AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequence of *Microbacterium* sp. Strain Nx66, Isolated from Waters Contaminated with Petrochemicals in El Saf-Saf Valley, Algeria

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ABSTRACT *Microbacterium* sp. strain Nx66 was isolated from waters contaminated by petrochemical effluents collected in Algeria. Its genome was sequenced using Illumina MiSeq (2 × 150-bp read pairs) and Oxford Nanopore (long reads) technologies and was assembled using Unicycler. It is composed of one chromosome of 3.42 Mb and one plasmid of 34.22 kb.

Actinobacteria are Gram-positive aerobic bacteria widely distributed in terrestrial and aquatic ecosystems and are known to produce a great variety of bioactive compounds (1, 2). They are mainly free living, commensals, or symbiotic, but some of them may cause infections in humans (3–8).

A total of 28 strains were isolated from a water sample collected in Skikda's El Saf-Saf Valley, Algeria (36.87981N, 6.93111E), receiving industrial releases from a petrochemical refinery (9). Aliquots of 100 μl up to 10^{-3} dilutions were inoculated onto Reasoner's 2A agar (R2A) agar plates incubated at 30°C for 24 h to 1 week, and bacterial colonies were purified by streaking three times onto fresh medium agar plates. Protein samples were prepared from colonies using a mix (50/50) of 70% (vol/vol) formic acid (Sigma, Lyon, France) and 50% (vol/vol) acetonitrile (Fluka, Buchs, Switzerland) and were analyzed by mass spectrometry as previously described (10). The Nx66 isolate was identified with low confidence as a *Microbacterium* strain.

Nx66 cells were grown for 72 h in R2A liquid medium at 30°C, and DNA was extracted using the MasterPure complete DNA and RNA purification kit (Epicentre). An Oxford Nanopore Technologies (ONT) library was prepared according to the manufacturer's instructions for 1D native barcoding genomic DNA (kits EXP-NBD103 and SQK-LSK109). DNA was quantified using the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Life Technologies), and purity was determined using a Nanodrop instrument (ThermoFisher). Size distribution and degradation were assessed using the fragment analyzer (AATI) high-sensitivity DNA fragment analysis kit. DNA was purified using AMPure XP beads (Beckman Coulter) and sheared at 20 kb using the Megaruptor system (Diagenode). One DNA damage repair, end repair, and dA tail step was performed before sample-specific index ligation. The library was loaded on an R9.4.1 revD flowcell and sequenced on a GridION instrument at 0.03 pmol within 48 h using MinKNOW v2.0.10-1 and Guppy v1.8.5-1 for base calling. Illumina 2 × 150-bp paired-end libraries were prepared according to

Citation Chéraiti N, Plewniak F, Tighidet S, Sayeh A, Gil L, Malherbe L, Memmi Y, Zilliox L, Vandecasteele C, Boyer P, Lopez-Roques C, Jaulhac B, Bensouilah M, Bertin PN. 2020. Complete genome sequence of *Microbacterium* sp. strain Nx66, isolated from waters contaminated with petrochemicals in El Saf-Saf Valley, Algeria. Microbiol Resour Announc 9:e01130-20. https://doi.org/10.1128/MRA.01130-20.

Editor Julia A. Maresca, University of Delaware

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Received 25 September 2020
Accepted 27 October 2020
Published 19 November 2020

FIG 1 Unrooted maximum likelihood phylogenetic tree of Nx66 and the 35 publicly available *Microbacterium* complete genome sequences. The tree was obtained with GToTree v1.4.16 with HMM source Actinobacteria.hmm (138 targets) and default parameters (JTT+CAT model) (20). The *Microbacterium* sp. Nx66 closest relatives were *Microbacterium* sp. strain China and *Microbacterium* sp. PAMC 28756 HSR44. The 35 complete genomes and their RefSeq accession numbers are *Microbacterium amylolyticum* (GCF_011046975.1), *Microbacterium aurum* (GCF_001974985.1), *Microbacterium chocolatum* (GCF_001652465.1), *Microbacterium endophyticum* (GCF_011047135.1), *Microbacterium foliorum* (GCF_003367705.1), *Microbacterium foliorum* (GCF_006385575.1), *Microbacterium hominis* (GCF_002843965.1), *Microbacterium hominis* (GCF_013282805.1), *Microbacterium lemovicicum* (GCF_003991875.1), *Microbacterium oleivorans* (GCF_001975955.2), *Microbacterium oleivorans* (GCF_013389665.1), *Microbacterium oxydans* (GCF_003991855.1), *Microbacterium oxydans* (GCF_004000565.1), *Microbacterium paludicola* (GCF_001887285.1), *Microbacterium protaetiae* (GCF_004135285.1), *Microbacterium sediminis* (GCF_004564075.1), *Microbacterium* sp. 1.5R (GCF_001889265.1), *Microbacterium* sp. 10M-3C3 (GCF_003931875.1), *Microbacterium* sp. 1S1 (GCF_008271365.1), *Microbacterium* sp. 4R-513 (GCF_011046485.1), *Microbacterium* sp. ABRD_28 (GCF_003850245.1), *Microbacterium* sp. BH-3-3-3 (GCF_001792815.1), *Microbacterium* sp. CGR1 (GCF_001266755.1), *Microbacterium* sp. L-031 (GCF_008727775.1), *Microbacterium* sp. No. 7 (GCF_001314225.1), *Microbacterium* sp. PAMC 28756 (GCF_001558975.1), *Microbacterium* sp. RG1 (GCF_005347485.1), *Microbacterium* sp. SGAir0570 (GCF_005491085.2), *Microbacterium* sp. ST-M6 (GCF_008727755.1), *Microbacterium* sp. strain China (GCF_002993305.1), *Microbacterium* sp. TPU 3598 (GCF_002356155.1), *Microbacterium* sp. XT11 (GCF_001513675.1), *Microbacterium* sp. Y-01 (GCF_003856715.1), *Microbacterium testaceum* (GCF_000202635.1), and *Microbacterium wangchenii* (GCF_004564355.1).

Illumina's protocols using the TruSeq Nano DNA high-throughput (HT) library prep kit. DNA was fragmented by sonication. Size selection was performed using sample purification beads (SPBs). Library quality was assessed using an Advanced Analytical fragment analyzer. Libraries were quantified by quantitative PCR (qPCR) using the Kapa library quantification kit. Sequencing was performed on an Illumina MiSeq instrument with V2 reagent kits.

Adaptors and low-quality extremities (Q, <20) were trimmed off short reads with BBDuk (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide). Read pairs with a Q value of <30 were discarded. Adaptors were trimmed off long reads using the Oxford Nanopore Technologies qcat program (https://github.com/nanoporetech/qcat). Long reads with a Q value of <9 were discarded using Nanofilt v2.5.0 (11). Assembly was performed with Unicycler v0.4.7 (12) using default parameters, yielding two circular replicons of 3,422,870 bp and 34,223 bp with GC contents of 70.17% and 66.07%, respectively. QUAST v5.0.2 (13) rated the assembly as good (mapping reads, 99.62%; coverage, 79× for Illumina and 117× for Nanopore). Analysis with CheckM v1.0.11 (14) returned 99.49% completeness, insignificant contamination (0.51%), and no strain heterogeneity.

Microbacterium genus assignment was confirmed by the RDP classifier (15) using 16S rRNA genes predicted with barrnap (https://github.com/tseemann/barrnap). The best average nucleotide identity computed with FastANI v1.2 (16) against the 35 complete public *Microbacterium* genomes was obtained with *Microbacterium* sp. strain China (93.45%, 1,062/1,151 fragments). This value, lower than observed intraspecies values (17, 18), suggests that Nx66 is close but not identical to strain China (Fig. 1).

Annotation with the MicroScope platform (19) predicted 3,506 genes in the chromosome (3,441 coding sequences [CDS], 47 tRNA genes, and 6 rRNA genes) and 41 CDS in the plasmid. The chromosome annotation showed the presence of a significant number of genes involved in resistance to toxic metals, such as arsenic and zinc, and organic compound degradation, such as xylan and chitin.

Data availability. The complete sequences of the *Microbacterium* sp. Nx66 genome and plasmid have been deposited in DDBJ/EMBL/GenBank under BioProject PRJEB39712 and assembly accession number GCA_904066215. Raw reads have been made available under the same BioProject number with accession numbers ERR4508043 for the MiSeq paired-end reads and ERR4508044 for the ONT long reads. The accession numbers for the annotated sequences of the chromosome and plasmid are LR880474.1 and LR880475.1, respectively.

ACKNOWLEDGMENTS

This work benefited from the financial support of the DMO/DMA project subsidized by the CNRS national program EC2CO-MicrobiEn. It was performed in collaboration with the GeT core facility, Toulouse, France (https://get.genotoul.fr/en/), and was supported by France Génomique National infrastructure, funded as part of "Investissement d'avenir" program managed by Agence Nationale pour la Recherche (contract ANR-10-INBS-09).

We are grateful to the Genoscope (Évry, France) for providing online access to MicroScope, the Microbial Genome Annotation and Analysis Platform (https://mage.genoscope.cns.fr/microscope/home/index.php).

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