





Whole-Genome Sequences of Eight Clinical Isolates of *Burkholderia pseudomallei* from Melioidosis Patients in Eastern Sri Lanka

Microbiology

Resource Announcements

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ABSTRACT Here, we report whole-genome sequences (WGS) of eight clinical isolates of *Burkholderia pseudomallei* obtained from melioidosis patients with sepsis in eastern Sri Lanka.

Whole-genome sequencing of *Burkholderia pseudomallei*, the causative agent of melioidosis, provides a better understanding about the phylogeography, transmission, evolution, virulence, epidemiology, and antibiotic resistance (1) of this organism. It is now clearly established that melioidosis is endemic in Sri Lanka with a wide geographic distribution (2). Whole-genome sequences (WGS) of *B. pseudomallei* are available for Southeast Asian (3) and northern Australian (4) strains. However, only a few WGS data sets have been published for the Indian subcontinent (5).

Here, we report eight complete genome sequences of clinical isolates of *B. pseudomallei* (BPs110, BPs111, BPs112, BPs114, BPs115, BPs116, BPs122, and BPs133) from melioidosis patients with acute sepsis in eastern Sri Lanka.

Strains were isolated from blood samples collected from melioidosis patients under sterile conditions, and blood agar base (Oxoid, UK) supplemented with 5% blood was used for the isolation of the organism. Subculturing was done several times on the same medium. One well-isolated single colony was restreaked on the fresh medium, a few well-isolated single colonies were pooled, and genomic DNA was extracted using a mini-QIAamp DNA isolation kit as recommended by the manufacturer (Qiagen, Germany). Multiple real-time PCR assays (*Yersinia*-like fimbrial/*Burkholderia thailandensis*-like flagellum and chemotaxis region [YLF/BTFC]) were performed (6, 7). Further, real-time *Ipxo* PCR was used for confirmation of presumptive *B. pseudomallei* (6). High-quality genomic DNA of each isolate was subjected to whole-genome sequencing from a paired end with 300 nucleotide reads (Nextera DNA library prep kit) using the MiSeq 2000 platform at Agiomix FZ LLC in the United Arab Emirates.

Raw sequence data were processed with Trimmomatic 0.36 (8) and FASTX-Toolkit 0.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/) to remove Illumina adaptor sequences and low-quality bases and reads. The quality of the raw sequence data was assessed using FastQC 0.11.4 (9) and MultiQC 1.0 (10). The Burrows-Wheeler Aligner (BWA) 0.7.12-r1039 (11) and Qualimap 2.2.1 (12) were used for raw read alignments and quality control of the alignment sequencing data. SPAdes 3.10.1 (13), ABACAS 1.3.1 (14), NCBI local BLAST 2.6.0, and online RAST (15) were used for genome assembly, annotation, and validation. All tools were used with default parameters, and cleaned sequences were used for downstream analysis. The assemblies were reorganized relative to the closed *B. pseudomallei* K96243 genome (GenBank accession numbers

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FABLE 1 Characteristics and accession numbers o	genomes of Burkholderia	pseudomallei isolates se	equenced in this study
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	Multilocus				YLF/		No. of	No. of				
Strain	sequence	Genome size (bp)	No. of	No. of	BTFC	No. of	RNA	tRNA	No. of	N ₅₀	No. of	GenBank
designation	type ^a	(GC content [%])	ncRNAs ^b	CDSs ^c	PCR^d	pseudogenes	genes	genes	contigs	(bp)	raw reads	accession no.
BPs110	1152	6,962,327 (68.39)	4	6,670	BTFC	514	77	61	132	115,980	2,192,501	CP036451, CP036452
BPs111	1364	6,721,089 (68.17)	4	6,670	YLF	513	76	60	147	113,263	2,007,588	CP036453, CP036454
BPs112	1442	6,258,284 (68.36)	4	6,608	YLF	537	78	62	163	83,750	1,993,557	CP037975, CP037976
BPs114	594	6,022,338 (68.39)	4	6,638	BTFC	508	77	77	158	101,821	2,617,163	CP037973, CP037974
BPs115	1413	6,756,482 (68.24)	4	6,663	YLF	504	77	61	160	103,836	2,427,392	CP037757, CP037758
BPs116	1179	6,693,503 (68.32)	4	6,593	BTFC	512	76	61	141	106,427	2,009,396	CP037759, CP037760
BPs122	594	6,242,888 (68.36)	4	6,709	BTFC	504	77	61	129	122,561	4,042,684	CP038194, CP038195
BPs133	594	6,106,529 (68.39)	4	6,647	BTFC	509	77	61	138	122,504	3,362,629	CP037971, CP037972

^a Based on the scheme at http://pubmlst.org/bpseudomallei.

^b ncRNAs, noncoding RNAs.

^c CDSs, protein-coding sequences.

^d YLF, Yersinia-like fimbrial region; BTFC, Burkholderia thailandensis-like flagellum chemotaxis region.

NC_006350 and NC_006351). All genomes reported here have been annotated using a best-placed reference protein set, GeneMarkS-2+, and the NCBI annotation provider (NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The genomes of the *B. pseudomallei* isolates reported here contain two chromosomes, and the features annotated are reported in Table 1.

Data availability. All of the whole-genome sequencing projects have been deposited in GenBank, and the accession numbers are given in Table 1. The raw data are also publicly accessible under the accession numbers SRR8658974, SRR8618097, SRR8741027, SRR8759108, SRR8661621, SRR8660934, SRR8867837, and SRR8867836.

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REFERENCES

- Price EP, Currie BJ, Sarovich DS. 2017. Genomic insights into the melioidosis pathogen, *Burkholderia pseudomallei*. Curr Trop Med Rep 4:95. https://doi.org/10.1007/s40475-017-0111-9.
- Corea EM, de Silva AD, Thevanesam V. 2018. Melioidosis in Sri Lanka. Trop Med Infect Dis 3:22. https://doi.org/10.3390/tropicalmed 3010022.
- Nandi T, Ong C, Singh AP, Boddey J, Atkins T, Sarkar-Tyson M, Essex-Lopresti AE, Chua HH, Pearson T, Kreisberg JF, Nilsson C, Ariyaratne P, Ronning C, Losada L, Ruan Y, Sung WK, Woods D, Titball RW, Beacham I, Peak I, Keim P, Nierman WC, Tan P. 2010. A genomic survey of positive selection in *Burkholderia pseudomallei* provides insights into the evolution of accidental virulence. PLoS Pathog 6:e1000845. https://doi.org/10 .1371/journal.ppat.1000845.
- Johnson SL, Baker AL, Chain PS, Currie BJ, Daligault HE, Davenport KW, Davis CB, Inglis TJ, Kaestli M, Koren S, Mayo M, Merritt AJ, Price EP, Sarovich DS, Warner J, Rosovitz MJ. 2015. Whole-genome sequences of 80 environmental and clinical isolates of *Burkholderia pseudomallei*. Genome Announc 3:e01282-14. https://doi.org/10.1128/ genomeA.01282-14.
- Mukhopadhyay C, Vandana KE, Chaitanya TAK, Shaw T, Bhat HV, Chakrabarty S, Paul B, Mallya S, Murali TS, Satyamoorthy K. 2015. Genome sequence of a *Burkholderia pseudomallei* clinical isolate from a patient with community-acquired pneumonia and septicemia. Genome Announc 3:e00915-15. https://doi.org/10.1128/genomeA.00915-15.
- Merritt A, Inglis TJ, Chidlow G, Harnett G. 2006. PCR-based identification of *Burkholderia pseudomallei*. Rev Inst Med Trop Sao Paulo 48:239–244. https://doi.org/10.1590/S0036-46652006000500001.

- Tuanyok A, Auerbach RK, Brettin TS, Bruce DC, Munk AC, Detter JC, Pearson T, Hornstra H, Sermswan RW, Wuthiekanun V, Peacock SJ, Currie BJ, Keim P, Wagner DM. 2007. A horizontal gene transfer event defines two distinct groups within *Burkholderia pseudomallei* that have dissimilar geographic distributions. J Bacteriol 189:9044–9049. https://doi.org/10 .1128/JB.01264-07.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10 .1093/bioinformatics/btu170.
- Andrews S. 2014. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/ fastqc.
- Ewels P, Magnusson M, Lundin S, Käller M. 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32:3047–3048. https://doi.org/10.1093/bioinformatics/ btw354.
- 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.
- Okonechnikov K, Conesa A, García-Alcalde F. 2016. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics 32:292–294. https://doi.org/10.1093/bioinformatics/ btv566.
- 13. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell se-

quencing. J Comput Biol 19:455-477. https://doi.org/10.1089/cmb .2012.0021.

- Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. 2009. ABACAS: algorithm-based automatic contiguation of assembled sequences. Bioinformatics 25:1968–1969. https://doi.org/10.1093/bioinformatics/ btp347.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.