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# Complete and Draft Genome Sequences of 12 Plant-Associated *Rathayibacter* Strains of Known and Putative New Species

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**ABSTRACT** Complete and draft genome sequences of 12 *Rathayibacter* strains were generated using Oxford Nanopore and Illumina technologies. The genome sizes of these strains are 3.21 to 4.61 Mb, with high G+C content (67.2% to 72.7%) genomic DNA. Genomic data will provide useful baseline information for natural taxonomy and comparative genomics of members of the genus *Rathayibacter*.

The genus *Rathayibacter* (*Actinobacteria*) comprises eight species with validly published names (1–6). In addition, some putative new species of this genus have been discovered, including “*Rathayibacter tanacetii*” (7–10). The species *R. rathayi*, *R. iranicus*, *R. tritici*, and *R. toxicus* are well-known plant pathogens causing a gumming disease of wheat and cereal grasses (4). *R. toxicus* is also responsible for toxicity of annual ryegrass and some other grasses, which often results in poisoning of grazing animals (7, 8). *Rathayibacter* species are transmitted to their host plants by seed gall nematodes of the genus *Anguina* (*Anguinidae*) (4, 11). Four additional *Rathayibacter* species were found in plant galls induced by the leaf gall nematode *Anguina graminis* (*R. festucae*) (3), in a diseased wheatgrass (*R. agropyri*) (6), and also in plants without any visible symptoms of bacterial diseases or nematode infestation (*R. caricis* and *R. oskolensis*) (3, 5).

Novel *Rathayibacter* strains were recovered from *Tanacetum vulgare* (*Asteraceae*) infested by the foliar nematode *Aphelenchoides fragariae* (*Aphelenchoididae*) and from plants with no visible disease symptoms (Table 1). All strains were isolated as described previously (5) and deposited in the All-Russian Collection of Microorganisms (VKM; <http://www.vkm.ru>). The universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AAGGAGGTGATCCAGCC-3') were used for 16S rRNA gene amplification and sequencing. The pairwise similarity between the 16S rRNA gene sequences was determined using TaxonDC (12). The strains showed 97.5% to 99.9% 16S rRNA gene sequence similarities with validly described *Rathayibacter* species.

For DNA extraction, biomass was grown in liquid peptone-yeast medium (13) 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 were prepared for long-read sequencing using the Nanopore rapid barcoding genomic DNA (gDNA) sequencing kit (catalog number SQK-RBK004; Oxford

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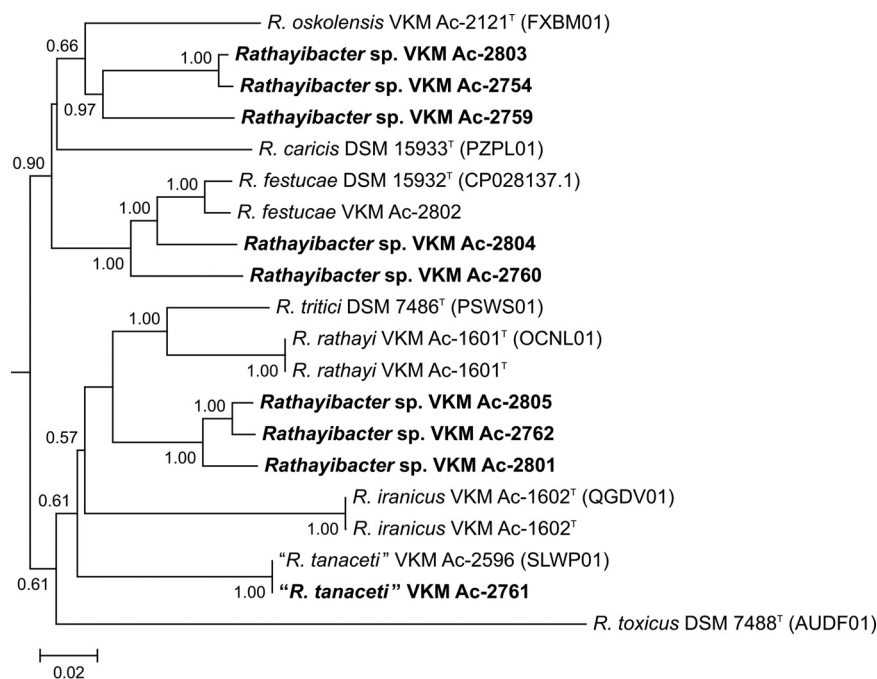
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**TABLE 1** Statistical information for genome sequences and DDBJ/ENA/GenBank accession numbers

| Organism                                    | Plant                             | Nematode               | No. of long reads | $N_{50}$ (bp) of long reads | No. of short reads <sup>a</sup> | Coverage (x) | No. of contigs | Contig $N_{50}$ (bp) | Genome size (Mbp) | G+C content (%) | No. of complete plasmids | No. of proteins | Completeness          | SRA accession no.        | GenBank accession no.  |
|---|-----------------------------------|------------------------|-------------------|-----------------------------|---------------------------------|--------------|----------------|----------------------|-------------------|-----------------|--------------------------|-----------------|-----------------------|--------------------------|--|
| <i>Rathayibacter</i> sp. VKM Ac-2759        | <i>Tanacetum vulgare</i>          | <i>A. fragariae</i>    | 105,881           | 8,604                       | 10,526,398                      | 442          |                |                      | 4.16              | 71.6            | 3                        | 3,814           | Complete              | SRR10912284, SRR10912285 | CP047176, CP047177, CP047178, CP047179, CP047175   |
| <i>Rathayibacter</i> sp. VKM Ac-2760        | <i>Tanacetum vulgare</i>          | <i>A. fragariae</i>    | 41,508            | 4,270                       | 12,240,072                      | 378          |                |                      | 4.61              | 72.1            | 2                        | 4,107           | Complete              | SRR10912303, SRR10912304 | CP047183, CP047184, CP047186, CP047187, CP047188, CP047189, CP047180, CP047181, CP047182 |
| " <i>R. tanacetii</i> " VKM Ac-2761         | <i>Tanacetum vulgare</i>          | <i>A. fragariae</i>    | 70,773            | 9,437                       | 23,061,818                      | 1,111        |                |                      | 3.21              | 70.7            |                          | 2,932           | Complete              | SRR10912305, SRR10912306 | CP047183, CP047184   |
| <i>Rathayibacter</i> sp. VKM Ac-2801        | <i>Androsace koso-polljanskii</i> | No                     | 52,705            | 8,511                       | 19,740,286                      | 791          |                |                      | 3.63              | 72.3            | 1                        | 3,317           | Complete              | SRR10912288, SRR10912289 | CP047183, CP047184   |
| <i>R. festucae</i> VKM Ac-2802              | <i>Androsace koso-polljanskii</i> | No                     | 80,390            | 4,226                       | 17,945,598                      | 572          |                |                      | 4.32              | 72.4            | 2                        | 3,871           | Complete              | SRR10912286, SRR10912287 | CP047180, CP047181, CP047182   |
| <i>Rathayibacter</i> sp. VKM Ac-2805        | <i>Gypsophila altissima</i>       | No                     | 175,323           | 4,603                       | 9,212,982                       | 431          |                |                      | 3.6               | 72.4            |                          | 3,285           | Complete              | SRR10912290, SRR10912294 | CP047185   |
| <i>Rathayibacter</i> sp. VKM Ac-2762        | <i>Limonium</i> sp.               | No                     | 36,401            | 3,682                       | 7,531,042                       | 302          |                |                      | 3.45              | 72.7            |                          | 3,151           | Complete <sup>b</sup> | SRR10912299, SRR10912300 | CP047419   |
| <i>Rathayibacter</i> sp. VKM Ac-2804        | <i>Koeleria macrantha</i>         | No                     | 91,359            | 5,322                       | 9,828,426                       | 374          |                |                      | 4.09              | 72.4            |                          | 3,686           | Complete <sup>b</sup> | SRR10912301, SRR10912302 | CP047420   |
| <i>R. rathayi</i> VKM Ac-1601 <sup>T</sup>  | <i>Dactylis glomerata</i>         | <i>Anguina</i> sp.     |                   |                             | 9,771,504                       | 401          | 60             | 256,770              | 3.21              | 69.3            |                          | 2,983           | Draft                 | SRR10912291              | WUCA000000000  |
| <i>R. iranicus</i> VKM Ac-1602 <sup>T</sup> | <i>Triticum aestivum</i>          | <i>Anguina tritici</i> | 3,667             | 4,472                       | 14,405,148                      | 542          | 62             | 193,466              | 3.38              | 67.2            |                          | 3,121           | Draft                 | SRR10912292, SRR10912293 | WUCB000000000  |
| <i>Rathayibacter</i> sp. VKM Ac-2754        | <i>Androsace koso-polljanskii</i> | No                     | 4,359             | 3,645                       | 3,293,486                       | 112          | 24             | 431,504              | 3.97              | 71.6            | 1                        | 3,660           | Draft                 | SRR10912295, SRR10912296 | WUCB000000000  |
| <i>Rathayibacter</i> sp. VKM Ac-2803        | <i>Androsace koso-polljanskii</i> | No                     | 57,177            | 5,352                       | 22,330,660                      | 753          | 4              | 3,988,627            | 4.29              | 71.3            | 2                        | 3,978           | Draft                 | SRR10912297, SRR10912298 | WUCD000000000  |

<sup>a</sup> 150-bp paired-end reads.

<sup>b</sup> Chromosome contains one gap.



**FIG 1** Phylogenomic tree based on 20 bacterial genomes of the genus *Rathayibacter*. The tree is drawn to scale, with branch lengths measured in the estimated number of substitutions per site. Branch support values (rate of elementary quartets) above 0.5 are indicated at the branch points. The following newly isolated strains of seven putative new species are given in bold: (i) VKM Ac-2803 and VKM Ac-2754, (ii) VKM Ac-2759, (iii) VKM Ac-2804, (iv) VKM Ac-2760, (v) VKM Ac-2805 and VKM Ac-2762, (vi) VKM Ac-2801, and (vii) VKM Ac-2761 (the second strain of "*R. tanacetii*"). The genomic sequence of *Clavibacter sepedonicus* ATCC 33113<sup>T</sup> (GenBank accession numbers [AM849034.1](https://www.ncbi.nlm.nih.gov/nuccore/AM849034.1) to [AM849036.1](https://www.ncbi.nlm.nih.gov/nuccore/AM849036.1)) served as an outgroup (not shown).

Nanopore Technologies) according to the manufacturer's protocol and were sequenced in-house using a MinION device.

DNA libraries of strains VKM Ac-2754, VKM Ac-2759, VKM Ac-2760, VKM Ac-2762, VKM Ac-2804, and VKM Ac-2805 were prepared for short-read sequencing using the Nextera DNA flex library prep kit (Illumina) and Nextera DNA CD indexes (Illumina) according to the manufacturer's instructions. DNA libraries of strains VKM Ac-1601<sup>T</sup>, VKM Ac-1602<sup>T</sup>, VKM Ac-2761, VKM Ac-2801, VKM Ac-2802, and VKM Ac-2803 were prepared using NEBNext Ultra II FS DNA library prep kit for Illumina (New England BioLabs) following the protocol for use with inputs of  $\geq 100$  ng with the following modifications: TruSeq DNA CD indexes (Illumina) were used in place of NEBNext adaptors to eliminate the need for PCR steps. The USER enzyme addition was skipped for this reason, and the volume was adjusted with water to reach the necessary sample volume for size selection steps. No PCR amplification was performed on these libraries. Pooled DNA libraries were sequenced by Novogene Co., Ltd.

Default parameters were used for all software unless otherwise specified. Nanopore basecalling was performed by Guppy basecalling software 2.3.5, available from the Oxford Nanopore Technology (ONT) community website (with the following parameters: --flowcell, FLO-MIN106; --kit, SQK-RBK004), and demultiplexed by Deepbinner 0.2.0 (14) (with parameter --rapid). Adapter sequences from long reads were removed using Porechop 0.2.4 (<https://github.com/rrwick/Porechop>) with parameter --discard\_middle. Adapter sequences and low-quality regions in short reads were cut using Trimmomatic 0.39 (15) with the following parameters: ILLUMINACLIP:adapters.fa:2:30:10; SLIDING-WINDOW:4:15; MINLEN:30, where adapters.fa is NexteraPE-PE.fa or TruSeq3-PE-2.fa depending on the kit used to prepare the library. Hybrid assembly was performed by Unicycler 0.4.8 (16). There was insufficient DNA quantity of VKM Ac-1601<sup>T</sup> to make a library for Nanopore sequencing; thus, the genome assembly of this organism was

performed on short reads only. The quality of assemblies was assessed with QUAST 5.0.2 (17). Assemblies were annotated with the NCBI PGAP (18) and the RAST Web server (19, 20). A phylogenomic tree was inferred by the balanced minimum evolution method using JolyTree (21). Statistical information for the complete and draft genome sequences is given in Table 1. It is worth noting that plasmids were identified in the genome assemblies of *Rathayibacter* strains for the first time.

The tree (Fig. 1) shows that 9 of the 10 novel strains cluster separately from the *Rathayibacter* species with validly published names. The calculated average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values (well below the borderlines for species differentiation [22]; not shown) indicated the presence of seven putative new species among the strains studied. Further comparative phenotypic study and genome-wide analyses of these strains and other members of the genus *Rathayibacter* will result in valid descriptions of the revealed new species and facilitate insight into the molecular mechanisms involved in interactions between plants and bacteria.

**Data availability.** These whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1. The versions reported here are the first versions. The accession numbers of the 16S rRNA gene sequences deposited in DDBJ/ENA/GenBank are [MT431563](#) to [MT431574](#).

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