Draft Genome Sequences of 13 Plant-Associated Actinobacteria of the Family Microbacteriaceae

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ABSTRACT  Draft genome sequences of 13 bacterial strains from the family Microbacteriaceae were generated using Illumina technology. The genome sizes varied from 3.0 to 4.8 Mb, and the DNA G+C content was 68.1 to 72.5%. The sequences obtained will contribute to the development of genome-based taxonomy and understanding of molecular interactions between bacteria and plants.

Members of the family Microbacteriaceae (class Actinobacteria) are widely distributed in various terrestrial and aquatic ecosystems and often occur in association with plants as endophytes and pathogens (1–3).

Novel strains of Microbacteriaceae were recovered from eight different plants of five families (Table 1) collected in various sites in California. Rathayibacter sp. strain VKM Ac-2835 was isolated from a Malus sp. with symptoms of bacterial wetwood disease by macerating several pieces of symptomatic superficial bark tissue in a sterile aqueous solution and then plating it onto Pseudomonas F agar (Becton, Dickinson, USA) amended with cycloheximide (100 mg/liter). The remaining strains were isolated from plants without visible symptoms of diseases, as described (3, 4), but Reasoner’s 2A (R2A) agar (Fluka Analytical, USA) was used as the plating medium for isolation. Rathayibacter agropyri CA-4T (=VKM Ac-2828T) was kindly provided by T. D. Murray. For preservation, strains were grown on R2A agar and lyophilized using standard techniques. All strains were deposited in the All-Russian Collection of Microorganisms (VKM; http://www.vkm.ru).

Biomass for DNA extraction was grown in liquid peptone-yeast medium (5) inoculated with cells from a single colony, followed by cultivation at 28°C for 18 to 20 h on a rotary shaker. Genomic DNA was extracted using a QIAamp DNA minikit (Qiagen, Germany). DNA libraries for strains VKM Ac-2828T, VKM Ac-2835, and VKM Ac-2836 were prepared in-house using a NEBNext Ultra II FS DNA library prep kit for Illumina (New England Biolabs, USA) following the protocol for use with inputs of ≥100 ng with modifications as described previously (6). Pooled DNA libraries were sequenced by Novogene Co., Ltd., on an Illumina HiSeq X Ten instrument to obtain 150-bp paired-end reads. For the remaining strains, DNA library construction and sequencing were conducted by Novogene Co., Ltd. Libraries were generated using a NEBNext DNA library prep kit for Illumina (New England Biolabs) following the manufacturer’s recommendations. Pooled DNA libraries were sequenced on an Illumina NovaSeq 6000 instrument to obtain 150-bp paired-end reads.
| Organism                          | Associated plant (family)       | No. of reads | Coverage (X) | No. of scaffolds | Scaffold \( N_{50} \) (bp) | Genome size (Mbp) | G+C content (%) | No. of proteins | SRA accession no. | GenBank accession no. |
|----------------------------------|---------------------------------|--------------|--------------|-----------------|----------------------------|-------------------|-----------------|-----------------|------------------|----------------------|
| **Rathayibacter agropyri**       | **Pascopyrum smithii** (Poaceae)| 14,362,336   | 578          | 25              | 656,029                   | 0.9               | 68.1            | 2,835           | SRX8466800         | JABRPL0000000000     |
| VKM Ac-2828^T                    |                                 |              |              |                 |                            |                   |                 |                 |                  |                      |
| **Rathayibacter** sp. VKM Ac-2835| **Malus domestica** (Rosaceae)  | 12,953,304   | 410          | 6               | 1,292,012                 | 4.3               | 72.2            | 3,849           | SRX8466801         | JABSNQ0000000000     |
| **Rathayibacter** sp. VKM Ac-2857| **Brachypodium distachyon** (Poaceae) | 21,681,636  | 692          | 8               | 1,409,124                 | 4.6               | 72.1            | 4,080           | SRX8466811         | JABMLE0000000000     |
| **Rathayibacter** sp. VKM Ac-2856| **Brachypodium distachyon** (Poaceae) | 20,040,560  | 689          | 9               | 783,576                   | 4.3               | 72.5            | 3,806           | SRX8466810         | JABMLF0000000000     |
| **Rathayibacter** sp. VKM Ac-2858| **Brachypodium distachyon** (Poaceae) | 22,149,976  | 762          | 9               | 783,576                   | 4.3               | 72.5            | 3,806           | SRX8466812         | JABMLD0000000000     |
| **Curtobacterium** sp. VKM Ac-2852| **Avena fatua** (Poaceae)       | 19,012,470   | 724          | 7               | 1,020,126                 | 3.9               | 70.8            | 3,580           | SXR8466807         | JABMUL0000000000     |
| **Curtobacterium** sp. VKM Ac-2861| **Marah** sp. (Cucurbitaceae)    | 17,600,848   | 642          | 15              | 624,972                   | 4.0               | 70.8            | 3,739           | SXR8466804         | JABMCLA0000000000     |
| **Frigoribacterium** sp. VKM Ac-2836| **Fragaria vesca** (Rosaceae)   | 28,426,948   | 1,100        | 10              | 1,120,753                 | 3.3               | 70.4            | 3,010           | SXR8466805         | JABRPK0000000000     |
| **Frigoribacterium** sp. VKM Ac-2859| **Brachypodium distachyon** (Poaceae) | 14,404,054  | 638          | 5               | 1,688,707                 | 3.3               | 71.3            | 3,027           | SXR8466812         | JABMCL0000000000     |
| **Frigoribacterium** sp. VKM Ac-2860| **Brachypodium distachyon** (Poaceae) | 14,248,970  | 632          | 5               | 1,688,688                 | 3.3               | 71.3            | 3,029           | SXR8466803         | JABMLB0000000000     |
| **Herbiconiux** sp. VKM Ac-2851 | **Soliva sessilis** (Asteraceae) | 18,251,960   | 623          | 9               | 1,342,178                 | 4.3               | 70.7            | 4,032           | SXR8466806         | JABMLO0000000000     |
| **Microbacteriaceae bacterium**  | **Myosotis** sp. (Boraginaceae) | 42,551,862   | 1,319        | 18              | 721,153                   | 4.8               | 69.6            | 4,359           | SXR8466808         | JABMLH0000000000     |
| **Microbacteriaceae bacterium**  | **Myosotis** sp. (Boraginaceae) |              |              |                 |                            |                   |                 |                 |                  |                      |
| VKM Ac-2854                      |                                 |              |              |                 |                            |                   |                 |                 |                  |                      |
| **Microbacteriaceae bacterium**  | **Myosotis** sp. (Boraginaceae) | 17,323,178   | 542          | 26              | 386,786                   | 4.7               | 68.3            | 4,255           | SXR8466809         | JABMLG0000000000     |
Default parameters were used for all software unless otherwise specified. The quality of the reads was checked with FastQC 0.11.8 (7). Adapter sequences and low-quality regions in the raw reads were cut with Trimmomatic 0.39 (8) with the following options: ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10, SLIDINGWINDOW:4:15, and MINLEN:50. Trimmed reads were assembled using SPAdes 3.14.1 (9) with the following options: --cov-cutoff, auto; and --careful. The quality of assembly was assessed with QUAST 5.0.2 (10). Assemblies were annotated with NCBI PGAP (11) and the RAST Web server (12, 13).

The pairwise similarity between the 16S rRNA gene sequences was determined using TaxonDC 1.3.1 (14). The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values were calculated using the JSpecies 1.2.1 (15) and GGDC 2.1 (16) tools, respectively.

Accession numbers and characteristics of the genomes are provided in Table 1. Figure 1 shows the phylogenomic positions of Rathayibacter strains sequenced in this work (in bold) and members of other validly published and some putative (6, 19, 20) Rathayibacter species. The tree was inferred by the balanced minimum evolution method using JolyTree 1.1.181205ac (21) with branch lengths scaled to the estimated number of substitutions per site. Branch support values (rate of elementary quartets) above 0.5 are indicated at the branch points. The genetic sequence of Clavibacter michiganensis subsp. sepedonicus ATCC 33113T (GenBank accession numbers AM849034.1 to AM849036.1) served as an outgroup (not shown) to root the tree.

A BLAST search confirmed the presence of a genomic cluster comprising a complete suite of tunicamyluracil-related biosynthetic genes in R. agropyri CA-4T as already reported by Tancos et al. (18) for this strain. This gene cluster is not present in any other genomes sequenced in this work.
Further whole-genome sequencing of other Microbacteriaceae along with comparative genomic and phenotypic analyses of putative and known species with validly published names will result in valid descriptions of the revealed new taxa, contributing to the development of the genome-based taxonomy of prokaryotes.

Data availability. These whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

We thank Andrei L. Osterman for his valuable help during work with this project, Timothy D. Murray for providing the Rathayibacter agropyri CA-47 strain, and Dean Kelch and Robert Price for plant identification.

This work was supported by the U.S. Department of Agriculture Animal and Plant Health Inspection Service according to the research project AP18PPQS&T00C159 (18-Kelch and Robert Price for plant identification.

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