi.org/10.1093/bioinformatics/btv638), BioSample accession number [SAMN35566361](https://doi.org/10.1093/bioinformatics/btv638), and SRA accession number [SRR25705839](https://doi.org/10.1093/bioinformatics/btv638). Sanger sequences of 16S rRNA and anaF genes of strain B0820 were deposited to GenBank with accession numbers [OR088434](https://doi.org/10.1093/bioinformatics/btv638) and [OR124837](https://doi.org/10.1093/bioinformatics/btv638), respectively.

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