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GENOME SEQUENCES

Draft Genome Sequence of *Ralstonia* sp. Strain SET104, Isolated from Root Nodules of *Aeschynomene indica*

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ABSTRACT Here, we report a draft genome sequence of *Ralstonia* sp. strain SET104, isolated from the root nodules of *Aeschynomene indica*. The assembled draft genome size was 4,796,748 bp, containing a predicted total of 4,464 protein-encoding sequences. SET104 appears to be a novel species of the genus *Ralstonia*.

Aeschynomene indica is known to form nitrogen-fixing root and stem nodules in association with bacteria in the family *Bradyrhizobiaceae* (1, 2) through intercellular infection (1, 3) that is independent of nodulation (Nod) factors (4, 5). Since this process is thought to be the basis of bacterial infection in leguminous plants (3, 6), an understanding of the mechanism involved will elucidate how legumes evolved these symbiotic relationships.

We collected *A. indica* seeds from wild plants growing in Sasayama, Japan, and confirmed their identity by sequencing the nuclear ribosomal DNA internal transcribed spacer (ITS)/5.8S region and the chloroplast DNA *trnL* intron (7). We germinated the seeds and grew the resulting seedlings in volcanic ash soil supplemented with nitrogen-free medium (8) and water in a paddy field in Sasayama. After 2 months, roots with nodules were harvested, surface sterilized, crushed, and streaked on arabinose-gluconate agar plates (9) to isolate the bacteria in the nodules. We then reinoculated the *A. indica* seedlings with the isolated bacteria under sterilized conditions. One of the isolated strains, named SET104, was identified as a *Ralstonia* sp. based on partial sequencing of the 16S rRNA gene. Since there have been no previous reports of nitrogen fixation by a *Ralstonia* sp., we focused on producing a draft genome for SET104.

Genomic DNA was extracted from strain SET104 cells using a genomic DNA purification kit (Promega, USA) following the manufacturer's protocol. DNA libraries were prepared using a TruSeq DNA sample prep kit (Illumina, USA) and sequenced with the NextSeq 500 sequencer (Illumina, USA). Paired-end sequencing generated a total of 28,752,817 reads with an average read length of 100 bp. The reads were quality trimmed using the FASTQ preprocessing program fastp (10). *De novo* assembly was then performed using the SPAdes genome assembler version 3.12 (11) with two options ("–k auto" and "–careful"), which yielded 58 contigs with an N_{50} value of 287,261 bp and 59-fold mean coverage. The resulting draft genome of strain SET104 consisted of 4,796,748 bases with a GC content of 63.3%. Annotation of the contigs was performed using the DFAST version 1.0.8 pipeline (12), which identified 4,464 putative coding sequences.

Genome annotation indicated an absence of the *nifH* gene, which encodes nitrogenase iron protein, and other genes that are required for nitrogen fixation. Furthermore, although the *nodI* and *nodJ* genes were found to be present, the canonical

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nodulation genes (*nodA*, *nodB*, and *nodC*) were not detected, indicating that strain SET104 does not produce Nod factors.

Application of the genome-based distance matrix calculator (http://enve-omics.ce.gatech.edu/g-matrix/index) revealed that SET104 had average nucleotide identity (ANI) and average amino acid identity (AAI) values of 88% and 91%, respectively, in relation to the closest species, *Ralstonia pickettii*. Since genomes with an ANI of >95% and/or an AAI of >95% are generally considered to have originated from the same species (13–15), we believe that SET104 represents a novel species of *Ralstonia*.

Data availability. The genome sequence of *Ralstonia* sp. strain SET104 was deposited in DDBJ/EMBL/GenBank under accession numbers BHVX01000001 to BHVX01000058. The raw sequencing reads have been submitted to the Sequence Read Archive (SRA) under the accession number DRA007473.

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