



Draft Genome Sequences of 28 Actinobacteria of the Family *Microbacteriaceae* Associated with Nematode-Infected Plants

 Sergey V. Tarlachkov,^a Irina P. Starodumova,^a Lubov V. Dorofeeva,^a Natalia V. Prisyazhnaya,^a Tatiana V. Roubtsova,^b Vladimir N. Chizhov,^c Steven A. Nadler,^d Sergei A. Subbotin,^{c,d,e} Lyudmila I. Evtushenko^a

^aAll-Russian Collection of Microorganisms (VKM), G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences, Pushchino, Russia

^bDepartment of Plant Pathology, University of California, Davis, California, USA

^cCenter of Parasitology of A. N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, Russia

^dDepartment of Entomology and Nematology, University of California, Davis, California, USA

^eCalifornia Department of Food and Agriculture, Sacramento, California, USA

ABSTRACT Draft genome sequences of 28 strains of *Microbacteriaceae* from plants infested by plant-parasitic nematodes were obtained using Illumina technology. The sequence data will provide useful baseline information for the development of comparative genomics and systematics of *Microbacteriaceae* and facilitate understanding of molecular mechanisms involved in interactions between plants and nematode-associated bacterial complexes.

Members of the family *Microbacteriaceae* (class *Actinobacteria*) inhabit various terrestrial and aquatic ecosystems and often occur in plants as endophytes and pathogens (1, 2). Plant-pathogenic species of the genus *Rhizobacter* belonging to this family are transmitted to their host plants by gall-forming nematodes of the genus *Anguina* (Anguinidae) (3, 4). Along with *Rhizobacter*, some other *Microbacteriaceae*, including members of the genera *Agreia*, *Leifsonia*, *Microbacterium*, and *Plantibacter*, were recovered from plant galls induced by Anguinidae and from leaf tissues of *Tanacetum vulgare* infested by *Aphelenchoides fragariae* (1, 5–8).

Novel nematode-associated strains of *Microbacteriaceae* were isolated from plant galls induced by different anguinids and from plant tissues affected by *Aphelenchoides* species (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 modified *Corynebacterium* agar (9) or Reasoner's 2A (R2A) agar (Fluka Analytical, USA) and incubated for 1 to 3 weeks at room temperature (18 to 24°C). The isolated strains were identified on the basis of matrix-assisted laser desorption ionization (MALDI) mass spectra and 16S rRNA gene sequences as described previously (10, 11) and deposited in the All-Russian Collection of Microorganisms (VKM; <http://www.vkm.ru>).

For genome sequencing, DNA was extracted with a QIAamp DNA minikit (Qiagen, Germany) from biomass grown in liquid peptone-yeast medium as described previously (7) or using cells incubated on R2A agar for 2 to 3 days at 28°C. DNA library construction and sequencing were conducted by Novogene Co., Ltd., using a NEBNext Ultra II 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.

Default parameters were used for all software unless otherwise specified. The quality of the reads was checked with FastQC 0.11.8 (12). Adapter sequences and low-quality regions in raw reads were cut with Trimmomatic 0.39 (13) with the following

Citation Tarlachkov SV, Starodumova IP, Dorofeeva LV, Prisyazhnaya NV, Roubtsova TV, Chizhov VN, Nadler SA, Subbotin SA, Evtushenko LI. 2021. Draft genome sequences of 28 Actinobacteria of the family *Microbacteriaceae* associated with nematode-infected plants. *Microbiol Resour Announc* 10:e01400-20. <https://doi.org/10.1128/MRA.01400-20>.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

Copyright © 2021 Tarlachkov et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Sergey V. Tarlachkov, sergey@tarlachkov.ru, or Sergei A. Subbotin, sergei.a.subbotin@gmail.com.

Received 6 December 2020

Accepted 3 February 2021

Published 4 March 2021

TABLE 1 Characteristics and DDBJ/ENA/GenBank accession numbers of genome sequences

Organism	Plant	Nematode	Geography	No. of reads	Coverage	No. of scaffolds	Scaffold N_{50} (bp)	Genome size (Mbp)	G+C content (%)	No. of proteins	SRA accession no.	GenBank accession no.
<i>Agria pratensis</i> VKM Ac-2874	<i>Poa annua</i>	<i>Anguina pacificae</i>	San Francisco, CA, USA	7,950,938	304	13	1,078,817	3.8	65.2	3,532	SRR13176583	JADKRRN0000000000
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> VKM Ac-1790	<i>Agrostis</i> sp.	<i>Anguina agrostis</i>	Sakhalin Island, Russia	16,949,770	737	9	808,662	3.3	72.7	3,036	SRR13176582	JADKRO0000000000
<i>Clavibacter michiganensis</i> subsp. <i>phaseoli</i> VKM Ac-2886	<i>Sambucus racemosa</i>	<i>Aphelenchoides ritzemabosi</i>	Moscow Region, Russia	18,464,076	785	21	2,249,679	3.4	73.2	3,186	SRR13176571	JADKRRP0000000000
<i>Clavibacter</i> sp. strain VKM Ac-2542	<i>Elymus repens</i>	<i>Anguina agropyri</i>	Moscow Region, Russia	15,327,488	656	5	1,206,839	3.3	73.0	3,103	SRR13176562	JADKRC0000000000
<i>Clavibacter</i> sp. strain VKM Ac-2872	<i>Poa annua</i>	<i>Anguina pacificae</i>	San Francisco, CA, USA	16,543,808	675	12	2,167,218	3.5	72.7	3,276	SRR13176561	JADKRRR0000000000
<i>Clavibacter</i> sp. strain VKM Ac-2873	<i>Agrostis capillaris</i>	<i>Anguina agrostis</i>	Washington, USA	17,375,186	751	14	534,200	3.3	73.2	3,064	SRR13176560	JADKRS0000000000
<i>Clavibacterium flaccumfaciens</i> VKM Ac-1386	<i>Agrostis</i> sp.	<i>Anguina agrostis</i>	Iturup Island, Russia	12,378,592	457	18	376,811	3.9	70.9	3,687	SRR13176559	JADKRT0000000000
<i>Clavibacterium flaccumfaciens</i> VKM Ac-1795	<i>Agrostis</i> sp.	<i>Anguina agrostis</i>	Sakhalin Island, Russia	11,498,758	417	36	428,105	3.9	70.7	3,692	SRR13176558	JADKRU0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-1376	<i>Poa annua</i>	<i>Subanguina radiciala</i>	Moscow Region, Russia	9,503,968	362	61	195,312	3.8	70.4	3,507	SRR13176557	JADKRV0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-1393	<i>Calamagrostis</i> sp.	<i>Heteroanguina graminiphila</i>	Kunashir Island, Russia	12,098,310	438	20	491,012	3.9	70.5	3,681	SRR13176556	JADKRW0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-1395	<i>Festuca rubra</i>	<i>Anguina graminis</i>	Moscow Region, Russia	9,679,080	355	18	628,495	3.9	71.1	3,670	SRR13176581	JADKRX0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-1796	<i>Centurea</i> sp.	<i>Mesoanguina picridis</i>	North Caucasus, Russia	12,573,324	503	9	630,474	3.6	71.0	3,364	SRR13176580	JADKRY0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-2865	<i>Agrostis capillaris</i>	<i>Anguina agrostis</i>	Washington, USA	13,029,810	500	15	428,310	3.7	71.4	3,461	SRR13176579	JADKRZ0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-2884	<i>Agrostis capillaris</i>	<i>Anguina agrostis</i>	Washington, USA	9,597,106	354	15	694,950	3.9	70.9	3,646	SRR13176578	JADKSA0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-2887	<i>Agrostis capillaris</i>	<i>Anguina agrostis</i>	Moscow Region, Russia	12,452,164	427	57	242,003	4.2	70.6	3,952	SRR13176577	JADKSB0000000000
<i>Curtobacterium</i> sp. strain VKM Ac-2889	<i>Agrostis</i> sp.	<i>Anguina agrostis</i>	Kunashir Island, Russia	11,453,674	457	9	940,853	3.6	71.0	3,357	SRR13176576	JADKSC0000000000
<i>Frigoribacterium</i> sp. strain VKM Ac-1396	<i>Festuca rubra</i>	<i>Anguina graminis</i>	Moscow Region, Russia	11,568,590	486	8	1,115,953	3.4	71.7	3,088	SRR13176575	JADKSD0000000000
<i>Frigoribacterium</i> sp. strain VKM Ac-2530	<i>Tanacetum vulgare</i>	<i>Aphelenchoides fragariae</i>	Moscow Region, Russia	19,836,426	834	5	1,902,771	3.4	72.7	3,110	SRR13176574	JADKSE0000000000
<i>Fronthabians</i> sp. strain VKM Ac-2883	<i>Fagus</i> sp.	<i>Litylenchus</i> sp.	New York State, USA	6,461,938	253	24	417,102	3.7	67.3	3,391	SRR13176573	JADKSF0000000000
<i>Herbiconiux</i> sp. strain VKM Ac-1786	<i>Elymus repens</i>	<i>Anguina agropyri</i>	Moscow Region, Russia	16,964,600	622	4	2,263,567	3.9	71.1	3,649	SRR13176572	JADKSG0000000000
<i>Microbacterium</i> sp. strain VKM Ac-2870	<i>Poa annua</i>	<i>Anguina pacificae</i>	San Francisco, CA, USA	11,489,968	526	12	704,918	3.1	68.3	2,905	SRR13176570	JADKSH0000000000
<i>Plantibacter</i> sp. strain VKM Ac-2876	<i>Poa annua</i>	<i>Anguina pacificae</i>	San Francisco, CA, USA	9,413,878	338	7	1,281,621	4.0	69.5	3,702	SRR13176569	JADKSI0000000000
<i>Plantibacter</i> sp. strain VKM Ac-2880	<i>Klasea latifolia</i>	<i>Mesoanguina picridis</i>	Iran	11,839,552	422	7	2,540,737	4.0	69.4	3,765	SRR13176568	JADKSJ0000000000
<i>Plantibacter</i> sp. strain VKM Ac-2885	<i>Festuca rubra</i>	<i>Anguina graminis</i>	Moscow Region, Russia	12,004,120	410	4	2,630,716	4.2	69.2	3,853	SRR13176567	JADKSK0000000000
<i>Pseudoclavibacter</i> sp. strain VKM Ac-2867	<i>Agrostis capillaris</i>	<i>Anguina agrostis</i>	Washington, USA	10,413,762	357	47	195,216	4.2	68.2	3,812	SRR13176566	JADKSL0000000000
<i>Pseudoclavibacter</i> sp. strain VKM Ac-2888	<i>Tanacetum vulgare</i>	<i>Aphelenchoides fragariae</i>	Moscow Region, Russia	11,942,026	409	7	3,268,120	4.2	68.5	3,851	SRR13176565	JADKSM0000000000
<i>Rathayibacter</i> sp. strain VKM Ac-2878	<i>Danthonia californica</i>	<i>Anguina danthoniae</i>	Fort Ross, CA, USA	11,690,574	569	9	860,400	2.9	69.9	2,692	SRR13176564	JADKSN0000000000
<i>Rathayibacter</i> sp. strain VKM Ac-2879	<i>Danthonia californica</i>	<i>Anguina danthoniae</i>	Fort Ross, CA, USA	9,176,840	448	9	860,400	2.9	69.9	2,690	SRR13176563	JADKSO0000000000

options: ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10; SLIDINGWINDOW:4:20; MINLEN:50. Trimmed reads were assembled using SPAdes 3.14.1 (14) with the following options: --cov-cutoff, auto, and --isolate. The quality of assembly was assessed with QUASt 5.0.2 (15). Assemblies were annotated with NCBI PGAP (16) and the RAST Web server (17, 18).

Additional comparative phenotypic study of the sequenced strains, along with genome-wide analyses of phylogenetically closely related plant endophytes and pathogens, will facilitate understanding of their role in bacterial-nematode complexes, including mechanisms of molecular interactions between members of these complexes and plants.

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

ACKNOWLEDGMENT

This work was sponsored by the United States Department of Agriculture Animal and Plant Health Inspection Service according to the research project AP18PPQS&T00C159 (18-0422-000-FR) “Enhancing Diagnostics of Plant Pathogenic Bacteria of the Genus *Rathayibacter*” (principal investigator [PI], S.A.S.).

REFERENCES

- Evtushenko LI. 2015. *Microbacteriaceae*, p 1–14. In Whitman WB, Rainey F, Kämpfer P, Trujillo M, Chun J, DeVos P, Hedlund B, Dedysh S (ed), Bergey’s manual of systematics of archaea and bacteria. Wiley, Hoboken, NJ. <https://doi.org/10.1002/9781118960608.fbm00035>.
- Murray TD, Schroeder BK, Schneider WL, Luster DG, Sechler A, Rogers EE, Subbotin SA. 2017. *Rathayibacter toxicus*, other *Rathayibacter* species inducing bacterial head blight of grasses, and the potential for livestock poisonings. *Phytopathology* 107:804–815. <https://doi.org/10.1094/PHYTO-02-17-0047-RWW>.
- Evtushenko LI, Dorofeeva LV. 2012. Genus XXII. *Rathayibacter* Zgurskaya, Evtushenko, Akimov and Kalakoutskii 1993, 147, p 953–964. In Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K-I, Ludwig W, Whitman WB (ed), Bergey’s manual of systematic bacteriology, 2nd ed, vol 5. Springer, New York, NY.
- Riley IT, McKay AC. 1990. Specificity of the adhesion of some plant pathogenic micro-organisms to the cuticle of nematodes in the genus *Anguina* (Nematoda: *Anguinidae*). *Nematologica* 36:90–103. <https://doi.org/10.1163/002925990X00068>.
- Evtushenko LI, Takeuchi M. 2006. The family *Microbacteriaceae*, p 1020–1098. In Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (ed), The prokaryotes: a handbook on the biology of bacteria. Archaea, Bacteria, Firmicutes, Actinomycetes, 3rd ed, vol 3. Springer, New York, NY.
- Starodumova IP, Tarlachkov SV, Prisyazhnaya NV, Dorofeeva LV, Ariskina EV, Chizhov VN, Subbotin SA, Evtushenko LI, Vasilenko OV. 2017. Draft genome sequence of *Rathayibacter* sp. VKM Ac-2630 isolated from leaf gall induced by the knapweed nematode *Mesoanguina picridis* on *Acroptilon repens*. *Genome Announc* 5:e00650-17. <https://doi.org/10.1128/genomeA.00650-17>.
- Tarlachkov SV, Starodumova IP, Dorofeeva LV, Prisyazhnaya NV, Leyn SA, Zlmal JE, Elane ML, Osterman AL, Nadler SA, Subbotin SA, Evtushenko LI. 2020. Complete and draft genome sequences of 12 plant-associated *Rathayibacter* strains of known and putative new species. *Microbiol Resour Announc* 9:e00316-20. <https://doi.org/10.1128/MRA.00316-20>.
- Vasilenko OV, Starodumova IP, Tarlachkov SV, Dorofeeva LV, Avtuhk AN, Evtushenko LI. 2016. Draft genome sequence of “*Rathayibacter tanacetii*” strain VKM Ac-2596 isolated from *Tanacetum vulgare* infested by a foliar nematode. *Genome Announc* 4:e00512-16. <https://doi.org/10.1128/genomeA.00512-16>.
- Dorofeeva LV, Krausova VI, Evtushenko LI, Tiedje JM. 2003. *Agromyces albus* sp. nov., isolated from a plant (*Androsace* sp.). *Int J Syst Evol Microbiol* 53:1435–1438. <https://doi.org/10.1099/ijs.0.02428-0>.
- Dorofeeva LV, Starodumova IP, Krauzova VI, Prisyazhnaya NV, Vinokurova NG, Lysanskaya VY, Tarlachkov SV, Evtushenko LI. 2018. *Rathayibacter oskolensis* sp. nov., a novel actinobacterium from *Androsace koso-poljanskii* Ovcz. (Primulaceae) endemic to the Central Russian Upland. *Int J Syst Evol Microbiol* 68:1442–1447. <https://doi.org/10.1099/ijsem.0.002681>.
- Tarlachkov SV, Starodumova IP. 2017. TaxonDC: calculating the similarity value of the 16S rRNA gene sequences of prokaryotes or ITS regions of fungi. *J Bioinform Genom* 3:1–4. <https://doi.org/10.18454/jbg.2017.3.5.1>.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.