## **UC Davis**

# **UC Davis Previously Published Works**

#### **Title**

Complete Genome Sequence of the Type Strain Pectobacterium punjabense SS95, Isolated from a Potato Plant with Blackleg Symptoms.

#### **Permalink**

https://escholarship.org/uc/item/578656m9

## **Journal**

Microbiology Resource Announcements, 9(32)

#### **Authors**

Sarfraz, Sohaib Oulghazi, Saïd Cigna, Jérémy et al.

#### **Publication Date**

2020-08-06

#### DOI

10.1128/MRA.00420-20

## **Copyright Information**

This work is made available under the terms of a Creative Commons Attribution License, available at <a href="https://creativecommons.org/licenses/by/4.0/">https://creativecommons.org/licenses/by/4.0/</a>

Peer reviewed







# Complete Genome Sequence of the Type Strain Pectobacterium punjabense SS95, Isolated from a Potato Plant with Blackleg Symptoms

Sohaib Sarfraz, a,b Saïd Oulghazi, a,d Jérémy Cigna, a,e Shahbaz Talib Sahi,c Kashif Riaz,c Muhammad Rizwan Tufail,c Amna Fayyaz, Khalid Naveed, Akhtar Hameed, Céline Lopez-Roques, Céline Vandecasteele, Denis Faure

<sup>a</sup>Institute for Integrative Biology of the Cell (I2BC), CEA CNRS University Paris-Saclay, Gif-sur-Yvette, France

ABSTRACT Pectobacterium punjabense is a newly described species causing blackleg disease in potato plants. Therefore, by the combination of long (Oxford Nanopore Technologies, MinION) and short (Illumina MiSeq) reads, we sequenced the complete genome of P. punjabense SS95<sup>T</sup>, which contains a circular chromosome of 4.793 Mb with a GC content of 50.7%.

he family Pectobacteriaceae encompasses pectinolytic plant pathogens that represent a threat to economically important vegetable crops and ornamental plants. Pectobacterium spp. are responsible for rotting diseases such as carrot or melon soft rot and potato blackleg and soft rot (1). Over the past decade, advances in genomics have allowed the scientific community to clarify the taxonomic position of many Pectobacterium species by either reexamining biological resources in the international collections or sampling a wider range of environments, from plants to surface waters (2-4). The type strain Pectobacterium punjabense SS95 (CFBP 8604, LMG 30622) was isolated from potato plants showing blackleg symptoms collected from Punjab, Pakistan, in 2017 (5). Serially diluted samples were plated onto crystal violet pectate (CVP) agar medium, and plates were incubated for 48 h at 28°C (6). Bacterial colonies producing pitting on CVP were purified on nutrient agar (beef extract [3 g], peptone [5 g], glucose [2.5 q], and agar [15 g per liter]). Genomic DNA was extracted using a MasterPure complete DNA purification kit (Epicentre, Madison, WI, USA). DNA quantification and quality control were performed using a Qubit 2.0 fluorometer and 1.0% agarose gel electrophoresis. Whole-genome shotgun DNA sequencing of P. punjabense SS95 was performed by a combination of Illumina MiSeq and Oxford Nanopore Technologies (ONT) MinION sequencing. The library was prepared using the Nextera DNA Flex kit (Illumina), and sequencing was performed using the MiSeq reagent kit v.2 with pairedend chemistry (2  $\times$  150 bp). The sequence reads were trimmed to remove adapter sequences with Cutadapt v.1.15, and quality control was performed using FastQC v.0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc).

The ONT library preparation and sequencing were performed at the GeT-PlaGe core facility (INRA, Toulouse), according to the manufacturer's instructions, by following the 1D native genomic DNA barcoding protocol (EXP-NBD103 and SQK-LSK108). At each step, the DNA was quantified using the Qubit double-stranded DNA (dsDNA) highsensitivity (HS) assay kit (Life Technologies). The DNA purity was tested using the NanoDrop spectrophotometer (Thermo Fisher), and the size distribution and degrada-

Citation Sarfraz S, Oulghazi S, Cigna J, Sahi ST, Riaz K, Tufail MR, Fayyaz A, Naveed K, Hameed A, Lopez-Roques C, Vandecasteele C, Faure D. 2020. Complete genome sequence of the type strain Pectobacterium punjabense SS95, isolated from a potato plant with blackleg symptoms. Microbiol Resour Announc 9:e00420-20. https://doi.org/10.1128/MRA.00420-20.

Editor Irene L. G. Newton, Indiana University, Bloomington

Copyright © 2020 Sarfraz et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Denis Faure, denis.faure@i2bc.paris-saclay.fr.

Received 18 May 2020 Accepted 15 July 2020 Published 6 August 2020

Department of Plant Pathology, University of Agriculture Faisalabad, Sub-campus Depalpur, Okara, Pakistan

<sup>&</sup>lt;sup>c</sup>Department of Plant Pathology, University of Agriculture Faisalabad, Faisalabad, Pakistan

<sup>&</sup>lt;sup>d</sup>Department of Biology, Faculty of Sciences, Moulay Ismaïl University, Meknes, Morocco

eFédération Nationale des Producteurs de Plants de Pomme de Terre (FN3PT), Paris, France

fINRA, US 1426, GeT-PlaGe, Genotoul, Castanet-Tolosan, France

Sarfraz et al. 

♠ Microbiologo

tion were assessed using the Fragment Analyzer high-sensitivity DNA fragment analysis kit (AATI). Purification steps were performed using AMPure XP beads (Beckman Coulter). Using the Megaruptor 1 system (Diagenode), 5 µg of DNA was sheared at 20 kb. A DNA damage repair step was performed on 3  $\mu$ g of sample. Then, end repair and dA tailing of double-stranded DNA fragments were performed on 1  $\mu$ g of sample. The library was generated; then, adapters were ligated onto the library, and it was loaded onto an R9.4.1 flow cell and sequenced on a MinION instrument at 0.15 pmol within 48 h. The raw sequence data (fast5 format) from ONT sequencing were obtained with MinKNOW v.1.10.23 and were base called with ONT Albacore Sequencing Pipeline Software v.2.1.10; reads passing the internal test were used for the subsequent analysis. Porechop v.0.2.1 (https://github.com/rrwick/Porechop) was used for adaptor trimming. After filtering (quality, >9; length, >3,000 nucleotides), the ONT data used for the assembly showed an average length of 10,431 bp and an  $N_{50}$  value of 11,971 bp. The 71,148 ONT reads were assembled using Canu v.1.7 (7) with the "genomeSize=5m" and "minReadLength=3000" options (genome coverage, 155×). The 1,144,952 Illumina reads were mapped onto the ONT assembly with Burrows-Wheeler Aligner MEM v.0.7.12 (8) for sequence and assembly error correction with Pilon v.1.22 (9). The contig was finally circularized using Circlator v.1.5.1 (https://github.com/sanger-pathogens/ circlator). The resulting sequence was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) (10), which predicted 4,307 coding sequences. The total genome size is 4,793,778 bp with a GC content of 50.7%.

The complete genome sequence of *P. punjabense* SS95<sup>T</sup> provides essential data for studying its genetic diversity and host range, as well as comparative genomic analyses between its closest relative species.

**Data availability.** The Illumina (accession number SRR11674121) and MinION (accession number SRR11788435) reads were deposited in the Sequence Read Archive (SRA). The complete genome sequence of *Pectobacterium punjabense* SS95 was deposited under GenBank accession number CP038498.1.

#### **ACKNOWLEDGMENTS**

This work was supported by the Higher Education Commission's IRSIP program (HEC, Pakistan) through Sohaib Sarfraz; CNRS (I2BC-SB2017), Agence Nationale de la Recherche (ANR-15-CE21-0003), and Fédération Nationale des Producteurs de Plants de Pomme de Terre (FN3PT) through Denis Faure; and Grand Challenges Canada (Stars in Global Health–Round 7, grant number 0664-01-10) through Kashif Riaz. This work was also supported by the France Génomique national infrastructure, funded as part of the program "Investissement d'avenir," managed by Agence Nationale pour la Recherche (contract ANR-10-INBS-09).

This work benefited from the facilities and expertise from the high-throughput sequencing platform of I2BC (Gif-sur-Yvette, France). This work was performed in collaboration with the GeT core facility (Toulouse, France).

We declare no conflict of interest.

#### **REFERENCES**

- Sarfraz S, Sahi ST, Oulghazi S, Riaz K, Rajput NA, Atiq M, Tufail MR, Hameed A, Faure D. 2020. Species diversity of *Dickeya* and *Pectobacte-rium* causing potato blackleg disease in Pakistan. Plant Dis 104: 1492–1499. https://doi.org/10.1094/PDIS-08-19-1743-RE.
- Portier P, Pédron J, Taghouti G, Fischer-Le Saux M, Caullireau E, Bertrand C, Laurent A, Chawki K, Oulgazi S, Moumni M, Andrivon D, Dutrieux C, Faure D, Hélias V, Barny M-A. 2019. Elevation of *Pectobacterium carotovorum* subsp. *odoriferum* to species level as *Pectobacterium odoriferum* sp. nov., proposal of *Pectobacterium brasiliense* sp. nov. and *Pectobacterium actinidiae* sp. nov., emended description of *Pectobacterium carotovorum* and description of *Pectobacterium versatile* sp. nov., isolated from streams and symptoms on diverse plants. Int J Syst Evol Microbiol 69:3207–3216. https://doi.org/10.1099/ijsem.0.003611.
- 3. Oulghazi S, Cigna J, Lau YY, Moumni M, Chan KG, Faure D. 2019. Transfer of the waterfall source isolate *Pectobacterium carotovorum* M022 to

- *Pectobacterium fontis* sp. nov., a deep-branching species within the genus *Pectobacterium*. Int J Syst Evol Microbiol 69:470 475. https://doi.org/10.1099/ijsem.0.003180.
- Pédron J, Bertrand C, Taghouti G, Portier P, Barny M-A. 2019. Pectobacterium aquaticum sp. nov., isolated from waterways. Int J Syst Evol Microbiol 69:745–751. https://doi.org/10.1099/ijsem.0.003229.
- Sarfraz S, Riaz K, Oulghazi S, Cigna J, Sahi ST, Khan SH, Faure D. 2018. Pectobacterium punjabense sp. nov., isolated from blackleg symptoms of potato plants in Pakistan. Int J Syst Evol Microbiol 68:3551–3556. https://doi.org/10.1099/ijsem.0.003029.
- Hélias V, Hamon P, Huchet E, Wolf JVD, Andrivon D. 2011. Two new effective semiselective crystal violet pectate media for isolation of *Pec*tobacterium and *Dickeya*. Plant Pathol 61:339–345. https://doi.org/10 .1111/j.1365-3059.2011.02508.x.
- 7. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017.

Volume 9 Issue 32 e00420-20 mra.asm.org **2** 



- Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
- 9. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated
- tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
- 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.

Volume 9 lssue 32 e00420-20 mra.asm.org **3**