

Genome Announcements

Complete Genome Sequence of *Rhodobacter sphaeroides* KD131[∇]

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***Rhodobacter sphaeroides* is a purple nonsulfur photosynthetic bacterium that is considered a possible source of H₂ production. *R. sphaeroides* KD131, which was isolated from sea mud in South Korea, was found to produce high levels of H₂. Here we report the complete and annotated genome sequence of *R. sphaeroides* KD131.**

Rhodobacter sphaeroides KD131 (KCTC12085) is a halophilic, purple nonsulfur photosynthetic bacterium that produces high levels of biohydrogen (H₂). The strain, KD131, was originally isolated from sea mud off the coast of DaeBu Island, South Korea (8). Recently, the strain was genetically developed for H₂ production under any conditions, regardless of the presence of light (6).

We have determined the complete genome sequence of strain KD131, which consists of two chromosomes and two plasmids, using the traditional shotgun whole-genome sequencing method. To accomplish this, two genome libraries (~2 kb and ~35 kb) were generated using randomly sheared genomic DNA of strain KD131. The sequences with as much as five times more genomic information were obtained from the 38,063 chromatograms produced by the libraries using ABI 3730xl autosequencers. The DNA-sequencing chromatograms were then analyzed automatically by base calling, fragment assembly, and contig editing using the Phred/Phrap/Consed software package (<http://www.phrap.org>) (4). For gap closing and to improve the quality of regions with a low Phred quality score or depth, noncloned PCR products amplified using the primers designed by Consed and genomic DNA as a template were sequenced. In addition, we performed manual curation based on the preannotated information in the draft genome sequence to ensure the quality of the genome database. The final assembly of the total genome was then performed manually based on the location and mate information to avoid improper assembly due to the presence of repeat sequences using Consed. The final predicted error rates were 0.07, 0.05, 0.15, and 0.04/10 kb in chromosome 1, chromosome 2, plasmid A, and plasmid B, respectively.

The complete sequences were analyzed using Glimmer3 (3)

for the protein-coding gene, tRNAscan-SE (9) for the tRNA, and RNAmmer (7) for the rRNA. The functions of predicted protein-coding genes were then annotated through comparisons with the databases of UniRef90 (11), NCBI-NR (1), COG (12), and KEGG (5). The genome of *R. sphaeroides* KD131 consists of chromosome 1, which contains 3,152,792 bp (3,101 open reading frames [ORFs], 39 tRNAs, and one 16S-23S-5S rRNA), chromosome 2, which contains 1,297,647 bp (1,224 ORFs, 15 tRNAs, and three 16S-23S-5S rRNAs), plasmid A, which contains 157,345 bp (142 ORFs), and plasmid B (102 ORFs), which contains 103,355 bp. In addition, the genome has a G+C content of ~68.7% to ~70.1%.

The aspect of distribution and content of the genes in strain KD131 were highly similar to those of a closely related cognate strain, 2.4.1, which has been analyzed by the Department of Energy Joint Genome Institute (2). However, the genome of *R. sphaeroides* KD131 may have a more-developed H₂-evolving system that consists mainly of an uptake hydrogenase and two nitrogenases. An uptake hydrogenase gene cluster and a molybdenum nitrogenase gene cluster are found in strain 2.4.1, but another iron nitrogenase gene cluster that is not found in strain 2.4.1 is highly similar to the homologue of *Rhodospseudomonas palustris* CGA009 (10). The information regarding the complete genome of strain KD131 that we report here will facilitate proteomic and metabolic studies designed to develop this strain for H₂ production.

Nucleotide sequence accession numbers. Genome information for the two chromosomes and two plasmids of *Rhodobacter sphaeroides* KD131 were deposited in GenBank under the accession numbers CP001150 to CP001153.

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