



# Genome Sequence of *Cupriavidus campinensis* Strain G5, a Member of a Bacterial Consortium Capable of Polyethylene Degradation

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**ABSTRACT** Nine different bacterial isolates were recovered from landfills. Each isolate was obtained in pure culture. As a consortium, the bacteria degrade polyethylene. The complete genome sequence of strain G5 was determined by PacBio sequencing. Using the TYGS for taxonomic classification, strain G5 was assigned to the species *Cupriavidus campinensis*.

As part of a bacterial consortium, *Cupriavidus campinensis* G5 degrades polyethylene and was isolated from a former plastic landfill (Niemegk-Neuendorf, Germany [52°04' 59"N, 12°41' 59"E]) using a modified iChip procedure (1). A bacterial suspension from the landfill was diluted in soft agar, loaded into an iChip, and incubated for 4 weeks (2, 3). The emerging microcolony of strain G5 was streaked on agar, and cells from a single colony were inoculated into casein-soy broth (casein peptone, 15 g L<sup>-1</sup>; soymeal peptone, 5 g L<sup>-1</sup>; yeast extract, 0.5 g L<sup>-1</sup>; NaCl, 5 g L<sup>-1</sup>; glucose, 10 mM [pH 7.2]). After growth for 24 h at 30°C, cells were centrifuged, washed twice with phosphate-buffered saline, and kept at -80°C.

The following procedures were performed at Genewiz/Azenta (Leipzig, Germany), including DNA extraction using the Genomic-tip 100/G kit (Qiagen, Hilden, Germany), DNA shearing to ~10 kb using the g-TUBE device without size selection, quality (NanoDrop and pulsed-field gel electrophoresis analyses) and quantity (Qubit 2.0) assessments, and sequencing library preparation using the SMRTbell library preparation kit v2.0 (Pacific Biosciences [PacBio], Menlo Park, CA, USA). The library was sequenced on a PacBio Sequel sequencer (4) using one single-molecule real-time (SMRT) cell. The sequencing yielded 68,653 reads, from which 389,796 sub-reads were obtained (average length, 5,724 bp; read  $N_{50}$ , 7,235 bp; total size, 2,231 Mbp). An assembly was performed within the SMRT Link Suite v5.0.0.6792 at Genewiz/Azenta, including checks for quality and coverage using default parameters. Subread coverage was reduced to 200-fold by random down-sampling, and the genome was automatically assembled using Canu v1.7 (5) with a coverage cutoff set to 40-fold and default parameters otherwise. The assembly was evaluated for overlapping contigs, which eventually were merged, and for potential assembly artifacts, which were dropped. Contigs were polished with raw sub-reads using Arrow v2.3.2 (<https://github.com/PacificBiosciences/pbbioconda>) with default parameters. Initial assembly generated 13 contigs, with a total length of 6,468,862 bp.

A supervised assembly using Geneious v10.2 (6) for mapping of PacBio reads to contigs, with subsequent integration and editing of contigs, was performed. Long duplications, such as two highly similar 27-kb prophages, were resolved by mapping adjacent unique regions to closely related genomes and by analyzing long PacBio reads; this resulted in two replicons. Chromosome 1 (GenBank accession number [CP097330](#)), which was obtained with 331-fold average coverage, is complete, circular, and 3,780,571 bp long, with a G+C content of 66.2%. Chromosome 2 (GenBank accession number [CP097331](#)), which was obtained with 339-fold average coverage, is complete, circular, and 2,524,655 bp long, with a G+C content of 66.7%. Start bases were set according to related chromosomes that are sufficiently similar

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and have been scrutinized for replication origin consensus sequences (7, 8). For GenBank accession number [CP097330](#), the start base is consistent with that of GenBank accession number [CP000352](#), upstream of *dnaA* (7). For GenBank accession number [CP097331](#), the start base is consistent with that of GenBank accession number [CU633750](#), upstream of *repA* (8). No additional replicons were detected.

The TYGS (9) assigned strain G5 to the species *Cupriavidus campinensis*, with a digital DNA-DNA hybridization (dDDH) value (d4) of 96.7% (95% confidence interval, 95.5 to 97.6%) (10). The small G+C content difference (0.20%) between the genome and the type strain, *Cupriavidus campinensis* LMG19282, supports the assignment. The genome was submitted to GenBank and annotated using PGAP v6.1 (11).

**Data availability.** The annotated genome has been deposited in GenBank under the BioProject accession number [PRJNA837128](#) and the BioSample accession number [SAMN28191058](#). The nucleotide sequence accession numbers are [CP097330](#) (major chromosome) and [CP097331](#) (minor chromosome). The Sequence Read Archive (SRA) accession number is [SRR19184626](#).

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