

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



LSHTM Research Online

Wagley, Sariqa; Scott, Andrew E; Ireland, Philip M; Prior, Joann L; Atkins, Timothy P; Bancroft, Gregory J; Studholme, David J; Titball, Richard W; (2019) Genome Resequencing of Laboratory Stocks of *Burkholderia pseudomallei* K96243. MICROBIOLOGY RESOURCE ANNOUNCEMENTS, 8 (9). ISSN 2576-098X DOI: <https://doi.org/10.1128/MRA.01529-18>

Downloaded from: <http://researchonline.lshtm.ac.uk/4652194/>

DOI: <https://doi.org/10.1128/MRA.01529-18>

Usage Guidelines:

Please refer to usage guidelines at <https://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by/2.5/>

<https://researchonline.lshtm.ac.uk>



Genome Resequencing of Laboratory Stocks of *Burkholderia pseudomallei* K96243

Sariqa Wagley,^a Andrew E. Scott,^b Philip M. Ireland,^b Joann L. Prior,^b Timothy P. Atkins,^b Gregory J. Bancroft,^c
David J. Studholme,^a Richard W. Titball^a

^aBiosciences, University of Exeter, Devon, United Kingdom

^bDefence Science and Technology Laboratory, Salisbury, Wiltshire, United Kingdom

^cLondon School of Hygiene & Tropical Medicine, London, United Kingdom

ABSTRACT We have resequenced the genomes of four *Burkholderia pseudomallei* K96243 laboratory cultures and compared them to the reported genome sequence that was published in 2004. Compared with the reference genome, these laboratory cultures harbored up to 42 single-nucleotide variants and up to 11 indels, including a 31.7-kb deletion in one culture.

B*Burkholderia pseudomallei* causes melioidosis, a bacterial disease of humans and animals (1). It is also a potential biothreat agent (2), and a panel of strains, including K96243, has been proposed to have potential countermeasures to melioidosis (2). Strain K96243 was originally isolated in 1996 from a 34-year-old female diabetic patient in Khon Kaen Hospital in Thailand (3). Since then, this strain has been extensively studied and passed between laboratories around the world. We genome sequenced cultures of strain K96243 with different passage histories held at different laboratories, namely, two from the Defense Science and Technology Laboratory (Dstl) and one each from the University of Exeter (UoE) and the London School of Hygiene and Tropical Medicine (LSHTM). Bacteria were grown with aeration in Luria-Bertani broth at 37°C for 24 h. DNA was extracted using a Genomic-tip 100/G kit (Qiagen Ltd.) following the manufacturer's instructions. DNA was concentrated using a GeneRead kit (lot no. 145025210), and end repair and adenylation of fragments were carried out using a NEXTflex rapid DNA-seq kit (catalog no. 5144-02) according to the manufacturer's instructions. Purification and concentration of the PCR-amplified library were carried out according to the GeneRead kit instructions.

The genome sequences shown in Table 1 were determined using 100-bp paired-end libraries with the Illumina HiSeq 2500 system. Quality and adapter trimming were performed using TrimGalore version 0.3.7 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) with the options “—q30 —paired.” TrimGalore uses CutAdapt version 1.15 (4). We used the “mem” algorithm in Burrow-Wheeler aligner (BWA) version 0.7.12-r1039 (5) to align the trimmed reads to the strain K96243 reference genome sequence already available (GenBank accession no. [BX571965](#) and [BX571966](#)) (6). The resulting sequence alignment map (SAM) file was converted to binary alignment map (BAM) format using SAMtools version 0.1.19-96b5f2294a (7) with the command line options “view -bS -q 1.” We called variants using Pilon version 1.22 (8) with the options “-Xmx16G —changes —vcf —tracks” and checked the variants using Integrated Genome Viewer version 2.3.78 (9) with its default settings.

In total, compared with the published sequence, we found 60 single-nucleotide variants (SNVs) across the 4 resequenced cultures (Table 1), and 29 of these SNVs were previously reported (2). At 21 sites, the same SNV was present in all resequenced cultures, suggesting errors in the reference genome. Many of the SNVs were

Citation Wagley S, Scott AE, Ireland PM, Prior JL, Atkins TP, Bancroft GJ, Studholme DJ, Titball RW. 2019. Genome resequencing of laboratory stocks of *Burkholderia pseudomallei* K96243. *Microbiol Resour Announc* 8:e01529-18. <https://doi.org/10.1128/MRA.01529-18>.

Editor Catherine Putonti, Loyola University Chicago

© Crown copyright 2019. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

Address correspondence to Sariqa Wagley, s.wagley@exeter.ac.uk.

Received 3 December 2018

Accepted 30 January 2019

Published 28 February 2019

TABLE 1 Characteristics of resequenced K96243 cultures^a

| Strain | SRA accession no. | No. of reads | Coverage (×) | No. of SNVs | No. of indels |
|---------------|----------------------------|--------------|--------------|-------------|---------------|
| K96243-UoE | SRS3855208 | 5,633,150 | 41 | 33 | 9 |
| K96243-LSTHM | SRS3855207 | 722,584 | 18 | 39 | 11 |
| K96243-Dstl-1 | SRS3855209 | 905,984 | 24 | 42 | 9 |
| K96243-Dstl-2 | SRS3855206 | 4,534,902 | 39 | 41 | 9 |

^a SNVs and indels are in comparison to the K96243 genome sequences in GenBank (accession no. [NC_006350](#) and [NC_006351](#)).

colocated in a 260-bp GC-rich region which may be difficult to sequence or may be hypermutable.

We identified 19 indels ranging from 1 nucleotide (nt) to 33.7 kb across the 4 resequenced cultures. At 5 sites, the same indel was present in all resequenced cultures, suggesting errors in the reference genome. A 31.7-kb region was deleted from chromosome 1 (nucleotide position 1439846 to 1471563; BPSL1247 to BPSL1269) of the UoE culture. This region did not correspond to any of the previously reported genome islands (3) and was not flanked by insertion sequence (IS) elements. It includes 5 hypothetical proteins and a cluster of 5 genes predicted to be involved in cytochrome oxidase-related functions (BPSL1256 to BPSL1257 and BPSL1259 to BPSL1261). It is possible that this region plays a role in electron transport.

Other workers have reported genome plasticity and diversity between different isolates of *B. pseudomallei* (10), and a recent study reported that, of a number of *B. pseudomallei* isolates resequenced, strain K96243 showed the greatest divergence from the deposited sequence (2).

The microevolution of *B. pseudomallei* during infection has previously been reported, with 8 SNVs and 6 small-scale (up to 56 nucleotides [nt]) indels differentiating these variants (11). In addition, derivatives from a single isolate, but with different colony morphologies, showed different virulences (12, 13) and different genetic makeups (14). However, it is reported that genetic differences, including SNVs, do not distinguish these different colony morphotypes (15).

Our findings show that the genetic makeups of laboratory stock cultures of *B. pseudomallei* strain K96243 are not identical. These findings highlight the need to sequence culture stocks of K96243 held in laboratories before carrying out work with this strain.

Data availability. These data have been deposited in DDBJ/ENA/GenBank under BioProject accession no. [PRJNA486512](#). The SRA accession numbers for each strain are [SRS3855208](#) (K96243-Exeter), [SRS3855207](#) (K96243-LSTHM), [SRS3855209](#) (K96243-Dstl-1), and [SRS3855206](#) (K96243-Dstl-2).

ACKNOWLEDGMENTS

This work was supported by Defense Science and Technology Laboratory grant DSTLX-1000060221 (WP2).

DNA library preparation and sequencing were performed by Karen Moore and colleagues in the University of Exeter Sequencing Facility.

REFERENCES

1. Limmathurotsakul D, Golding N, Dance DAB, Messina JP, Pigott DM, Moyes CL, Rolim DB, Bertherat E, Day NPJ, Peacock SJ, Hay SI. 2016. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nat Microbiol* 1:15008. <https://doi.org/10.1038/nmicrobiol.2015.8>.
2. Sahl JW, Allender CJ, Colman RE, Califf KJ, Schupp JM, Currie BJ, Van Zandt KE, Gelhaus HC, Keim P, Tuanyok A. 2015. Genomic characterization of *Burkholderia pseudomallei* isolates selected for medical countermeasures testing: comparative genomics associated with differential virulence. *PLoS One* 10:e0121052. <https://doi.org/10.1371/journal.pone.0121052>.
3. Holden MTG, Titball RW, Peacock SJ, Cerdeno-Tarraga AM, Atkins T, Crossman LC, Pitt T, Churcher C, Mungall K, Bentley SD, Sebahia M, Thomson NR, Bason N, Beacham IR, Brooks K, Brown KA, 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, Rabinowitsch 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>.
4. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* 17:10–12. <https://doi.org/10.14806/ej.17.1.200>.
5. 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>.
6. Afgan E, Baker D, van den Beek M, Blankenberg D, Bouvier D, Čech M, Chilton J, Clements D, Coraor N, Eberhard C, Grüning B, Guerler A, Hillman-Jackson J, Von Kuster G, Rasche E, Soranzo N, Turaga N, Taylor J, Nekrutenko A, Goecks J. 2016. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Res* 44:W3–W10. <https://doi.org/10.1093/nar/gkw343>.
 7. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
 8. 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>.
 9. Thorvaldsdottir H, Robinson JT, Mesirov JP. 2013. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform* 14:178–192. <https://doi.org/10.1093/bib/bbs017>.
 10. Tumapa S, Holden MT, Vesaratchavest M, Wuthiekanun V, Limmathurotsakul D, Chierakul W, Feil EJ, Currie BJ, Day NP, Nierman WC, Peacock SJ. 2008. *Burkholderia pseudomallei* genome plasticity associated with genomic island variation. *BMC Genomics* 9:190. <https://doi.org/10.1186/1471-2164-9-190>.
 11. Limmathurotsakul D, Holden MT, Coupland P, Price EP, Chantratita N, Wuthiekanun V, Amornchai P, Parkhill J, Peacock SJ. 2014. Microevolution of *Burkholderia pseudomallei* during an acute infection. *J Clin Microbiol* 52:3418–3421. <https://doi.org/10.1128/JCM.01219-14>.
 12. Shea AA, Bernhards RC, Cote CK, Chase CJ, Koehler JW, Klimko CP, Ladner JT, Rozak DA, Wolcott MJ, Fetterer DP, Kern SJ, Koroleva GI, Lovett SP, Palacios GF, Toothman RG, Bozue JA, Worsham PL, Welkos SL. 2017. Two stable variants of *Burkholderia pseudomallei* strain MSHR5848 express broadly divergent *in vitro* phenotypes associated with their virulence differences. *PLoS One* 12:e0171363. <https://doi.org/10.1371/journal.pone.0171363>.
 13. Bernhards RC, Cote CK, Amemiya K, Waag DM, Klimko CP, Worsham PL, Welkos SL. 2017. Characterization of *in vitro* phenotypes of *Burkholderia pseudomallei* and *Burkholderia mallei* strains potentially associated with persistent infection in mice. *Arch Microbiol* 199:277–301. <https://doi.org/10.1007/s00203-016-1303-8>.
 14. Hsueh PT, Chen YS, Lin HH, Liu PJ, Ni WF, Liu MC, Chen YL. 2015. Comparison of whole-genome sequences from two colony morphovars of *Burkholderia pseudomallei*. *Genome Announc* 3:e01194-15. <https://doi.org/10.1128/genomeA.01194-15>.
 15. Vipond J, Kane J, Hatch G, McCarrison J, Nierman WC, Losada L. 2013. Sequence determination of *Burkholderia pseudomallei* strain NCTC 13392 colony morphology variants. *Genome Announc* 1:e00925-13. <https://doi.org/10.1128/genomeA.00925-13>.