AMERICAN SOCIETY FOR MICROBIOLOGY genomeAnnouncements™

PROKARYOTES

Draft Genome Sequences of New Genomospecies "*Candidatus* Pectobacterium maceratum" Strains, Which Cause Soft Rot in Plants

Fedor V. Shirshikov,[a,b] Aleksei A. Korzhenkov,[c] Kirill K. Miroshnikov,[d] Anastasia P. Kabanova,[a,b] Alla P. Barannik,[a] Alexander N. Ignatov,[b] Konstantin A. Miroshnikov[a,b]

[a]Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

[b]PhytoEngineering R&D Center LLC, Rogachevo, Moscow Region, Russia

[c]Immanuel Kant Baltic Federal University, Kaliningrad, Russia

[d]Winogradsky Institute of Microbiology, Research Center of Biotechnology RAS, Moscow, Russia

ABSTRACT Investigation of collections of phytopathogenic bacteria has revealed some strains distinct from known *Pectobacterium* spp. We report here the draft genome sequences of five such strains, isolated during the period of 1947 to 2012. Based on comparative genomics, we propose a new candidate genomospecies of the genus *Pectobacterium*, "*Candidatus* Pectobacterium maceratum."

The genus *Pectobacterium* is a genetically diverse group of pectinolytic phytopathogens (1) with a broad range of plant hosts (2). Within a collection of 200 bacterial isolates, we have found several strains closely related to *Pectobacterium carotovorum*, which were repeatedly isolated from macerated plant tissue of cabbage and potato tubers in the Moscow Region, Russia. These five strains caused large outbreaks of soft rot in fields and storage during the period of 1947 to 2012 (Table 1).

Bacterial strains were cultivated overnight in liquid LB medium at 27°C. Genomic DNA extraction was performed using the phenol-chloroform method. The NEBNext Ultra DNA library prep kit for Illumina (New England BioLabs, Ipswich, MA) was used for DNA library construction. DNA samples were sequenced to generate 100-bp paired-end reads using the Illumina MiSeq platform. Paired-end reads were filtered and trimmed using CLC Genomics Workbench software (Qiagen, Aarhus, Denmark). The genome sequences were assembled using SPAdes software (3) and annotated using the Prokka annotation pipeline (4).

Recently, the general guidelines for using genome data in prokaryotic taxonomy were published (5). We applied these guidelines to determine the systematic positions of our strains. Pairwise calculations of average nucleotide identity (ANI) (6) and digital DNA-DNA hybridization (dDDH) using formula 2 for incompletely sequenced genomes (7) were performed. We used a set of 50 genome sequences, including those of our five strains and representative strains from all currently proposed taxa of the genera *Pectobacterium* and *Dickeya*. Based on the ANI distance dendrogram, our strains form a new, distinct clade that shares a common ancestor with *P. carotovorum* subsp. *odoriferum*. To distinguish members of the new clade from other subspecies of *P. carotovorum*, we calculated corresponding maximal ANI and dDDH values, as follows: *P. carotovorum* subsp. *actinidiae*, 93% and 50%, respectively; *P. carotovorum* subsp. *brasiliense*, 92% and 50%; *P. carotovorum* subsp. *carotovorum*, 95% and 65%; and *P. carotovorum* subsp. *odoriferum*, 95% and 62%. The ANI and dDDH values in comparison with those of the new species *Pectobacterium polaris* (8), which is closely related to *P. carotovorum* subsp. *brasiliense*, are 94% and 55%, respectively. The minimal intragroup ANI and dDDH values for our strains are 98% and 82%, respectively.

Received 28 February 2018 **Accepted** 17 March 2018 **Published** 12 April 2018

Citation Shirshikov FV, Korzhenkov AA, Miroshnikov KK, Kabanova AP, Barannik AP, Ignatov AN, Miroshnikov KA. 2018. Draft genome sequences of new genomospecies "*Candidatus* Pectobacterium maceratum" strains, which cause soft rot in plants. Genome Announc 6:e00260-18. https://doi.org/10.1128/genomeA.00260-18.

Address correspondence to Fedor V. Shirshikov, shrshkv@ya.ru.

F.V.S., A.A.K., and K.K.M. contributed equally to this work.

TABLE 1 Statistics for the draft *Pectobacterium* genome sequences

Strain	Origin	Yr	Size (bp)	Coverage (×)	No. of contigs	No. of CDSs[a]	GenBank accession no.
F018	Cabbage	1947	4,911,793	58	27	4,311	PDVV00000000
F131	Potato	1993	4,786,484	50	87	4,173	PDVW00000000
F135	Potato	2012	4,891,351	53	75	4,316	PDVX00000000
PB69	Potato	2012	4,993,011	44	21	4,382	PDVY00000000
PB70	Potato	2012	4,992,983	44	28	4,388	PDVZ00000000

[a] CDS, coding sequence.

Thresholds of species delineation by ANI and dDDH are 95 to 96% and 70%, respectively (5); the cutoff to distinguish subspecies by dDDH is 79% (9).

Thus, we propose a new candidate genomospecies for the new strains, "*Candidatus* Pectobacterium maceratum." Based on high similarity with our strains (minimal ANI and dDDH values of 98% and 83%, respectively), the Finnish *Pectobacterium* strain SCC1 (10) also belongs to "*Ca.* Pectobacterium maceratum." Considering that SCC1 is a model plant pathogen with a complete genome sequence, we propose it as a type strain of the candidate genomospecies. An interesting genome feature of all these strains is the presence of an *evf* gene, which encodes a virulence factor for persistence in the gut of *Drosophila* (11, 12). Perhaps, acquisition of the gene by some *P. carotovorum* subsp. *odoriferum* cells caused the divergence of "*Ca.* Pectobacterium maceratum" and evolution of the associated symbiosis with flies.

Further formal description of "*Ca.* Pectobacterium maceratum" is currently in progress.

Accession number(s). This whole-genome sequencing project has been deposited at DDBJ/ENA/GenBank under the accession numbers listed in Table 1. The BioProject accession numbers are PRJNA414969 (strain F018) and PRJNA415106 (strains F131, F135, PB69, and PB70).

ACKNOWLEDGMENTS

This work was supported by the Russian Scientific Foundation (grant 16-16-00073). F.V.S. was supported by fellowships from the Russian Federation Government (2011 to 2012) and the Republic of Tatarstan (2014).

We thank Stepan V. Toshchakov for fruitful discussion and access to a sequencing platform hosted by Immanuel Kant Baltic Federal University. This research was made possible through support provided by Tatiana V. Ovchinnikova.

REFERENCES

1. Zhang Y, Fan Q, Loria R. 2016. A re-evaluation of the taxonomy of phytopathogenic genera *Dickeya* and *Pectobacterium* using whole-genome sequencing data. Syst Appl Microbiol 39:252–259. https://doi.org/10.1016/j.syapm.2016.04.001.
2. Czajkowski R, Pérombelon MCM, Jafra S, Lojkowska E, Potrykus M, van der Wolf JM, Sledz W. 2015. Detection, identification and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: a review. Ann Appl Biol 166:18–38. https://doi.org/10.1111/aab.12166.
3. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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.
4. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
5. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0.002516.
6. Rodriguez-R LM, Konstantinidis KT. 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ Preprints 4:e1900v1. https://doi.org/10.7287/peerj.preprints.1900v1.
7. Auch AF, von Jan M, Klenk HP, Göker M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2:117–134. https://doi.org/10.4056/sigs.531120.
8. Dees MW, Lysøe E, Rossmann S, Perminow J, Brurberg MB. 2017. *Pectobacterium polaris* sp. nov., isolated from potato (*Solanum tuberosum*). Int J Syst Evol Microbiol. https://doi.org/10.1099/ijsem.0.002448.
9. Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V, Fiebig A, Rohde C, Rohde M, Fartmann B, Goodwin LA, Chertkov O, Reddy TBK, Pati A, Ivanova NN, Markowitz V, Kyrpides NC, Woyke T, Göker M, Klenk H-P. 2014. Complete genome sequence of DSM 30083^T, the type strain (U5/41^T) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. Stand Genomic Sci 9:2. https://doi.org/10.1186/1944-3277-9-2.
10. Niemi O, Laine P, Koskinen P, Pasanen M, Pennanen V, Harjunpää H, Nykyri J, Holm L, Paulin L, Auvinen P, Palva ET, Pirhonen M. 2017. Genome sequence of the model plant pathogen *Pectobacterium carotovorum* SCC1. Stand Genomic Sci 12:87. https://doi.org/10.1186/s40793-017-0301-z.
11. Basset A, Tzou P, Lemaitre B, Boccard F. 2003. A single gene that promotes interaction of a phytopathogenic bacterium with its insect vector, *Drosophila melanogaster*. EMBO Rep 4:205–210. https://doi.org/10.1038/sj.embor.embor730.
12. Vallet-Gely I, Lemaitre B, Boccard F. 2008. Bacterial strategies to overcome insect defences. Nat Rev Microbiol 6:302–313. https://doi.org/10.1038/nrmicro1870.