Draft Genome Sequence of the Naturally Competent Bacillus simplex Strain WY10

Eric C. Keen,a Valery V. Bliskovsky,b Sankar L. Adhya,c Gautam Dantasd,e,f

The Edison Family Center for Genome Sciences & Systems Biology, Washington University School of Medicine, St. Louis, Missouri, USAa; CCR Genomics Core, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, Missouri, USA; Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USAa; Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri, USA

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.

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; N50 = 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 \emph{B. simplex}. To our knowledge, WY10 is the first \emph{B. simplex} strain to be functionally characterized as naturally competent and the first Gram-positive bacterium to demonstrate compatibility with the \emph{Escherichia coli} plasmid \textit{pR6K} (R6K origin) \cite{Srivastava2007}. The draft genome sequence of \emph{B. simplex} WY10 will facilitate further analysis of phage-mediated plasmid transformation and, by extension, horizontal gene transfer and bacterial evolution.

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

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