BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Genome Announcement

Draft genome of a South African strain of *Pectobacterium carotovorum* subsp. *brasiliense*

Khayalethu Ntushelo[a,*], *Joseph Mafofo*[b,1]

[a] *University of South Africa, Science Campus, Department of Agriculture and Animal Health, Florida, South Africa*
[b] *Formerly, Agricultural Research Council – Biotechnology Platform, Onderstepoort, South Africa*

ARTICLE INFO

Article history:
Received 23 March 2016
Accepted 9 June 2016
Available online 30 November 2016
Associate Editor: John Anthony McCulloch

Keywords:
Pectobacterium carotovorum subsp. *brasiliensis*
Genome sequencing
Illumina

ABSTRACT

The draft genome of *Pectobacterium carotovorum* subsp. *brasiliense* (*Pcb*) which causes blackleg of potato was submitted to the NCBI and released with reference number NZ_LGRF00000000.1. The estimated genome size based on the draft genome assembly is 4,820,279 bp from 33 contigs ranging in length from 444 to 1,660,019 nucleotides. The genome annotation showed 4250 putative genes, 4114 CDS and 43 pseudo-genes. Three complete rRNA gene species were detected: nine 5S, one 16S and one 23S. Other partial rRNA gene fragments were also identified, nine 16S rRNA and three 23S rRNA. A total of 69 tRNA genes and one ncRNA gene were also annotated in this genome.

A new bacterium species similar to *Pectobacterium carotovorum* subsp. *carotovorum* that was recently discovered has been shown to be the cause of blackleg in Brazil, USA, South Africa, Canada, New Zealand and Korea.[1–5] It has been classified as *P. carotovorum* subsp. *brasiliense*.[6,7] Very few studies have focussed on studying the complete genome of *P. carotovorum* subsp. *brasiliense* and as such, the pathogenicity of this subspecies of *P. carotovorum* cannot be predicted. Moreover, not much work has been done to characterise the *P. carotovorum* subsp. *brasiliense* strain found in South Africa. The work presented here focused on preliminary characterisation of the complete genome of *P. carotovorum* subsp. *brasiliense* found in South Africa.

Strain BD255 of *P. carotovorum* subsp. *brasiliense*, collected from rotting watermelon in 2002, was selected for this study. Total DNA was isolated using the QIAamp® DNA Mini Kit (Qiagen) and quantified using the Qubit® dsDNA BR Assay kit (Thermo Fisher). The DNA was used to prepare sequencing libraries using the Nextera XT DNA Library Prep Kit (Illumina). Two insert size selections were then performed at 250 and 800 bp respectively and sequenced paired end; 2 × 100 on the Illumina HiScanSQ and 2 × 300 on the Illumina MiSeq respectively. A total of 1 GB of sequencing data was obtained for the 2 × 100 and 3 GB for the 2 × 300 formats. The sequence reads were trimmed for adapters using Trimmomatic,[8] filtered for quality using the FASTX-Toolkit

* *Corresponding author.*
E-mail: ntushk@unisa.ac.za (K. Ntushelo).
[1] Presently, Alliance Global Group FZ LLC, Dubiotech, Dubai, United Arab Emirates.
http://dx.doi.org/10.1016/j.bjm.2016.06.016

suite of tools (http://hannonlab.cshl.edu/fastx_toolkit/) and the *de novo* assembly was performed using Velvet v. 1.2.08.[9] The assembly gave an average coverage across the genome of 117X.

The draft genome assembly was submitted to NCBI (www.ncbi.nlm.nih.gov) and can be accessed under project accession number NZ_LGRF01000000 and consists of sequences with accession numbers LGRF01000001-LGRF01000033. The estimated genome size is 4,820,279 nucleotides, about the same size as the complete genome sequence of *P. carotovorum* subsp. *carotovorum* NCBI reference NC_012917 submitted in www.ncbi.nlm.nih.gov, and consists of a total of 33 contigs ranging in size from 444 to 1,660,019 nucleotides. Annotation was performed using the NCBI PGAP using the Best-placed reference protein set; GeneMarkS+ method. Results show 4250 genes, 4114 CDS, 43 pseudogenes, 9 complete 5S rRNA genes, 1 complete 16S rRNA gene and 1 complete 23S rRNA gene. There are also partial rRNA genes 9 (16S rRNA) and 3 (23S rRNA). Additionally, a total of 69 tRNA 1 ncRNA genes were also detected.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The strain of *Pectobacterium carotovorum* subsp. *brasiliense* sequenced was generously donated by Dr Reinette Gouws when she was a plant pathologist at the Agricultural Research Council – Vegetable and Ornamental Plant Institute of South Africa.

REFERENCES

1. Choi O, Kim J. *Pectobacterium carotovorum* subsp. *brasiliense* causing soft rot on paprika in Korea. *J Phytopathol.* 2013;161:125–127.
2. De Boer SH, Li X, Ward LJ. *Pectobacterium* spp. associated with bacterial stem rot syndrome of potato in Canada. *Phytopathology*. 2012;102:937–947.
3. Kim H-S, Ma B, Perna NT, Charkowski AO. Phylogeny and virulence of naturally occurring type III secretion system-deficient *Pectobacterium* strains. *Appl Environ Microbiol.* 2009;75:4539–4549.
4. Panda P, Fiers MA, Armstrong K, Pitman AR. First report of blackleg and soft rot of potato caused by *Pectobacterium carotovorum* subsp. *brasiliensis* in New Zealand. *New Dis Rep.* 2012;26:15.
5. van der Merwe JJ, Coutinho TA, Korsten L, van der Waals JE. *Pectobacterium carotovorum* subsp. *brasiliensis* causing blackleg on potatoes in South Africa. *Eur J Plant Pathol.* 2010;126:175–185.
6. Duarte V, De Boer SH, Ward LJ, De Oliveira AMR. Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. *J Appl Microbiol.* 2004;96:535–545.
7. Nahan S, De Boer SH, Maiss E, Wydra K. Taxonomic relatedness between *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium carotovorum* subsp. *odoriferum* and *Pectobacterium carotovorum* subsp. *brasiliense* subsp. nov. *J Appl Microbiol.* 2012;113:904–913.
8. Glasner JD, Marquez-Villavicencio M, Kim HS, et al. Niche-specificity and the variable fraction of the *Pectobacterium* pan-genome. *Mol Plant Microbe Interact.* 2008;21(12):1549–1560.
9. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 2008;18:1821–1829.