



Whole-Genome Sequence of the Novel *Rubrobacter taiwanensis* Strain Yellowstone, Isolated from Yellowstone National Park

Shawn Freed, Jr.,^a Robert F. Ramaley,^b John A. Kyndt^a

^aCollege of Science and Technology, Bellevue University, Bellevue, Nebraska, USA

^bDepartment of Biochemistry and Molecular Biology, UNMC, Omaha, Nebraska, USA

ABSTRACT The family *Rubrobacteraceae* is often represented by its thermophilic and radiotolerant species. *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus* have been extensively studied, contributing to defining the characteristics of the family. We have now sequenced the genome of a *Rubrobacter taiwanensis* strain, isolated from Yellowstone National Park, to further characterize the family.

The key feature of *Rubrobacter* species is their thermophilic behavior. This was first observed in isolates of *R. xylanophilus* and *R. radiotolerans* (1, 2) and has since been documented in two newer species, *R. calidifluminis* and *R. naiadicus* (3). They commonly exhibit halotolerance and have been found to live in hot springs around the world, but their most unique characteristic is their high tolerance to gamma radiation. This has been hypothesized to be a by-product of defense against desiccation, similar to that in the radiation-tolerant genus *Deinococcus*, which is found in high gamma radiation environments (2, 4). The documented species in this family are recorded as having modest genome sizes (around 2 to 3 Mbp) and commonly contain genes for reduction of nitrates to nitrite, as well as cytochrome oxidase and catalase (1, 4). A more recent study suggests the presence of proteins involved in chlorophyll biosynthesis, suggesting a photosynthetic pathway (5).

A pink isolate was obtained from the Mushroom Hot Spring runoff in Yellowstone National Park, with a sampling point temperature of 50°C (lat 44.5387, long -110.798). The sample was obtained from the microbial mat and its surrounding water. The isolate was purified by streaking on solid *Thermus* medium with 0.3% tryptone and 0.3% yeast extract (6). A partial 16S rRNA gene was sequenced (530 bp) by MIDI Labs (Newark, DE). A BLAST (NCBI) comparison showed 99% and 100% similarity with *Rubrobacter taiwanensis* LS286 and LS293, respectively, 96% similarity with *Rubrobacter spartanus*, 95% similarity with *Rubrobacter xylanophilus*, and 94% similarity with *Rubrobacter radiotolerans*. No genome sequence is currently available for either of the closely related *R. taiwanensis* strains, which were isolated previously from hot springs in Taiwan (7). We have sequenced the genome of this new isolate to further characterize the species identity.

We isolated DNA of our *R. taiwanensis* isolate from living culture grown on *Thermus* medium with 0.3% yeast extract and 0.15% fructose and ribose. Genomic DNA was isolated using the GeneJET DNA purification kit (Thermo Scientific) to perform a genome comparison and explore the similarities and differences from more extensively studied species. Utilizing Qubit and NanoDrop technologies, we determined the quality and quantity of DNA, showing a 260/280 ratio of 1.79. The genome was sequenced by an Illumina MiniSeq system using 500 μ l of a 1.8 pM library. Paired-end (2 \times 150-bp) sequencing generated 3,883,502 reads and 330 Mbp. Quality control of the reads was performed using FastQC within BaseSpace version 1.0.0 (Illumina) using a k-mer size of

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Address correspondence to John A. Kyndt, jkyndt@bellevue.edu.

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5 and contamination filtering. We performed *de novo* assembly using Velvet version 1.2.10 (8) through BaseSpace using a minimum k-mer size of 21 and a maximum k-mer size of 121 and including reverse complement reads. This assembly yielded 53 contigs ($\geq 1,000$ bp), the largest being 350,459 bp, and an N_{50} value of 112,895 bp. The GC content was 67.2%. We then annotated the genome sequence using Rapid Annotations using Subsystems Technology (RAST) version 2.0 (9), using the RASTk default annotation pipeline, including the automated error fix and gap backfill settings. This showed our strain to be 3,035,128 bp long with 3,170 coding sequences and 48 RNAs identified.

A JSpecies comparison (10) of average percentage nucleotide identities (ANI) between this *R. taiwanensis* genome and other published *Rubrobacteraceae* genomes gave the following percentages: 74.2% (*R. xylanophilus* DSM9941), 71.4% (*R. aplysinae*), 71.0% (*R. radiotolerans* RSPS-4), and 69.6% (*R. indicocanis*). Thus, *R. taiwanensis* appears to be approximately equidistant to the other four *Rubrobacter* species that have been sequenced. They are all more distant from *Kineococcus radiotolerans*, with an ANI of 65.0%. The *R. taiwanensis* ANI numbers are clearly below the proposed 95% cutoff for the genome definition of a species (10), suggesting its own species identity.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [SKBU00000000](https://doi.org/10.1093/bioinformatics/btv681). The version described in this paper is version SKBU01000000. The raw sequencing reads have been submitted to the SRA, and the corresponding accession number is [SRR8670886](https://doi.org/10.1093/bioinformatics/btv681).

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REFERENCES

- Carreto L, Moore E, Nobre MF, Wait R, Riley PW, Sharp RJ, da Costa MS. 1996. *Rubrobacter xylanophilus* sp. nov., a new thermophilic species isolated from a thermally polluted effluent. *Int J Syst Evol Microbiol* 46:460–465. <https://doi.org/10.1099/00207713-46-2-460>.
- Ferreira AC, Nobre MF, Moore E, Rainey FA, Battista JR, da Costa MS. 1999. Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*. *Extremophiles* 3:235–238. <https://doi.org/10.1007/s007920050121>.
- Albuquerque L, Johnson MM, Schumann P, Rainey FA, da Costa MS. 2014. Description of two new thermophilic species of the genus *Rubrobacter*, *Rubrobacter calidifluminis* sp. nov. and *Rubrobacter naiadicus* sp. nov., and emended description of the genus *Rubrobacter* and the species *Rubrobacter bracarensis*. *Syst Appl Microbiol* 37:235–243. <https://doi.org/10.1016/j.syapm.2014.03.001>.
- Egas C, Barroso C, Froufe HJC, Pacheco J, Albuquerque L, da Costa MS. 2014. Complete genome sequence of the radiation-resistant bacterium *Rubrobacter radiotolerans* RSPS-4. *Stand Genomic Sci* 9:1062–1075. <https://doi.org/10.4056/sigs.5661021>.
- Gupta RS, Khadka B. 2016. Evidence for the presence of key chlorophyll-biosynthesis-related proteins in the genus *Rubrobacter* (phylum Actinobacteria) and its implications for the evolution and origin of photosynthesis. *Photosynth Res* 127:201–218. <https://doi.org/10.1007/s11120-015-0177-y>.
- Ramaley RF, Hixson J. 1970. Isolation of a nonpigmented, thermophilic bacterium similar to *Thermus aquaticus*. *J Bacteriol* 103:527–528.
- Chen M-Y, Wu S-H, Lin G-H, Lu C-P, Lin Y-T, Chang W-C, Tsay S-S. 2004. *Rubrobacter taiwanensis* sp. nov., a novel thermophilic, radiation-resistant species isolated from hot springs. *Int J Syst Evol Microbiol* 54:1849–1855. <https://doi.org/10.1099/ijs.0.63109-0>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Richter M, Rosselló-Móra R, Glöckner FO, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.