Draft Genome Sequences of 9 Actinobacteria from the Family Microbacteriaceae Associated with Insect- and Nematode-Damaged Plants

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ABSTRACT  Draft genome sequences of 9 strains of known and putative new species of Microbacteriaceae isolated from insect- and nematode-damaged plants were generated using Illumina technology. The data obtained will contribute to the development of the genome-based prokaryote taxonomy and the knowledge on the biology of the microbial group investigated.

Actinobacteria of the family Microbacteriaceae are distributed widely in various terrestrial and aquatic environments and often inhabit plants as endophytes or pathogens (1–3). Many putative new species of several genera of this family, along with representatives of known species, were found in plants infested by different nematodes (4, 5).

Novel strains were isolated from affected and unaffected parts of leaves of different herbaceous and woody plants infected by nematodes, leaf-mining insects, and plant-parasitic mites (Table 1).

The air-dried plant samples were soaked in distilled water for 1 h, washed twice with sterile distilled water, placed in 0.85% NaCl solution, and milled. One drop of the obtained suspension was plated onto Reasoner’s 2A (R2A) agar (Fluka Analytical, USA) and incubated for 1 to 3 weeks at room temperature (18 to 24°C). The universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGATCCAGGCC-3′) with standard cycling conditions (95°C for 5 min; 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min 20 s; and 72°C for 4 min) were used for 16S rRNA gene PCR amplification followed by sequencing. Preliminary identification based on nearly entire 16S rRNA gene sequences revealed that the newly isolated strains belong to four genera of the family Microbacteriaceae, exhibiting high sequence identities to the known species (Table 1).

For genome sequencing, biomass was grown in liquid peptone-yeast-glucose (PYG) medium (5 g peptone, 3 g yeast extract, 5 g glucose, 0.2 g KH₂PO₄, and 1 L distilled water [pH 7.2]) or on R2A agar inoculated with cells from a single colony, followed by cultivation at 28°C for 2 to 3 days. Genomic DNA was extracted using a QIAamp DNA minikit (Qiagen, Germany). DNA library construction and sequencing were performed by using Novogene Co., Ltd. Libraries were generated with the NEBNext DNA library prep kit for Illumina (New England Biolabs, USA) following the manufacturer’s recommendations. Pooled DNA libraries were sequenced on an Illumina NovaSeq 6000 instrument to obtain 150-bp paired-end reads.

The quality of the reads was checked with FastQC v0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapter sequences and low-quality regions in raw reads
TABLE 1 Strain data, characteristics, and DDBJ/ENA/GenBank accession numbers of genome sequences

| Organism | Plant | Plant parasite | Geography | No. of reads | Coverage (%) | No. of scaffolds | Scaffold N₅₀ (bp) | Genome size (Mb) | G+C content (%) | No. of proteins | Closest species by 16S rRNA gene (identity %) | Closest species by ANI (identity %) | Closest species by dDDH (identity %) | 16S rRNA gene GenBank accession no. | Genome GenBank accession no. | Genome SRA accession no. |
|----------|-------|----------------|-----------|--------------|--------------|-----------------|-----------------|-----------------|----------------|----------------|----------------------------------------|-------------------------------|---------------------------------|----------------------------------|--------------------------|--------------------------|
| Clavibacter michiganensis subsp. phaseoli (100) | | | | | | | | | | | | | | | | | |
| Guthrobacterum mohejodari (97.30) | | | | | | | | | | | | | | | | | |
| Clavibacter michiganensis subsp..phaseoli (100) | | | | | | | | | | | | | | | | | |
| Rathayibacter caraci (99.79) | | | | | | | | | | | | | | | | | |
| Rathayibacter caraci (97.71) | | | | | | | | | | | | | | | | | |
| Rathayibacter caraci (82.16) | | | | | | | | | | | | | | | | | |
| Rathayibacter sp. | | | | | | | | | | | | | | | | | |
| Rathayibacter sp. | | | | | | | | | | | | | | | | | |
| Rathayibacter sp. | | | | | | | | | | | | | | | | | |
| Rathayibacter sp. | | | | | | | | | | | | | | | | | | Unaffected parts of leaves were used for the isolation of strains. |
were cut with Trimmomatic 0.39 (6). Trimmed reads were assembled using SPAdes 3.15.4 (7). Assemblies were annotated with NCBI PGAP 6.1 (8). The pairwise similarity between the 16S rRNA gene sequences was determined using TaxonDC 1.3.1 (9). The genome relatedness indices, viz., the average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values, were calculated using JSpecies 1.2 (10) and GGDC 2.1 (11) tools, respectively. Accession numbers and characteristics of genomes are provided in Table 1.

The ANI and dDDH values obtained for strains VKM Ac-2922 and VKM Ac-2923 and their closest relatives were well below the accepted species threshold (12), indicating the strains to be members of two putative new species in the genera Curtobacterium and Microbacterium. Four strains (VKM Ac-2926, VKM Ac-2927, VKM Ac-2928, and VKM Ac-2929) may represent a new species as well. The genome relatedness indices for these strains toward their closest relative, namely, Rathayibacter festucae, were at the borderline for species definition or slightly below (12). From the genome relatedness indices, three remaining strains (VKM Ac-2921, VKM Ac-2924, and VKM Ac-2925) belonged to the known Clavibacter and Rathayibacter species.

Data availability. The whole-genome shotgun projects and the 16S rRNA gene sequences have been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1.

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