

Circularly assembled genome sequences of *Asticcacaulis* sp. strain MM231 and *Brevundimonas subvibrioides* MM232, isolated from a pond at Bielefeld University campus

Bianca Laker,^{1,2} Tim Schulze,^{1,2} Mara Reis,^{1,2} Sophia-Maria Bormann,³ Gianluca Radke,³ Levin Joe Klages,^{2,4} Tobias Busche,^{2,4,5} Lutz Wobbe,² Andrea Bräutigam,^{1,2} Marion Eisenhut^{1,2}

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Photosynthetic organisms often interact with heterotrophic microbes. We here report the complete genome sequences of the bacterial strains *Asticcacaulis* sp. MM231 and *Brevundimonas subvibrioides* MM232. Both bacteria were co-isolated from a single green colony originating from an aquatic sample taken from a pond at the Bielefeld University campus.

KEYWORDS genomes, aquatic habitat

In aquatic systems, photosynthetic organisms live together with bacteria, as they exude sugars that serve as a carbon source for the heterotrophic bacteria. For the genus *Brevundimonas*, living in symbiotic consortia with microalgae was shown (1), while for a member of the *Asticcacaulis*, an interaction with the aquatic plant duckweed was observed (2). To enhance the knowledge of bacteria co-living with photosynthetic aquatic autotrophs, we cultivated waterborne samples on a medium without a carbon source and isolated DNA from the green, chlorophyll-containing colonies.

The aquatic sample was collected from the surface water of a pond located at the Bielefeld University campus, Germany (52°2'6.4"N 8°29'43.3"E) and centrifuged. The cell pellet was resuspended in fresh 0.9% NaCl solution. Dilutions were plated on solidified BG11 medium (3) and incubated on a windowsill at 22°C for 7 days. A single green colony was picked and streaked on an agar medium. After 7 days of growth, DNA was isolated from the streaked biological material on the plate with the NucleoSpin Microbial DNA kit (Macherey-Nagel, Düren, Germany), and RNA was digested. The ligation sequencing kit V14 [SQK-LSK114; Oxford Nanopore Technologies (ONT), Oxford, UK] following the manufacturer's protocol without DNA shearing and size selection was used for the preparation of sequencing libraries. One R10.4.1 flow cell was run for 21 hours on a GridION (ONT). The super-accurate base-calling model from MinKNOW v23.07.5 (ONT) was used for base-calling producing 390,379 raw reads with an N_{50} of 16,082 bp. All programs were run with default parameters unless otherwise specified. Adapters were trimmed with Porechop v0.2.4 (4), and the reads were used in a blastn search (BLAST 2.14.0+) (5) with parameters "-evalue 0.0001 -max_hsps 1" against NCBI's prokaryotic RefSeq genome database (6). Reads matching the genus *Asticcacaulis* or *Brevundimonas* were assembled with Canu v2.2 (7) using a genome size of 5 or 3.2 Mbp, respectively. The assemblies resulted in a single genome contig each, with two (MM231) or one (MM232) additional sequence (Table 1). Yet, these additional sequences were not circularly assembled. Contigs were polished two times with racon v1.5.0 (8) and minimap v2.26 with parameter "-ax map-ont" (9) and two times with Medaka v1.8.0 applying "-m r1041_e82_400bps_sup_v4.2.0" (ONT). Overlapping ends were removed with Berokka v0.2.3 (<https://github.com/tseemann/berokka>), and sequences were rotated such that

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine, Baltimore, Maryland, USA

Address correspondence to Marion Eisenhut, marion.eisenhut@uni-bielefeld.de.

The authors declare no conflict of interest.

Received 25 June 2024

Accepted 11 September 2024

Published 27 September 2024

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TABLE 1 Sequencing and assembly statistics for *Asticcacaulis* sp. strain MM231 and *Brevundimonas subvibrioides* MM232^a

Parameter	Finding for	
	<i>Asticcacaulis</i> sp. strain MM231	<i>Brevundimonas subvibrioides</i> MM232
Genomic sequence		
Length (bp)	3,458,694	3,281,735
GC content (%)	59.00	68.40
Genome coverage (x)	43.65	38.45
Gene annotation		
Total no. of genes	3,432	3,247
No. of protein-coding genes	3,377	3,183
No. of rRNAs	6	6
No. of tRNAs	49	59
BUSCO results (%)		
Complete	94.6	96.8
Single copy	94.4	96.3
Duplicated	0.2	0.5
Fragmented	4.4	2.1
Missing	1.0	1.1
Other contigs		
No. of sequences	2	1
Length (bp)	(a) 618,632 (b) 570,284	17,430
GC content (%)	(a) 57.30 (b) 56.80	56.80
Coverage (x)	(a) 41.86 (b) 36.52	10.70
Total no. of genes	(a) 500 (b) 441	10
No. of protein-coding genes	(a) 499 (b) 435	10
No. of rRNAs	(a) 0 (b) 3	0
No. of tRNAs	(a) 1 (b) 3	0

^aThe used database and the number of searched BUSCOs were alphaproteobacteria_odb10 with 432 BUSCO genes. For the other sequences in the *Asticcacaulis* assembly, (a) refers to sequence "MM231_p1" and (b) to "MM231_p2."

they start with *dnaA* using Circlator v1.5.5 (10). Phylogenetic classification was performed with the Type (Strain) Genome Server (TYGS) (11). Genome completeness of the circular genomic contigs was validated each with benchmarking universal single-copy orthologs (BUSCO) v5.5.0 setting "--mode geno" (12). Genes were annotated with Prokka v1.14.6 (13). Relevant statistics for the assemblies are reported in Table 1.

The genome sequences of *Asticcacaulis* sp. strain MM231 and *Brevundimonas subvibrioides* MM232 can be classified as complete. Both were circularly assembled, and they have a high number of complete BUSCO genes. According to TYGS, the here represented *Asticcacaulis* sp. has *Asticcacaulis benevestitus* DSM 16100 as the closest relative (GenBank accession number [GCA_000376105.1](https://www.ncbi.nlm.nih.gov/nuccore/GCA_000376105.1)), and the *Brevundimonas* sp. has *Brevundimonas subvibrioides* ATCC 15264 as the closest relative (GenBank accession number [GCA_000144605.1](https://www.ncbi.nlm.nih.gov/nuccore/GCA_000144605.1)).

ACKNOWLEDGMENTS

M.E. acknowledges funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—SFB1535—Project ID 458090666. We acknowledge support for the publication costs by the Open Access Publication Fund of Bielefeld University and the DFG.

This work was supported by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI).

AUTHOR AFFILIATIONS

¹Computational Biology, Faculty of Biology, Bielefeld University, Bielefeld, Germany

²Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany

³Biology, Bielefeld University, Bielefeld, Germany

⁴Microbial Genomics and Biotechnology, Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany

⁵Bielefeld University, Medical School East Westphalia-Lippe, Bielefeld University, Bielefeld, Germany

AUTHOR ORCID*s*

Bianca Laker  <http://orcid.org/0000-0002-5792-0102>

Lutz Wobbe  <https://orcid.org/0000-0001-5141-9897>

Andrea Bräutigam  <http://orcid.org/0000-0002-5309-0527>

Marion Eisenhut  <http://orcid.org/0000-0002-2743-8630>

DATA AVAILABILITY

The MM231 and MM232 assemblies, gene annotations, and reads are available in GenBank/ENA under BioProject accession number [PRJEB75201](https://www.ncbi.nlm.nih.gov/bioproject/PRJEB75201). The GenBank/ENA accession number for the MM231 raw reads is [ERR13189148](https://www.ncbi.nlm.nih.gov/ena/entry/ERR13189148) and [GCA_964186625.1](https://www.ncbi.nlm.nih.gov/ena/entry/GCA_964186625.1) for the MM231 assembly and annotation. The GenBank/ENA accession number for the MM232 raw reads is [ERR12945363](https://www.ncbi.nlm.nih.gov/ena/entry/ERR12945363) and [GCA_964034995.1](https://www.ncbi.nlm.nih.gov/ena/entry/GCA_964034995.1) for the MM232 assembly and annotation.

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