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The Genome Sequence of the *Halobacterium salinarum* Type Strain Is Closely Related to That of Laboratory Strains NRC-1 and R1

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ABSTRACT High-coverage long-read sequencing of the *Halobacterium salinarum* type strain (91-R6) revealed a 2.17-Mb chromosome and two large plasmids (148 and 102 kb). Population heterogeneity and long repeats were observed. Strain 91-R6 and laboratory strain R1 showed 99.63% sequence identity in common chromosomal regions and only 38 strain-specific segments. This information resolves the previously uncertain relationship between type and laboratory strains.

Halobacterium salinarum is a well-studied model haloarchaeon first isolated from cured cod in 1922 (1). The source of this organism was found to be salt. This original type strain was lost (2), and a neotype was assigned, *H. salinarum* isolate 91-R6 (3) (NRC 34002 = ATCC 33171 = DSM 3754), which is referred to hereafter as strain 91-R6. It was isolated in Canada from the red discoloration on a salted cow hide (3). Little experimental work has been reported for the type strain, but two laboratory strains of *H. salinarum* have been sequenced, namely, strain NRC-1 (4) and strain R1 (5). Their relationship to the type strain has previously remained uncertain.

A fresh culture of strain 91-R6 (DSM 3754^T) was obtained from the DSMZ and inoculated directly into liquid medium, omitting any colony purification. DNA from the resulting cells was used for genome sequencing using high-coverage PacBio long-read technology (5 single-molecule real-time [SMRT] cells; 253,044 reads; average length, 5,400 bp; and 1.3 Gbp total, using kits from PacBio, including template preparation, MagBead loading, and sequencing). For assembly, we used the SMRTanalysis pipeline (RS_HGAP_assembly.2 v2.3.0, Pacific Biosciences, with default parameters) which runs HGAP (DAGCON-based hierarchical genome assembly process) in three steps (6), namely, preassembly, *de novo* assembly with the Celera assembler, and final polishing with Quiver. Despite high coverage, the assembly gave 43 contigs. A supervised genome assembly was performed using Canu v1.7 (7) for assembly and Geneious v10.2 (8) for integration and editing of contigs. Considerable population heterogeneity (transposon integrations and transposon-triggered genome inversions) was encountered, which explained the failure of the automated assembly procedure. The representative genome sequence consists of 1 chromosome (2,178,608 bp, 67.1% G+C content, and 400-fold coverage) and 2 large plasmids (pHSAL1, 148,406 bp and 60.6% G+C content; pHSAL2, 102,666 bp and 56.5% G+C content; 500-fold coverage for plasmids). The plasmids share a 39,230-bp duplication devoid of any sequence difference.

The chromosomes of strains 91-R6 and R1 were compared in detail by methods we previously described for strain comparisons of *Haloquadratum walsbyi* and *Photorhabdus laumondii* (9, 10). They showed very high DNA sequence similarity (99.63% se-

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quence identity covering 84.9% of 2.17 Mb in strain 91-R6 and 92.5% of 2.00 Mb in strain R1) and complete colinearity. Only 38 strain-specific regions were identified. As the chromosomes of strains R1 and NRC-1 are nearly identical, there is also very high similarity of the chromosomes from strains 91-R6 and NRC-1. The plasmids of strains 91-R6 and R1 exhibited patches with very high interstrain similarity (107 kb, pHSAL1/pHS3; 42.5 kb, chromosome/pHS3; and 13.3 kb, pHSAL2/pHS1).

Given the close genomic similarity of the strains, the annotation of strain R1 was used as a reference for that of strain 91-R6. Strain 91-R6 codes for 2,451 regular proteins, of which 2,092 are shared with strain R1, with only a minority (73) having less than 98% protein sequence identity. Strain R1 is 1 of 12 haloarchaeal genomes which have been reliably annotated by our gold standard protein-based strategy (5, 11). Our efforts also include regular systematic correlation with high-level databases (Swiss-Prot and KEGG).

During this project, we revised the annotation of the *H. salinarum* NRC-1 genome and submitted it to NCBI as a third-party annotation (NCBI:TPA).

Data availability. The genome sequence of strain 91-R6 has been deposited in GenBank under the accession numbers [CP038631](#) (chromosome), [CP038632](#) (pHSAL1), and [CP038633](#) (pHSAL2). Raw reads have been deposited in the SRA archive under BioProject accession number [PRJNA530823](#). The third-party annotation of the NRC-1 genome has been deposited in the Third Party Annotation section of GenBank (accession numbers BK010829, BK010830, and BK010831).

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