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GENOME SEQUENCES

Metagenome-Assembled Genome Sequence of *Dolichospermum circinale* Strain Clear-D4, Assembled from a Harmful Cyanobacterial Bloom Enrichment Culture

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ABSTRACT *Dolichospermum circinale* (formerly *Anabaena circinale*) is a significant harmful algal bloom species. We report the draft metagenome-assembled genome (MAG) for a strain of *D. circinale* (Clear-D4) obtained from an enrichment culture. The genome sequence comprises 5,029,933 bp in 560 contigs with a GC content of 37%.

Cyanobacterial harmful algal blooms (cyanoHABs) pose environmental, ecological, and economic threats to freshwaters. Species of *Dolichospermum*, a diazotrophic, bloom-forming, heterocystous, cyanobacterial genus, have the potential to produce cyanotoxins, including microcystin, anatoxin, and saxitoxin (1–3). Increasing *Dolichospermum* bloom frequency in recent decades necessitates an improved understanding of this prolific cyanoHAB genus (4). Clear Lake, CA, is 303d-listed for nutrients and experiences annual cyanoHABs (5). To better understand the microbial communities involved in *Dolichospermum* cyanoHABs, we performed metagenomic sequencing on a *Dolichospermum* enrichment culture, resulting in a new metagenome-assembled genome (MAG) for *Dolichospermum circinale*.

Bucket tow surface water samples were collected from Clear Lake (lat 38.973166, long 122.72809) in August 2019. Free *Dolichospermum* trichomes, visualized with a dissecting scope, were pipettor hand-picked and enriched in 50% BG-11_0 medium at 25°C with 100 μmol Q/m^2/s light on a 12:12-h light/dark cycle. Additions of 50% BG-11_0 were introduced every other week to maintain growth. Prior to sequencing, we identified the genus *Dolichospermum* morphologically in the above enrichments with a Zeiss AxioStar epifluorescence microscope (Oberkochen, Germany) (6). A single enrichment culture (Clear-D4) was chosen for metagenomic sequencing. Cell material from the Clear-D4 enrichment (50 ml) was filtered onto 8-μm polycarbonate filters and rinsed into 2-ml bead-beating tubes using lysing solution from the Qiagen DNeasy PowerBiofilm kit (Hilden, Germany). The cells were lysed using 5 liquid N_2 freeze-thaw cycles, followed by the addition of proteinase K and incubation at 55°C overnight. Genomic DNA was then extracted using the aforementioned Qiagen kit protocol. The quality of isolated DNA was verified using Tris-borate-EDTA (TBE) gel electrophoresis and quantified via NanoDrop UV-visible (UV-Vis) spectroscopy and Qubit spectrofluorimetry (Thermo Fisher Scientific, Waltham, MA). Library preparation (i.e., NEBNext DNA library prep kit via the manufacturer's recommendations) and 1 Gbp of Illumina paired-end (PE) 2 × 150-bp sequencing were conducted by Novogene (Nanjing, China) with 300-bp size-selected inserts, generating 19,844,532 reads. KBase and modules therein were used for *de novo* assembly with default settings unless otherwise noted (7). Prior to *de novo* assembly, the quality of the paired-end reads was checked with FastQC v0.11.5 (8), and the reads were trimmed to enhance quality using Trimmomatic v0.36 (9). *De novo* genome assembly was done using metaSPAdes v3.13.0 (10). Binning was completed using MaxBin2 v2.2.4 (11), with refinement using anvi-refine in Anvi'o (12). Genome annotation was completed using

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PGAP with default settings (13). Initial bin taxonomy was determined using GTDB-tk in the PhyloSanity wrapper (14).

Clear-D4 consisted of 560 contigs, 5,029,933 bp, a GC content of 37%, and an N_{50} value of 52,616 bp. A total of 4,813 coding genes were identified (12). CheckM v1.0.18 (15) (with default settings) estimated this initial MAG to be 91.85% complete with 6.47% contamination. The refined genome was phylogenetically confirmed as *Dolichospermum circinale* using GTDB-tk v1.1.1 db_r95 using "classify_wf." The predicted metabolism of Clear-D4 was analyzed using the FuncSanity module of MetaSanity (14) with default settings, which projected that this organism is a diazotrophic, oxygenic photoautotroph that can reduce arsenate and produce sulfolipids. Screening for secondary metabolites with antiSMASH revealed the predicted presence of geosmins; however, no cyanobacterial toxins were detected by this analysis (16).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JACVZX000000000. The version described in this paper is the first version, JACVZX010000000. The BioProject number is PRJNA657201, and the reads are available at the SRA under accession number SRX8961729.

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