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Revised Sequence and Annotation of the *Rhodobacter sphaeroides* 2.4.1 Genome

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The DNA sequences of chromosomes I and II of *Rhodobacter sphaeroides* strain 2.4.1 have been revised, and the annotation of the entire genomic sequence, including both chromosomes and the five plasmids, has been updated. Errors in the originally published sequence have been corrected, and ~11% of the coding regions in the original sequence have been affected by the revised annotation.

Rhodobacter sphaeroides is a purple nonsulfur photosynthetic bacterium belonging to the alpha class of proteobacteria. *R. sphaeroides* is metabolically diverse, capable of growing photo- or chemoheterotrophically or -autotrophically and of fixing dinitrogen.

The *R. sphaeroides* 2.4.1 genome was originally sequenced in 2001 (3) (www.rhodobacter.org) and consists of two chromosomes (I and II) and five plasmids (A, B, C, D, and E). Recently, after sequencing the genome of a mutant strain, we identified a number of potential errors in the published 2.4.1 sequence. Here, we report revised sequences of chromosomes I and II of *Rhodobacter sphaeroides* 2.4.1 and a revised annotation of the entire genomic sequence, including the five plasmids.

Genomic DNA was randomly sheared into ~200-bp fragments. The library created from these fragments was sequenced on an Illumina GAIIX system. The resulting 36-bp paired-end reads were aligned to the original 2.4.1 genomic sequence using the software program maq-0.7.1. (1) (www.maq.sourceforge.net). An iterative process utilizing maq-0.7.1 and manual inspection was used to identify single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) between the reads and the reference sequence. We also used whole-genome sequencing data from a 454 FLX Titanium platform and a combination of PCR and Sanger sequencing to verify areas of low read coverage in the Illumina data.

Compared to the original genomic sequence, there are 106 SNPs in the revised genomic sequence. There are 37 insertions, comprising 141 bp total (the largest spanning 46 bp), and 73 deletions, comprising 200 bp total (the largest spanning 45 bp).

The entire genomic sequence (including the five plasmids, which retained their original sequences) was annotated using the JGI microbial genome automated annotation pipeline (5), followed by manual curation using the GenePRIMP software program (6). The predicted coding sequences (CDSs) were translated and used to search the NCBI nonredundant database and the UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction and functional annotation were performed within the IMG-ER platform (4).

The revised sequence contains 4,515 predicted CDSs (versus 4,383 in the original sequence). Of the CDSs in the original sequence, 89% remain unmodified. Of the modified CDSs, 173 were

extended and 202 were truncated in length; these modified CDSs retained their original locus tags. In 42 cases, two or more CDSs from the original sequence were combined into a single CDS, which retained the locus tag of the original CDS furthest upstream. Twenty-three CDSs were replaced by new CDSs (with new locus tags) on the opposite DNA strand, and seven CDSs were eliminated. All CDSs new to the revised genomic sequence have been given locus tags beginning with RSP_7500. All modified CDSs contain a description of the modification as a note in their GenBank and IMG entries. Most modifications resulted in CDSs that more closely match those of other sequenced *R. sphaeroides* strains (2, 7).

Nucleotide sequence accession numbers. The newly sequenced and annotated genomic chromosomes and the newly annotated genomic plasmids have been deposited in GenBank under the same accession numbers as those for the original *R. sphaeroides* 2.4.1 genome (CP000143 to CP000147, DQ232586, and DQ232587).

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