



## Draft Genome Sequence of a Rare Israeli Clinical Isolate of Burkholderia pseudomallei

Ofir Israeli,<sup>a</sup> Inbar Cohen-Gihon,<sup>a</sup> Tal Brosh-Nissimov,<sup>b,c</sup> Shay Weiss,<sup>d</sup> Anat Zvi,<sup>a</sup> Adi Beth-Din,<sup>a</sup> Ohad Shifman,<sup>a</sup> Ma'ayan Israeli,<sup>a</sup> Uri Elia,<sup>a</sup> Shirley Lazar,<sup>a</sup> Erez Bar-Haim,<sup>a</sup> Ofer Cohen,<sup>a</sup> Theodor Chitlaru<sup>a</sup>

<sup>a</sup>Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness Ziona, Israel <sup>b</sup>Infectious Diseases Unit, Assuta Ashdod University Hospital, Ashdod, Israel <sup>c</sup>Faculty of Health Sciences, Ben-Gurion University in the Negev, Beersheba, Israel <sup>d</sup>Department of Infectious Diseases, Israel Institute for Biological Research, Ness Ziona, Israel

**ABSTRACT** We report here the draft genome sequence of *Burkholderia pseudomallei* MAA2018. This highly virulent strain was isolated in 2018 from the first melioidosis case in Israel associated with recreational travel to Goa, India.

The Gram-negative bacterium *Burkholderia pseudomallei* is the etiological cause of melioidosis, a severe zoonotic infectious disease (165,000 global estimated annual morbidities, including 89,000 deaths [1]) that is endemic mainly in Southeast Asia and northern Australia (2). *B. pseudomallei* is considered a potential bioweapon (CDC category B) due to the prevalence in soil and water, multiple routes of infection, and especially high virulence associated with inhalation exposure, low infectious dose, high mortality rates, native resistance to a wide range of antibiotics, and nonavailability of a vaccine (3, 4). Melioidosis is manifested by nonspecific symptoms that hinder identification of the disease and consequently may inadvertently be diagnosed as tuberculosis or a common form of pneumonia (1, 5, 6). The *B. pseudomallei* genome, consisting of two chromosomes, is highly plastic, resulting in a high number of strains exhibiting significant variability in genetic features (7).

We report here the draft genome sequence of a clinical B. pseudomallei strain isolated from a melioidosis case diagnosed in Israel and associated with recreational travel to Goa, India. This is the third documented clinical isolate of melioidosis in Israel (8, 9) and the first case originating from India. The isolate, referred to as B. pseudomallei MAA2018 (melioidosis case from Assuta Hospital, Ashdod, Israel, in 2018) was isolated from sputum collected from a 29-year-old male returning from Goa, India, suffering from a subacute upper-lobe pneumonia of a 6-month duration. The identification of the infectious agent as B. pseudomallei was initially suggested by a metabolism-specific bacterial identification system (Vitek XL2) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) spectrum (Vitek mass spectrometer [MS]) and subsequently confirmed by PCR analysis, as described previously (10). Virulence analysis of B. pseudomallei MAA2018 in the murine model of melioidosis established an intranasal 50% lethal dose (LD<sub>50</sub>; determined by following for 30 days the survival of mice inoculated with increasing doses of bacteria and calculated by linear regression using the GraphPad Prism version 5 statistical analysis software [described in references 11 and 12]) of 6 CFU and 30 CFU in the BALB/c and C57BL/6J strains of mice, respectively, suggesting that it belongs to a group of highly virulent *B. pseudomallei* strains (13).

For the genome sequencing of *B. pseudomallei* MAA2018, DNA was extracted from a 40-h-old colony grown on Luria broth (LB) agar using the QIAamp DNA blood minikit (Qiagen). A Nextera XT paired-end library (Illumina) was prepared from 1 ng DNA. The library was sequenced on a MiSeq platform (Illumina) using paired-end sequencing

Nissimov T, Weiss S, Zvi A, Beth-Din A, Shifman O, Israeli M, Elia U, Lazar S, Bar-Haim E, Cohen O, Chitlaru T. 2019. Draft genome sequence of a rare Israeli clinical isolate of *Burkholderia pseudomallei*. Microbiol Resour Announc 8:e00281-19. https://doi.org/10.1128/MRA .00281-19.

Citation Israeli O, Cohen-Gihon I, Brosh-

**Editor** Frank J. Stewart, Georgia Institute of Technology

**Copyright** © 2019 Israeli et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Theodor Chitlaru, theodorc@iibr.gov.il.

O.I. and I.C.-G. contributed equally to this work.

**Received** 31 March 2019 **Accepted** 19 April 2019 **Published** 9 May 2019

[This article was published on 9 May 2019 with a byline that lacked Shay Weiss. The byline was updated in the current version, posted on 5 September 2019.]

with 150 nucleotides (nt) and a mean insertion size of 503 nt. This produced 5,148,334 reads, with 130 coverage (length of the reads  $\times$  read number/genome length) mapping to B. pseudomallei. For Nanopore sequencing, libraries were prepared from 5 ng of genomic DNA (gDNA) using a rapid PCR barcoding kit (SQK-RPB004; Oxford Nanopore), without fragmentation, and sequenced on the MinION using a MK1 R9.4 flow cell, following the protocol for 1D genomic gDNA, producing 16,809 reads. A hybrid Nanopore-Illumina de novo assembly was determined using SPAdes (14), with the following parameters: -t 4 -m 32 -k 31, 51, 71. The number of assembled contigs larger than 1,000 nt was 183. The  $N_{50}$  contig size was 80,391 nt. FastQC was used to check the raw sequence data for quality control (http://www.bioinformatics.babraham.ac.uk/ projects/fastqc/). The draft genome of the MAA2018 strain consisted of 7,423,108 bp (G+C content, 67%). The genomic sequence of the MAA2018 isolate was compared to B. pseudomallei genomes available in the NCBI database, revealing that it differs from its most closely related strain, B. pseudomallei 2008724758 (GenBank accession number CP018382), a clinical isolate collected in 2010 in California from a melioidosis case with unknown travel history (15), by 8,708 and 8,166 single-nucleotide polymorphisms (SNPs) on chromosomes 1 and 2, respectively.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers SLUE01000001 to SLUE01000183 (BioProject number PRJNA525961 and BioSample number SAMN11081001). The raw reads were submitted and are available in the Sequence Read Archive at the NCBI as Fast5 files (SRA accession number PRJNA525961).

## REFERENCES

- Wiersinga WJ, Virk HS, Torres AG, Currie BJ, Peacock SJ, Dance DAB, Limmathurotsakul D. 2018. Melioidosis. Nat Rev Dis Primers 4:17107. https://doi.org/10.1038/nrdp.2017.107.
- Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, Rolim DB, Bertherat E, Day NP, Peacock SJ, Hay SI. 2016. Predicted global distribution of Burkholderia pseudomallei and burden of melioidosis. Nat Microbiol 11:15008. https://doi.org/10.1038/ nmicrobiol.2015.8.
- Gilad J, Schwartz D, Amsalem Y. 2007. Clinical features and laboratory diagnosis of infection with the potential bioterrorism agents *Burkholderia mallei* and *Burkholderia pseudomallei*. Int J Biomed Sci 3:144–152.
- Gilad J, Harary I, Dushnitsky T, Schwartz D, Amsalem Y. 2007. Burkholderia mallei and Burkholderia pseudomallei as bioterrorism agents: national aspects of emergency preparedness. Isr Med Assoc J 9:499–503.
- Loveleena, Chaudhry R, Dhawan B. 2004. Melioidosis; the remarkable imitator: recent perspectives. J Assoc Physicians India 52:417–420.
- Le Tohic S, Montana M, Koch L, Curti C, Vanelle P. 2019. A review of melioidosis cases imported into Europe. Eur J Clin Microbiol Infect Dis 1–14. https://doi.org/10.1007/s10096-019-03548-5.
- Holden TGM, Titball RW, Peacock SJ, Cerdeño-Tárraga AM, Atkins T, Crossman LC, Pitt T, Churcher C, Mungall K, Bentley SD, Sebaihia M, Thomson NR, Bason N, Beacham IF, Karen Brooks K, Katherine A, Brown KA, Nat F, Brown NF, Challis GL, Cherevach I, Chillingworth T, Cronin A, Crossett B, Davis P, DeShazer D, Feltwell T, Fraser A, Hance Z, Hauser H, Holroyd S, Jagels K, Keith KE, Maddison M, Moule S, Price C, Quail MA, Rabbinowitsch E, Rutherford K, Sanders M, Simmonds M, Songsivilai S, Stevens K, Tumapa S, Vesaratchavest M, Whitehead S, Yeats C, Barrell BG, Oyston PCF, Parkhill J. 2004. Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei*. Proc Natl Acad Sci U S A 101: 14240–14245. https://doi.org/10.1073/pnas.0403302101.
- Almog Y, Yagel Y, Geffen Y, Yagupsky P. 2016. A Burkholderia pseudomallei infection imported from Eritrea to Israel. Am J Trop Med Hyg 95: 997–998. https://doi.org/10.4269/ajtmh.16-0481.

- Cahn A, Koslowsky B, Nir-Paz R, Temper V, Hiller N, Karlinsky A, Gur I, Hidalgo-Grass C, Heyman SN, Moses AE, Block C. 2009. Imported melioidosis, Israel, 2008. Emerg Infect Dis 15:1809–1811. https://doi.org/10 .3201/eid1511.090038.
- Price EP, Dale JL, Cook JM, Sarovich DS, Seymour ML, Ginther JL, Kaufman EL, Beckstrom-Sternberg SM, Mayo M, Kaestli M, Glass MB, Gee JE, Wuthiekanun V, Warner JM, Baker A, Foster JT, Tan P, Tuanyok A, Limmathurotsakul D, Peacock SJ, Currie BJ, Wagner DM, Keim P, Pearson T. 2012. Development and validation of *Burkholderia pseudomallei*specific real-time PCR assays for clinical, environmental or forensic detection applications. PLoS One 7:e37723. https://doi.org/10.1371/journal .pone.0037723.
- Chitlaru T, Israeli M, Bar-Haim E, Elia U, Rotem S, Ehrlich S, Cohen O, Shafferman A. 2016. Next-generation *Bacillus anthracis* live attenuated spore vaccine based on the htrA<sup>-</sup> (high temperature requirement A) Sterne strain. Sci Rep 6:18908. https://doi.org/10.1038/srep18908.
- Bar-On L, Cohen H, Elia U, Rotem S, Bercovich-Kinori A, Bar-Haim E, Chitlaru T, Cohen O. 2017. Protection of vaccinated mice against pneumonic tularemia is associated with an early memory sentinel-response in the lung. Vaccine 35:7001–7009. https://doi.org/10.1016/j.vaccine.2017 .10.053.
- 13. Warawa JM. 2010. Evaluation of surrogate animal models of melioidosis. Front Microbiol 1:141. https://doi.org/10.3389/fmicb.2010.00141.
- Bankevich A, Nurk S, Antipov D, Gurevich A, Dvorkin M, Kulikov AS, Lesin V, Nikolenko S, Pham S, Prjibelski A, Pyshkin A, Sirotkin A, 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.
- Gee JE, Gulvik CA, Elrod MG, Batra D, Rowe LA, Sheth M, Hoffmaster AR. 2017. Phylogeography of *Burkholderia pseudomallei* isolates, Western Hemisphere. Emerg Infect Dis 23:1133–1138. https://doi.org/10.3201/ eid2307.161978.