



Genome Sequences of Bacteria Associated with the Diatom *Cyclotella cryptica* Strain CCMP332

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ABSTRACT We report draft genomes of two bacterial strains in the genera *Hyphobacterium* and *Reichenbachiella*, which are associated with the diatom *Cyclotella cryptica* strain CCMP332. Genomes from these strains were 2,691,501 bp and 3,325,829 bp, respectively, and will be useful for understanding interactions between diatoms and bacteria.

The phycosphere (1) describes the consortium of bacteria associated with diatoms and other algae. We assembled and annotated draft genomes from two bacteria associated with *Cyclotella cryptica* strain CCMP332, a diatom of longstanding interest for its biology and promising biotechnological applications (2, 3).

We acquired strain CCMP332 from the National Center for Marine Algae and Microbiota. CCMP332 was originally isolated in 1956 in Martha's Vineyard, Massachusetts, by R. Guillard. We grew CCMP332 in L1 medium (4) at 22°C on a 12-h/12-h light/dark cycle. We harvested cells by centrifugation and extracted DNA using the cetyltrimethylammonium bromide (CTAB) protocol (5). We prepared one sequencing library using the Oxford Nanopore Technologies (ONT) ligation sequencing kit (SQK-LSK108) for sequencing on the ONT MinION platform. We prepared short-read Illumina libraries using the Kapa HyperPlus kit (Roche) with 300- to 400-bp insert sizes for sequencing on the Illumina HiSeq 4000 platform.

For all analyses, default software parameters were used unless noted otherwise. We used Guppy (v.2.3.5) for base calling of the MinION reads and Canu (v.1.7) (6) for error correction of the raw MinION reads. We assembled the raw MinION reads with Flye (v.2.4.2, using the parameter --genome-size 165m) (7) and visualized the assembly with BlobTools (v.1.1.1) (8) to identify the bacterial genomes (Fig. 1). We mapped the corrected MinION reads to draft contigs with minimap2 (v.2.10-r761) (9) for contig correction with Racon (v.1.3.3) (10). We then mapped the Illumina reads to the contigs with BWA-MEM (v.0.7.17-r1188) (11) and performed three rounds of contig polishing with Pilon (v.1.2.2, using the parameter "fix bases") (12). We confirmed the circularity of contig 298 with Circlator (v.1.5.5) (13) but could not circularize contig 353. We used Prokka (v.1.14.6) (14) for genome annotation and EPA-NG (v.0.3.5) (15) and Gappa (v.0.5.0) (16) for phylotaxonomic placement of each genome. Genome completeness was assessed at each assembly stage with benchmarking universal single-copy orthologs (BUSCO) (v.4.0.6, using the alphaproteobacteria_odb10 and bacteroidetes_odb10 data sets) (17).

Draft contig correction and polishing increased BUSCO completeness to 94.6% and 98.0% for contigs 298 and 353, respectively. The characteristics and annotation summaries for each genome are listed in Table 1. Contig 298 is closely related to an undescribed species of *Hyphobacterium* (*Alphaproteobacteria*). This assignment is plausible based on known environmental preferences of other *Hyphobacterium* species (18, 19). Two 16S rRNA genes in contig 353 were 98.1% identical, and both

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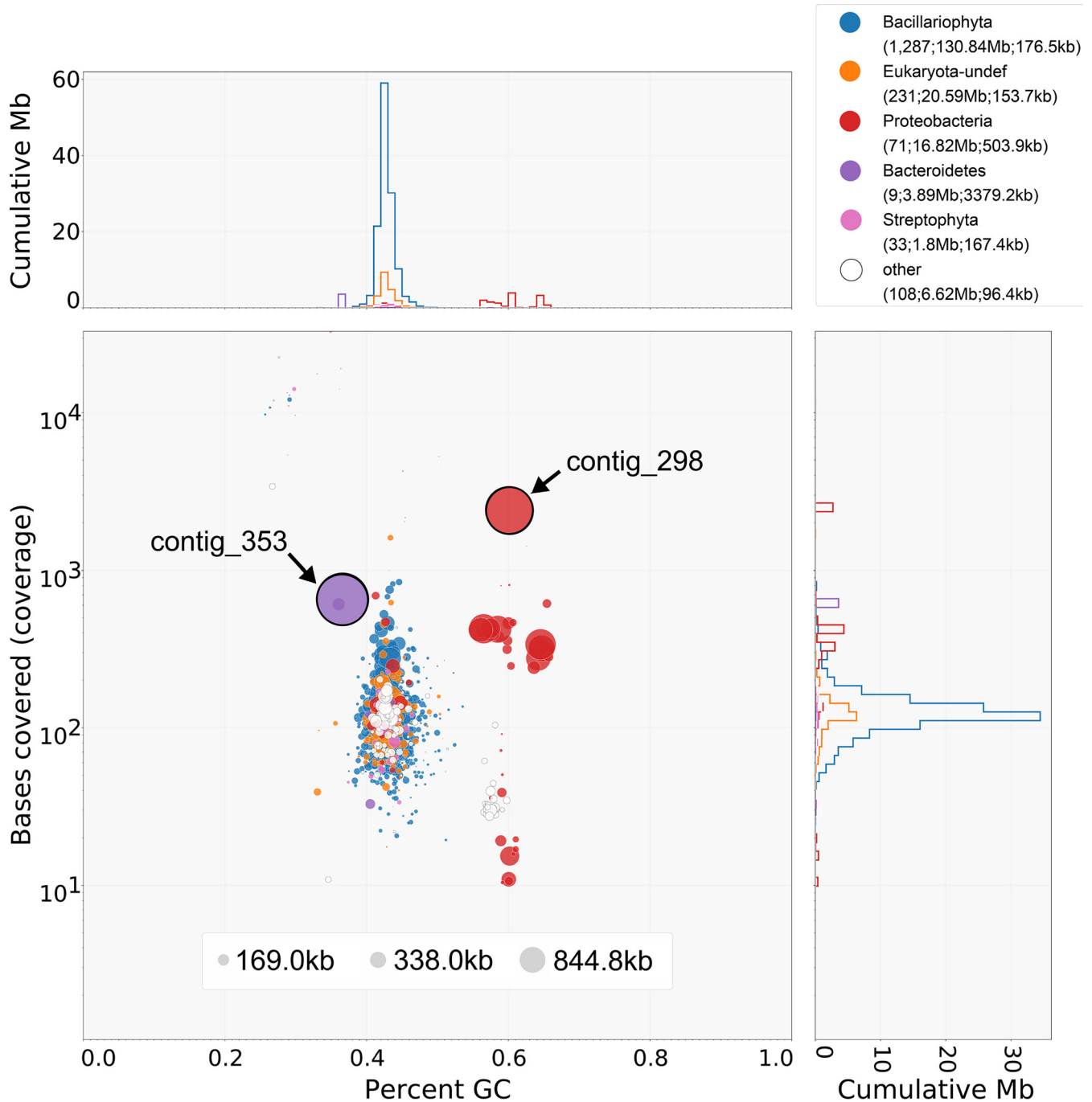


FIG 1 Blob plot showing the taxon-annotated GC content and read coverage of the assembly contigs. Each circle represents one contig, and the circle size corresponds to the contig length (in base pairs). Colors correspond to the taxonomic assignment of each contig via BLASTX searches against the UniProt Reference Proteomes database. Histograms along each axis show the cumulative megabases for each phylum. Read coverage was calculated from the alignment of both MinION and Illumina reads. The circles corresponding to contigs 298 and 353 are labeled and outlined. The data shown in the key have the following format: phylum (number of contigs; length of contigs; contig N_{50} value).

were placed in the family *Cyclobacteriaceae*, with one assigned only at the family level and the other assigned to the genus *Reichenbachiella*. *Reichenbachiella* species live in marine habitats (20–23), and one species has been associated with the red alga *Gracilaria blodgettii* (23). Low support for the placement of contig 353 in *Reichenbachiella* might indicate that the genome belongs to an undescribed genus that is closely related to *Reichenbachiella*.

TABLE 1 Characteristics, annotations, and taxonomy of bacterial genomes associated with *C. cryptica* CCMP332

Characteristic	Genome data for:	
	Contig 298	Contig 353
GenBank accession no.	CP058669	CP058647
GC content (%)	60.15	37.09
Avg read coverage (×)	1,225	464
Circularity	Confirmed ^a	Linear
Genome size (bp)	2,690,927	3,325,829
No. of long reads ^b	11,448	25,404
No. of short reads (single/paired) ^c	52,515,998/22,560,574	6,305,304/18,014,964
Bases confirmed by Illumina (%)	99.63	99.94
Complete BUSCO count (%)	397 (94.6) ^d	394 (98.0) ^e
No. of CDS ^f (functional/hypothetical)	1,394/1,207	1,310/1,629
No. of pseudogenes	56	12
No. of tRNAs	103	37
No. of tmRNAs	1	1
EPA-NG taxonomy ^g	<i>Hyphobacterium</i> sp. 002783385	<i>Cyclobacteriaceae/Reichenbachiella</i> sp.
EPA-NG LWR ^{g,h}	86.84	65.57/49.99

^a“Confirmed” indicates that both Flye and Circlator confirmed circularity of a genome.

^bOut of 5,951,322 total MinION reads. MinION N_{50} , 4,395 bp.

^cOut of 449,895,635 total Illumina reads.

^dGenome mode against the alphaproteobacteria_odb10 data set.

^eGenome mode against the bacteroidetes_odb10 data set.

^fCDS, coding sequences.

^gMultiple values or taxonomic names indicate differences in taxonomic assignment or LWR values between 16S copies.

^hLength weight ratio (LWR) is a support value for EPA-NG phylogenetic assignments; higher values indicate higher support for that assignment.

Data availability. Genome sequences have been deposited in GenBank under accession numbers CP058669 and CP058647, as part of BioProject PRJNA642781. Sequencing reads are available under BioProject PRJNA628076. Please note that the *Cyclobacteriaceae* sp. is listed on NCBI as a *Hyphobacterium* sp.

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