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 tanaceti" (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. carcis and R. oskolensis) (3, 5).

Novel Rathayibacter strains were recovered from Tanacetum vulgare (Asteraceae) infested by the foliar nematode Aphelenchoides fragariae (Aphelenchoidea) 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...
| Organism                          | Plant          | Nematode          | No. of long reads | No. of short reads | Coverage (×) | No. of contigs | Confg N50 (bp) | Genome size (Mb) | G+C content (%) | No. of complete plasmids | No. of proteins | Completeness | SRA accession no. | GenBank accession no. |
|----------------------------------|----------------|-------------------|-------------------|-------------------|--------------|----------------|----------------|-----------------|----------------|------------------------|----------------|--------------|--------------------|--------------------|
| Rathayibacter sp. VKM Ac-2759   | Tanacetum vulgare | A. fragariae     | 105,881           | 8,604             | 10,526,398   | 442            | 4.16           | 71.6            | 3              | 3,814                  | Complete       | SRR10912284, SRR10912285 | CP047176, CP047177, CP047178, CP047179 |
| Rathayibacter sp. VKM Ac-2760   | Tanacetum vulgare | A. fragariae     | 41,508            | 4,270             | 12,240,072   | 378            | 4.61           | 72.1            | 2              | 4,107                  | Complete       | SRR10912303, SRR10912304 | CP047173, CP047174, CP047175 |
| *R. tanaceti* VKM Ac-2761        | Tanacetum vulgare | A. fragariae     | 70,773            | 9,437             | 23,061,818   | 1,111          | 3.21           | 70.7            | 2              | 2,932                  | Complete       | SRR10912305, SRR10912306 | CP047186 |
| Rathayibacter sp. VKM Ac-2801   | Androsace koso-poljanskii | No       | 52,705            | 8,511             | 19,740,286   | 791            | 3.63           | 72.3            | 1              | 3,317                  | Complete       | SRR10912288, SRR10912289 | CP047183, CP047184 |
| R. festucae VKM Ac-2802         | Androsace koso-poljanskii | No       | 80,390            | 4,226             | 17,945,598   | 572            | 4.32           | 72.4            | 2              | 3,671                  | Complete       | SRR10912286, SRR10912287 | CP047180, CP047181, CP047182 |
| Rathayibacter sp. VKM Ac-2805   | Gypsophila altilisima | No       | 175,323           | 4,603             | 9,212,982    | 431            | 3.6            | 72.4            | 3              | 3,285                  | Complete       | SRR10912290, SRR10912294 | CP047185 |
| Rathayibacter sp. VKM Ac-2762   | Limonium sp.      | No               | 36,401            | 3,682             | 7,351,042    | 302            | 3.45           | 72.7            | 3              | 3,151                  | Complete       | SRR10912299, SRR10912300 | CP047419 |
| Rathayibacter sp. VKM Ac-2804   | Koeleria macrantha | No               | 91,359            | 5,322             | 9,828,426    | 374            | 4.09           | 72.4            | 2              | 3,666                  | Complete       | SRR10912301, SRR10912302 | CP047420 |
| R. rathayi VKM Ac-1601T          | Dactylis glomerata | Anguina sp.      | 9,771,504         | 401              | 256,770      | 3.21           | 69.3           | 2              | 2,983                  | Draft          | SRR10912291 | WUC900000000 |
| R. iranicus VKM Ac-1602T         | Triticum aestivum | Anguina tritici | 3,667             | 4,472             | 14,405,148   | 542            | 3.38           | 67.2            | 3              | 3,121                  | Draft          | SRR10912292, SRR10912293 | WUC800000000 |
| Rathayibacter sp. VKM Ac-2754   | Androsace koso-poljanskii | No       | 4,359             | 3,645             | 3,293,486    | 112            | 3.97           | 71.6            | 1              | 3,660                  | Draft          | SRR10912295, SRR10912296 | WUC900000000 |
| Rathayibacter sp. VKM Ac-2803   | Androsace koso-poljanskii | No       | 57,177            | 5,352             | 22,330,660   | 753            | 4.29           | 71.3            | 2              | 3,978                  | Draft          | SRR10912297, SRR10912298 | WUC900000000 |

*a* 150-bp paired-end reads.

*b* Chromosome contains one gap.
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-1601T, VKM Ac-1602T, 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 ≥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; SLIDINGWINDOW: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-1601T 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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