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. brasilienne, 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. brasilienne, 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.

Copyright © 2018 Shirshikov et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

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

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

Table 1

| Strain | Origin | Yr | Size (bp) | Coverage (×) | No. of contigs | No. of CDSs | GenBank accession no. |
|--------|--------|----|-----------|--------------|---------------|-------------|----------------------|
| F018   | Cabbage| 1947 | 4,911,793 | 58           | 27            | 4,311       | PDV000000000         |
| F131   | Potato | 1993 | 4,786,484 | 50           | 87            | 4,173       | PDV000000000         |
| F135   | Potato | 2012 | 4,891,351 | 44           | 21            | 3,382       | PDV000000000         |
| PB69   | Potato | 2012 | 4,993,011 | 53           | 75            | 4,166       | PDV000000000         |
| PB70   | Potato | 2012 | 4,992,983 | 44           | 28            | 3,388       | PDV000000000         |

<CDs, coding sequence.>