



Whole-Genome Sequence of *Burkholderia pseudomallei* Strain HNBPO01, Isolated from a Melioidosis Patient in Hainan, China

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ABSTRACT Here, we report the complete genome sequence of *Burkholderia pseudomallei* HNBPO01, an epidemic strain isolated from a melioidosis patient with pneumonia in Hainan, China.

Burkholderia pseudomallei is a Gram-negative bacterium that causes melioidosis, a serious human and animal infection commonly reported in parts of Africa, South America, and Asia (1, 2). This organism was possibly used as a biological weapon (3). The limited treatment options combined with the difficulty in diagnosis and the current lack of a vaccine make this organism important to public health in tropical regions worldwide (4). In China, melioidosis is primarily reported in the southeast coastal regions, including Hainan, Guangdong, Fujian, and Taiwan (5, 6). The organism is widespread in water and soil of its regions of endemicity (7). Infection is commonly acquired via contact with a contaminated environmental source (8). Deciphering whole-genomic sequences provides insight into the pathogen's phylogeny or diversity and the biogeographical contribution of virulence; however, the majority of the currently available sequences of *B. pseudomallei* are from strains isolated in northern Australia or Thailand (9, 10). Here, we report the genome sequence of *B. pseudomallei* strain HNBPO01, isolated from the blood of a melioidosis patient with pneumonia using a previous method (6). Sampling procedures were approved by the ethics committee of the Hainan Medical University.

For the draft genome sequence, *B. pseudomallei* HNBPO01 was grown on a Luria-Bertani (LB) agar plate with gentamicin for 24 h at 37°C (11). Genomic DNA was extracted using a bacterial DNA extraction kit (Omega Bio-tek, USA) and then sequenced using the PacBio RS II platform at the Beijing Genomics Institute (BGI) in Shenzhen, China. PacBio subreads with a length of <1 kb were removed in order to obtain more accurate and reliable subreads in the subsequent bioinformatics analysis. For PacBio sequencing, approximately 10 g of DNA was fragmented to 10 to 20 kbp using the g-TUBE apparatus (Covaris). The sequencing library was constructed using the SMRTbell template prep kit 1.0 according to the PacBio 20-kb library protocol. Sequencing was performed on the PacBio RS II instrument in one single-molecule real-time (SMRT) cell (v3) for 6 h. Illumina libraries were constructed with 270-bp inserts using standard methods. The paired-end sequencing library was prepared using a Genomic DNA prep kit (Qiagen, USA) and then sequenced using an Illumina HiSeq 4000 platform. A total of 1,115 Mb of raw data were produced from 7,438,126 150-bp reads. After filtering out low-quality reads with SOAPnuke using default parameters (12), we obtained 1,024 Mb clean short reads. The genome was assembled with the Hierarchical Genome Assembly Process 3 (HGAP3) (13) and Quiver in SMRT analysis v2.3.0, GATK (<https://www.broadinstitute.org/gatk/>), SOAPsnp (14), and SOAPindel (15) to correct small indels, resulting in 2 contigs representing the 2 chromosomes in *B. pseudomallei* HNBPO01. The genome was annotated with

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Glimmer3 v3.02 using default parameters (16). There were 59,381 polymerase reads after filtering. The data size of all the polymerase reads was 877,413,219 bp, and their average length was 14,775 bp. The average quality of all polymerase reads was 0.68 read quality (RQ). There were 102,965 filtered subreads with a total data size of 873,175,857 bp. The average length of the subreads was 8,480 bp with an N_{50} value of 10,525 bp. The average quality of all subreads was 0.86 RQ.

The *B. pseudomallei* HNBP001 genome consists of two chromosomes. The newly sequenced complete genome sequence of HNBP001 comprises a large chromosome and a small chromosome of 7,163,595 bp in total. Chromosome 1 is 3,800,466 bp in size (GC content, 68.04%) with 3,807 protein-coding sequences, 6 rRNA genes, and 45 tRNA genes. Chromosome 2 is 3,363,129 bp in size (GC content, 68.32%) with 3,342 protein-coding sequences, 6 rRNA genes, and 14 tRNA genes. These numbers are similar to those of previously published genome annotations of *B. pseudomallei* (17, 18). The genome sequence of strain HNBP001 will serve as a reference for additional assemblies of *B. pseudomallei* isolates from China.

Data availability. The finished annotated sequences of the two chromosomes of *B. pseudomallei* have been deposited at NCBI GenBank under the accession numbers CP038805 (chromosome 1) and CP038806 (chromosome 2). The raw data were deposited in the Sequence Read Archive (SRA) for the PacBio RS II (SRA number SRR9027110) and Illumina (SRA number SRR9691870) read data.

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REFERENCES

- Wiersinga WJ, Currie BJ, Peacock SJ. 2012. Melioidosis. *N Engl J Med* 367:1035–1044. <https://doi.org/10.1056/NEJMra1204699>.
- 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>.
- Lafontaine ER, Balder R, Michel F, Hogan RJ. 2014. Characterization of an autotransporter adhesin protein shared by *Burkholderia mallei* and *Burkholderia pseudomallei*. *BMC Microbiol* 14:92. <https://doi.org/10.1186/1471-2180-14-92>.
- Aziz A, Sarovich DS, Harris TM, Kaestli M, McRobb E, Mayo M, Currie BJ, Price EP. 2017. Suspected cases of intracontinental *Burkholderia pseudomallei* sequence type homoplasy resolved using whole-genome sequencing. *Microb Genom* 3. <https://doi.org/10.1099/mgen.0.000139>.
- Dong SF, Wu LX, Long FQ, Wu Q, Liu X, Pei H, Xu K, Lu YJ, Wang Y, Lin YZ, Xia QF. 2018. The prevalence and distribution of *Burkholderia pseudomallei*, in rice paddy within Hainan, China. *Acta Trop* 187:165–168. <https://doi.org/10.1016/j.actatropica.2018.08.007>.
- Fang Y, Chen H, Li YL, Li Q, Ye ZJ, Mao XH. 2015. Melioidosis in Hainan, China: a retrospective study. *Trans R Soc Trop Med Hyg* 109:636–642. <https://doi.org/10.1093/trstmh/trv065>.
- Fang Y, Huang Y, Li Q, Chen H, Yao Z, Pan J, Gu J, Tang B, Wang HG, Yu B, Tong YG, Zou QM, Mao XH. 2012. First genome sequence of a *Burkholderia pseudomallei* isolate in China, strain BPC006, obtained from a melioidosis patient in Hainan. *J Bacteriol* 194:6604–6605. <https://doi.org/10.1128/JB.01577-12>.
- Barnes JL, Ketheesan N. 2005. Route of infection in melioidosis. *Emerg Infect Dis* 11:638–639. <https://doi.org/10.3201/eid1104.041051>.
- Cheng AC, Currie BJ. 2005. Melioidosis: epidemiology, pathophysiology, and management. *Clin Microbiol Rev* 18:383–416. <https://doi.org/10.1128/CMR.18.2.383-416.2005>.
- Currie BJ, Dance DA, Cheng AC. 2008. The global distribution of *Burkholderia pseudomallei* and melioidosis: an update. *Trans R Soc Trop Med Hyg* 102:S1–S4. [https://doi.org/10.1016/S0035-9203\(08\)70002-6](https://doi.org/10.1016/S0035-9203(08)70002-6).
- Peacock SJ, Chieng G, Cheng AC, Dance DA, Amornchai P, Wongsuvan G, Teerawattanasook N, Chierakul W, Day NP, Wuthiekanun V. 2005. Comparison of Ashdown's medium, *Burkholderia cepacia* medium, and *Burkholderia pseudomallei* selective agar for clinical isolation of *Burkholderia pseudomallei*. *J Clin Microbiol* 43:5359–5361. <https://doi.org/10.1128/JCM.43.10.5359-5361.2005>.
- Chen YX, Chen YS, Shi CM, Huang ZB, Zhang Y, Li SK, Li Y, Ye J, Yu C, Li Z, Zhang XQ, Wang J, Yang HM, Fang L, Chen Q. 2017. SOAPnuke: a MapReduce acceleration-supported software for integrated quality control and preprocessing of high-throughput sequencing data. *GigaScience* 7:gix120. <https://doi.org/10.1093/gigascience/gix120>.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Li RQ, Li YR, Fang XD, Yang HM, Wang J, Kristiansen K, Wang J. 2009. SNP detection for massively parallel whole-genome resequencing. *Genome Res* 19:1124–1132. <https://doi.org/10.1101/gr.088013.108>.
- Li ST, Li RQ, Li H, Lu JL, Li YR, Bolund L, Schierup MH, Wang J. 2013. SOAPindel: efficient identification of indels from short paired reads. *Genome Res* 23:195–200. <https://doi.org/10.1101/gr.132480.111>.
- Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679. <https://doi.org/10.1093/bioinformatics/btm009>.
- Johnson SL, Bishop-Lilly KA, Ladner JT, Daligault HE, Davenport KW, Jaissle J, Frey KG, Koroleva GI, Bruce DC, Coyne SR, Broomall SM, Li PE, Teshima H, Gibbons HS, Palacios GF, Rosenzweig CN, Redden CL, Xu Y, Minogue TD, Chain PS. 2015. Complete genome sequences for 59 *Burkholderia* isolates, both pathogenic and near neighbor. *Genome Announc* 3:e00159-15. <https://doi.org/10.1128/genomeA.00159-15>.
- Holden MTG, Titball RW, Peacock SJ, Cerdeno-Tarraga AM, Atkins T, Crossman LC, Pitt T, Churcher C, Mungall K, Bentley SD, Sebaihia 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, Rabinowitz E, Rutherford K, Sanders M, Simmonds M, Songvilai S, Stevens K, Tumapa S, Vesaratchavest M, Whitehead S, Yeats C, Barrell BG, Oyston PC, 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>.