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Complete Genome Sequences of Four Microbacterium Strains Isolated from Metal- and Radionuclide-Rich Soils

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ABSTRACT Here, we present the genome sequences of four Microbacterium strains, which were isolated at different locations in Europe from metal- or radionuclide-rich soils. High-quality complete genome sequences were obtained with PacBio and Illumina data sets with an original two-step procedure.

The Microbacterium genus is composed of ubiquitous high-GC-content Gram-positive Actinobacteria. Despite its presence in many environments and its potential in bioremediation processes (1–7), the bacterial genus Microbacterium is underrepresented in the genome databases and is still poorly studied. At the time of writing, there were more than 100 Microbacterium species with a valid name (http://www.bacterio.net/microbacterium.html) and 323 genomes, including 28 complete genomes, available in the databases (https://www.ncbi.nlm.nih.gov/assembly/?term/microbacterium). Here, we report the complete genome sequences of four members of this genus, which were isolated from metal-rich or radionuclide-rich soil samples. The Microbacterium oleivorans strain A9 was isolated from radionuclide-contaminated soil in Chernobyl (8) and exhibits a high uranium tolerance (9). Its draft genome sequence was previously published (10). The strains ViU2A and ViU22T were isolated from natural uranium-rich soil samples collected in France (the soil composition is described in reference 11; the strain ViU22T is the type strain of the species Microbacterium lemovicicum [12]). The strain HG3 was cultured from metal-rich black sand from Iceland and has been established as mercury tolerant (13).

Bacteria were cultured in LB at 30°C until late exponential growth phase. High-quality genomic DNA was extracted from cells using the DNeasy blood and tissue kit (Qiagen) following the manufacturer’s instructions for Gram-positive bacteria. DNA integrity was checked using agarose gel electrophoresis, and DNA purity and concentration were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Whole-genome shotgun sequencing was carried out with PacBio long-read technology and the Illumina short-read technology. Library preparation and single-end sequencing with 100-base read lengths and a HiSeq 2000 instrument (Illumina) were performed by GenoScreen (Lille, France). Library preparation and long-read sequencing were performed by Eurofins Genomics Europe Shared Services GmbH (formerly GATC Biotech AG, Germany). Library preparation incorporated adaptor sequences compatible with PacBio RS II sequencing technology (single-molecule real-time [SMRT] sequencing) using proprietary methods of Eurofins Genomics Europe Shared Services GmbH. Sequencing was carried out on a PacBio RS II system with SMRT technology. For both sequencing methods, the total number of reads is indicated in Table 1.

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In the first step, genome de novo assembly was performed on the PacBio reads using Canu software version 1.0 with default parameters (14). Trimming and circularization in a single genome assembly were done using this tool. Then, to improve the quality of the genome sequence, a correction was made using the Illumina reads with the MIRA assembler version 4.0.2 (15) on the complete genome sequence (assembled using Canu and oriented on the origin of replication \( \text{oriC} \) by an in-house pipeline) as reference. We used the default configuration for MIRA except for the option job, which was set to “genome,” “mapping,” and “accurate,” and for the option parameters, which were set to SOLEXA_SETTINGS -CL:pec /H11005 yes COMMON_SETTINGS -NW:cac /H11005 no -SK:mmhr 1.

The accession numbers, assembly metrics, and genome characteristics for each genome are listed in Table 1. All chromosomal sequences were circularized and oriented with the predicted \( \text{oriC} \) region as the beginning of the sequences. Taxonomic assignment at the species level was provided by the NCBI using their quality control test for bacterial genomes. This test uses average nucleotide identity (ANI), which compares the submitted genome sequence against the genomes of the type strains and proxytype strains that are already in GenBank, as described in reference 16.

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Data availability. The raw data and whole-genome sequences of the four \textit{Microbacterium} strains have been deposited in the GenBank database under the accession numbers listed in Table 1.

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| Characteristic | Data for strain: | A9 | Hg3 | ViU2a | ViU22 |
|---------------|-----------------|----|-----|-------|-------|
| GenBank accession no. | CP031421 | CP031422 | CP031338 | CP031423 |
| Raw data accession no. (Illumina) | SRR8416123 | SRR8416223 | SRR8416225 | SRR8417352 |
| No. of reads (Illumina) | 18,500,154 | 19,895,949 | 15,648,367 | 13,317,740 |
| Raw data accession no. (PacBio) | SRR8416122 | SRR8416222 | SRR8416224 | SRR8417351 |
| No. of reads (PacBio) | 144,950 | 154,761 | 172,715 | 164,482 |
| Mean read length (bp) (PacBio) | 8,474 | 8,380 | 7,755 | 8,270 |
| Genome length (Mb) | 2.99 | 3.9 | 3.8 | 3.6 |
| G+C content (%) | 69 | 68.2 | 68.3 | 70.8 |
| No. of protein-coding genes | 2,880 | 3,764 | 3,708 | 3,297 |
| No. of tRNA genes | 50 | 51 | 54 | 52 |
| No. of rRNA genes | 6 | 6 | 6 | 6 |
| No. of TCSs | 62 | 70 | 75 | 55 |
| No. of TFs | 156 | 274 | 280 | 191 |
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