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Draft genomes of two rhizosphere associated bacterial isolates from Tims Branch, a heavy metal contaminated wetland

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ABSTRACT Two bacterial isolates were recovered from wetland sediments from Tims Branch, a heavy metal contaminated wetland located at the Savannah River Site. Draft genomes of the two recovered isolates, *Rhodoblastus* strain 17X3 and *Comamonas* strain 17RB, were generated from Illumina MiSeq sequencing data.

KEYWORDS *Rhodoblastus*, *Comamonas*, rhizosphere-inhabiting microbes

Tims Branch is a uranium (U) contaminated stream located at the Savannah River Site (SRS), a DOE site located in Aiken, SC, USA. Previous studies indicated the importance of the wetland rhizosphere in immobilizing U (1, 2). Two bacterial strains were isolated from root-associated sediment from an uncontaminated portion of Tims Branch (33.331429N, 81.719287W). Draft genomes were generated for strains 17RB and 17X3 to aid with understanding the role of microbes in iron, carbon, and nitrogen cycling which may affect mobility of U in Tims Branch. Sediment was collected from 15 cm depth, associated with roots of soft rush plants (*Jancus effuses*) and processed as described previously (3). Sediment (0.5 g) was suspended in phosphate-buffered saline (PBS) (10 mL). Strain 17RB was initially enriched using FeS gradient agar tubes inoculated with 100 µL of sediment slurry (4). Strain 17RB was isolated by spread plating 100 µL from FeS gradient agar tubes followed by repeated streaking for isolation on R2A agar. Strain 17X3 was isolated by spread plating 100 µL of sediment slurry (serially diluted by 1/10 dilutions in PBS to 10^{-4}) using 1.5% agar plates containing 1 mL per L Wolfe's mineral media with 0.5% xylose and 0.1% yeast extract as carbon sources (5). Strains were deemed to be axenic by three rounds of streaking for isolation with uniform colony morphologies and observation of uniform cell morphology by phase contrast microscopy. Both strains were grown aerobically at 25°C for 5–7 days between transfers.

Cultures for DNA extraction were grown in the same manner as used to maintain strains. DNA was extracted using a MoBio Powersoil DNA extraction kit (Qiagen) according to the manufacturer's instructions except 100 mg of cell material collected from plate cultures grown under aforementioned cultivation conditions was used instead of soil. Sequencing was performed at the University of Delaware Center DNA Sequencing and Genotyping Center (Newark, DE). Library preparation was performed using Illumina DNA Prep, Tagmentation kit (Illumina) with 20 ng of input DNA. Sequencing was performed using an Illumina MiSeq instrument (2 × 300 bp paired reads) with MiSeq Reagent Kit v3 (Illumina). Default parameters were used for all software unless otherwise specified. Reads were quality filtered using Trim Galore v0.6.10 (https://github.com/FelixKrueger/TrimGalore) to remove adapters and sequences with lengths under 160 bp and quality scores under 25. Paired reads that were retained after quality filtering were used for genome assembly using SPAdes v3.15.4 (6). Resulting assemblies (Table 1) were uploaded to kBase (https://www.kbase.us/) and contigs <600 bp were removed from assemblies (7). Quality of draft genomes was assessed using CheckM

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TABLE 1 Summary of genome statistics

Parameter	Sequenced strain	
	***Rhodoblastus* strain 17X3**	***Comamonas* strain 17RB**
Number of pairs of Illumina MiSeq sequences	17,391,591	13,472,895
Coverage (×)	861	620
Genome size (bp)	4,190,025	4,790,057
G+C content (%)	63.68%	65.29%
Number of contigs	69	23
N_{50} (bp)	161,112	332,848
Number of protein encoding genes	3,850	4,170
CheckM completeness (%)	98.75	98.76
CheckM contamination (%)	1.25	0.36
Closest average nucleotide identity (ANI) match, genome accession ID, and % ANI match	*Rhodoblastus* sp903903185 (GCA_903903185.1) (80.79%)	*Comamonas terrigena* (GCF_900461435.1) (96.42%)
Genome assembly accession ID	GCA_030056575.1	GCA_030056555.1
SRA accession ID	SRR24582421	SRR24582422

v1.0.18 (8). Taxonomy of draft genome assemblies was determined using GTDB-Tk v1.7.0 (9).

Genome annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.5 (10). Both genome assemblies included predicted nitrate reductases. The strain 17X3 genome assembly also included a putative methanol dehydrogenase suggesting strain 17X3 may be capable of coupling methylotrophy to nitrate reduction. Strains may link denitrification to carbon or iron oxidation, processes which may affect the immobilization of U at Tims Branch.

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AUTHOR CONTRIBUTIONS

Nathaniel A. Losey, Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft | Wendy W. Kuhne, Supervision, Writing – original

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DATA AVAILABILITY

The associated BioProject for both genomes is ID PRJNA971589, the BioSample ID for strain 17X3 is SAMN35039236 and 17RB is SAMN35039235. Accession IDs for draft assemblies of strains 17X3 and 17RB are GCA_030056575.1 and GCA_030056555.1 respectively. SRA accession IDs for Illumina sequences for strains 17X3 and 17RB are SRR24582421 and SRR24582422, respectively.

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