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GENOME SEQUENCES

Draft Genome Sequences of *Thiorhodococcus mannitoliphagus* and *Thiorhodococcus minor*, Purple Sulfur Photosynthetic Bacteria in the Gammaproteobacterial Family *Chromatiaceae*

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ABSTRACT We have determined the draft genome sequences of *Thiorhodococcus mannitoliphagus* and *Thiorhodococcus minor* for comparison with those of *T. drewsii* and *Imhoffiella purpurea*. According to average nucleotide identity (ANI) and whole-genome phylogenetic comparisons, these two species are clearly distinct from the *Imhoffiella* species and *T. drewsii*.

The purple photosynthetic bacterial family *Chromatiaceae* is fairly large, with more than 72 named species, and the genus *Thiorhodococcus* alone contains about 10 species. The first *Thiorhodococcus* species was described by Guyoneaud et al. (1) as *T. minus*, which was later changed to *T. minor* (2). *Thiorhodococcus* species, including *T. drewsii* (3), were reported to be most closely related to the species of *Allochromatium* and *Thiocystis* (4). *Thiorhodococcus bheemlicus* was split off as *Imhoffiella bheemlica*, which along with *Imhofiella purpurea* AK35 are the only species in the genus (5). The genome sequences currently reported are those of *T. drewsii* (6) and *I. purpurea* AK35 (7). We have now produced draft genome sequences for *T. minor* and *T. mannitoliphagus* (8) to further define the genus and to clarify the position of the *Imhoffiella* species.

Cells of *Thiorhodococcus minor* DSM 11518 and *T. mannitoliphagus* DSM 18266 were grown and genomic DNA was prepared by the DSMZ culture collection. DNA analysis using Qubit and NanoDrop showed an $A_{260/280}$ ratio of 2.1 for *T. mannitoliphagus* and 1.74 for *T. minor*. The sequencing libraries were prepared using the Illumina Nextera DNA Flex library prep kit. The genomes were sequenced with an Illumina MiniSeq using 500 μl of a 1.8 pM library. Paired-end (2 $\times$ 150-bp) sequencing generated 2,534,816 reads and 198.3 Mbp for *T. minor* (32$\times$ coverage) and 2,624,596 reads and 207.5 Mbp for *T. mannitoliphagus* (33$\times$ coverage). Quality control of the reads was performed using FastQC within BaseSpace version 1.0.0 (Illumina), using a k-mer size of 5 and contamination filtering. We assembled the genome *de novo* using SPAdes version 3.10.0 (9) through PATRIC (10). This assembly yielded 389 contigs (>300 bp) and an N_{50} value of 60,973 bp for *T. minor*, while *T. mannitoliphagus* was assembled into 525 contigs with an N_{50} value of 38,298 bp. The *T. minor* genome had a GC content of 64.8% and was 6,204,734 bp long, and the *T. mannitoliphagus* genome had 62.5% GC content and was 6,310,033 bp long. The genomes were annotated using the RAST tool kit (RASTtk; 11) within PATRIC (10). This showed *T. minor* to have 6,147 coding sequences (CDSs) and 50 tRNAs, and *T. mannitoliphagus* contained 6,471 CDSs and 48 tRNAs. The genomes, when annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), resulted in 5,426 CDSs for *T. minor* and 5,468 CDSs for *T. mannitoliphagus*. Default parameters were used for all software applications unless otherwise noted.

A JSpecies comparison (12) of average percent nucleotide identity (ANIb) showed 82.3% identity between *T. minor* and *T. mannitoliphagus*. Both genomes gave an ANI of 75% with *Thiorhodococcus drewsii* DSM15006 and 75 to 76% with *Imhoffiella purpurea*

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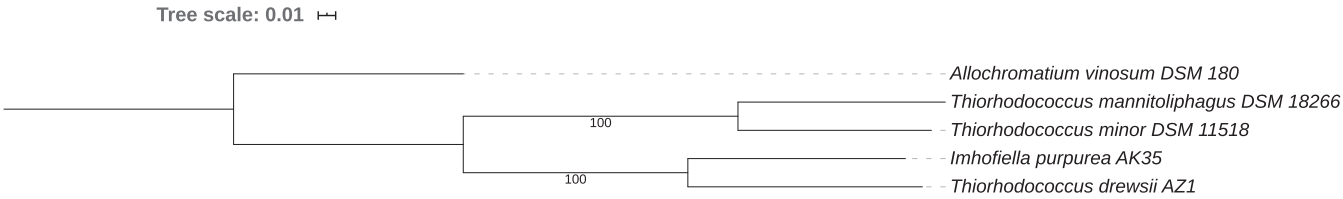

FIG 1 Whole-genome-based phylogenetic tree of all sequenced *Thiorhodococcus* and *Imhoffiella* species. The phylogenetic tree was generated using the codon tree method within PATRIC (10), which used cross-genus families (PGFams) as homology groups. Among these selected genomes, 500 PGFams were found using the CodonTree analysis, and the aligned proteins and coding DNA from single-copy genes were used for RAxML analysis (13, 14). The support values for the phylogenetic tree were generated using 100 rounds of the "Rapid bootstrapping" option in RaxML (13). *Allochromatium vinosum* was used as an outgroup. iTOL was used for the tree visualization (15).

AK35. The ANI numbers for all these genomes are clearly below the proposed 95% cutoff for genome definition of a species (12). *T. drewsii* and *I. purpurea* are also 82% identical to one another, which does not support the separation of these two as separate genera. Whole-genome-based phylogenetic analysis of all of the *Thiorhodococcus* genomes using RAxML within PATRIC (13, 14) showed two distinct groups, with *T. minor* and *T. mannitoliphagus* as the closest relatives and the group of *T. drewsii* and *I. purpurea* more distantly related, consistent with the ANI analysis (Fig. 1). These whole-genome analyses suggest that while *T. minor* and *T. mannitoliphagus* group together, *T. drewsii* could be included in the genus *Imhoffiella* if further sequence, morphological, and physiological studies warrant it, although our studies suggest that all four should remain in the genus *Thiorhodococcus*, which is clearly differentiated from *Allochromatium*.

Data availability. These whole-genome shotgun projects have been deposited at DDBJ/ENA/GenBank under the accession numbers JAAIJQ000000000 for *Thiorhodococcus minor* and JAAIJR000000000 for *Thiorhodococcus mannitoliphagus*. The versions described in this paper are versions JAAIJQ010000000 and JAAIJR010000000. The raw sequencing reads have been submitted to SRA, and the corresponding accession numbers are SRR11068433 for *Thiorhodococcus minor* and SRR11068465 for *Thiorhodococcus mannitoliphagus*.

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