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PROKARYOTES

Draft Genome Sequence of the Naturally Competent *Bacillus simplex* Strain WY10

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ABSTRACT We sequenced a naturally competent bacterial isolate, WY10, cultured from a Wyoming soil sample. Sequence analysis revealed that WY10 is a novel strain of *Bacillus simplex*. To our knowledge, WY10 is the first *B. simplex* strain to be characterized as naturally competent for DNA uptake by transformation.

Received 17 October 2017 **Accepted** 23 October 2017 **Published** 16 November 2017

Citation Keen EC, Bliskovsky VV, Adhya SL, Dantas G. 2017. Draft genome sequence of the naturally competent *Bacillus simplex* strain WY10. Genome Announc 5:e01295-17. https://doi.org/10.1128/genomeA.01295-17.

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The Gram-positive bacterial genus *Bacillus* comprises dozens of species and includes obligate human pathogens (*B. anthracis*), facultative human pathogens (*B. cereus*), and nonpathogenic model organisms (*B. subtilis*) (1). Various *Bacillus* species are ubiquitous in soils and other natural environments, and some strains are notable for their natural competence for DNA uptake *in vitro* and *in situ* (2). As part of a previously published study (3), we obtained a bacterial isolate from a soil sample collected in Cheyenne, WY, USA, which formed mucoid off-white colonies on LB plates and spontaneously took up exogenous plasmid DNA in liquid culture. 16S rRNA profiling placed this isolate, then called WY10, within *Bacillus*; here, we report that WY10 constitutes a novel strain of *Bacillus simplex*.

DNA was extracted from a WY10 overnight culture with phenol-chloroform, processed into sequencing libraries (Nextera XT kit; Illumina), and sequenced using 2 × 250 paired-end reads (MiSeq; Illumina). The resulting 4,066,400 paired-end reads were trimmed of Illumina adapters and human-like sequences with Trimmomatic (4) and Deconseq (5), respectively. The remaining 3,924,890 reads (96.5% of original) were assembled with SPAdes (6), yielding a draft genome assembly of 117 contigs (largest contig = 1,148,191 bp; N_{50} = 322,734 bp) and 106 scaffolds. All 81 scaffolds >200 bp, comprising 40.3% G+C content across 5,516,951 bp, were functionally annotated with Rapid Annotations using Subsystems Technology (RAST) (7). RAST identified 78 RNAs and 5,739 coding sequences, 44% of which could be functionally categorized. Because individual WY10 scaffolds displayed the greatest sequence similarity by BLASTn to *B. simplex*, JSpeciesWS (8) was used to calculate average nucleotide identity (ANI) based on MUMmer (9) between the WY10 draft genome sequence and all existing *B. simplex* genomes. WY10 shares 93.7% and 90.9% ANI with two completely sequenced *B. simplex* strains, DSM 1321 and SH-B26, respectively, and 85.9% to 97.3% ANI (median ANI = 96.7%) with nine additional *B. simplex* whole-genome shotgun assemblies. Thus, WY10 does not represent a unique type species (conventionally <95% ANI [10]) of *Bacillus* but rather a novel strain of *B. simplex*.

We recently reported that certain bacteriophages, dubbed "superspreaders," do not efficiently degrade bacterial plasmids during infection and instead release intact plasmid DNA upon lysis, thereby promoting horizontal gene transfer by transformation (3). In this previous work, WY10 was shown to acquire and manifest antibiotic resistance via

the uptake of phage-released plasmid DNA. Here, we announce the draft genome sequence of WY10 and its placement within *B. simplex*. To our knowledge, WY10 is the first *B. simplex* strain to be functionally characterized as naturally competent and the first Gram-positive bacterium to demonstrate compatibility with the *Escherichia coli* plasmid p$\pi\gamma$ (R6K origin) (11). The draft genome sequence of *B. simplex* WY10 will facilitate further analysis of phage-mediated plasmid transformation and, by extension, horizontal gene transfer and bacterial evolution.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number NWQJ00000000. The version described in this paper is the first version, NWQJ01000000.

ACKNOWLEDGMENTS

We thank Robert Potter, Alaric D'Souza, and Andrew Gasparrini for discussion and Steve Shema, Eric Martin, and Brian Koebbe for technical assistance.

This research was supported by the Intramural Research Program of the National Cancer Institute and by a National Science Foundation Graduate Research Fellowship (DGE-1143945) to E.C.K. The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

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