

# Report Documentation Page

Form Approved  
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE <b>AUG 2010</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2010 to 00-00-2010</b>	
4. TITLE AND SUBTITLE <b>High-Redundancy Draft Sequencing of 15 Clinical and Environmental Burkholderia Strains</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Naval Medical Research Center, Biological Defense Research Directorate, Silver Spring, MD, 20910</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

## GENOME ANNOUNCEMENTS

### High-Redundancy Draft Sequencing of 15 Clinical and Environmental *Burkholderia* Strains<sup>∇</sup>

Sanghamitra Mukhopadhyay,<sup>1†</sup> Maureen K. Thomason,<sup>1‡</sup> Shannon Lentz,<sup>1</sup> Nichole Nolan,<sup>1</sup> Kristin Willner,<sup>1§</sup> Jay E. Gee,<sup>2</sup> Mindy B. Glass,<sup>2</sup> Timothy J. J. Inglis,<sup>3</sup> Adam Merritt,<sup>2</sup> Avram Levy,<sup>3</sup> Shanmuga Sozhamannan,<sup>1</sup> Al Mateczun,<sup>1</sup> and Timothy D. Read<sup>1\*</sup>

*Biological Defense Research Directorate, Naval Medical Research Center, Silver Spring, Maryland 20910<sup>1</sup>; Bacterial Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, Georgia 30333<sup>2</sup>; and Division of Microbiology and Infectious Diseases, PathWest Laboratory Medicine WA, Nedlands, WA 6009, Australia<sup>3</sup>*

Received 20 August 2010/Accepted 30 August 2010

**The Gram-negative *Burkholderia* genus includes several species of intracellular bacterial pathogens that pose substantial risk to humans. In this study, we have generated draft genome sequences of 15 strains of *B. oklahomensis*, *B. pseudomallei*, *B. thailandensis*, and *B. ubonensis* to an average sequence read coverage of 25- to 40-fold.**

The Gram-negative *Burkholderia* genus includes several species of intracellular bacterial pathogens that pose substantial risk to humans. The high virulence of the *B. pseudomallei*/*B. mallei* species by the respiratory route and the fact that the bacteria can be aerosolized has caused them to be considered bioterrorists (1); both *B. pseudomallei* and *B. mallei* have been designated category B select agents by the Centers for Disease Control and Prevention (CDC) (18).

Genomes of 15 strains of *B. oklahomensis*, *B. pseudomallei*, *B. thailandensis*, and *B. ubonensis* were sequenced using the Roche/454 Sequencing GS-20 instrument (13). The average read length obtained from the 15 libraries was 97 nucleotides (nt). Raw sequence data assembled into 450 to 1,000 contigs of more than 1,000 nt per genome, with an average redundancy of coverage of 25 to 40 reads per base. The GC contents of the nucleotide sequences of the strains were 63 to 67%.

High-redundancy draft genome sequencing is an economic way of assessing species diversity and is used to screen strains for subsequent genome sequence completion. The data generated in this project have already proved useful in helping to

identify conserved vaccine targets (2), have been incorporated into global comparative genomics analyses of the *Burkholderia* genus (16, 20), and have been used for identification of candidate loci for multilocus variable-number tandem-repeat typing schemes (19).

**Brief strain descriptions.** *B. pseudomallei* strain B7210 is a human isolate from Australia, obtained in a case of empyema infection in 1970 (4, 6). Strain BCC 215 is an isolate obtained in a fatal case of septicemic melioidosis in a family located in rural northeastern Brazil. Three of four affected children, all siblings, died. An indirect hemagglutination assay (IHA) indicated seroconversion subsequent to clinical recovery for the one survivor (17). DM98, a strain with a persistently mucoid phenotype, was isolated in a single case of septicemic melioidosis in tropical northern Australia and was obtained from a blood culture (8). NCTC 13177 (also known as BCC 6) was isolated in an outbreak of fatal septicemic melioidosis cases in northwestern Australia that were linked to a contaminated water supply (9–11). PHLS 9 (CDC2002721637) is a human isolate from Pakistan, obtained in 1988 (4, 6). PHLS 14 was isolated from a monkey liver in the Philippines in 1990 (4, 6). PHLS 91 is a sheep lung isolate from Australia, obtained in 1984 (4, 6). PHLS 112 is a human isolate obtained from north-east Thailand in 1992 (4, 6). Strain 7894 is a human isolate obtained from Ecuador in 1962 (4, 6).

*B. oklahomensis* strain C6786 was isolated from an infected human in Oklahoma (7, 14). E0147 was isolated in a case of human infection in Georgia (7, 15).

*B. thailandensis* Bt4 is an environmental isolate from northern Australia, obtained during an investigation of *Burkholderia* ecology in potable water supply systems (12). MSMB43 is an arabinose-assimilating environmental strain from a borehole near Darwin, Northern Territories, Australia, and falls into an intermediate phylogenetic clade relative to *B. pseudomallei* and

\* Corresponding author. Present address: Department of Human Genetics, Emory University School of Medicine, 615 Michael Street, Atlanta, GA 30322. Phone: (404) 727-9706. Fax: (404) 727-2199. E-mail: tread@emory.edu.

† Present address: Biological Threat Reduction Program, SAIC-Threat Reduction Support Center, Alexandria, VA 22310.

‡ Present address: Cell Biology and Metabolism Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD 20892, and Department of Biochemistry and Molecular and Cell Biology, Georgetown University Medical Center, Washington, DC 20007.

§ Present address: Chemical and Biological Division, Science and Technology Directorate, Department of Homeland Security, Washington, DC 20005.

<sup>∇</sup> Published ahead of print on 24 September 2010.

*B. thailandensis* (3). CDC3015869 (TXDOH) was isolated from the blood of a 2-year-old boy infected after a near-drowning incident (5).

*B. ubonensis* strain Bu was isolated from a rhizosphere sample from a mine site in northern Australia (12).

**Nucleotide sequence accession numbers.** The sequences determined in this study have been deposited in the National Center for Biotechnology Information (NCBI) RefSeq database under the following accession numbers: for *B. pseudomallei* strains B7210, BCC 215, DM98, NCTC 13177, PHLS 9, PHLS 14, PHLS 91, PHLS 112, and 7894, NZ\_ABBN00000000, NZ\_ABBR00000000, NZ\_ABBI00000000, NZ\_ABBQ00000000, NZ\_ABBL00000000, NZ\_ABBJ00000000, NZ\_ABBK00000000, NZ\_ABBP00000000, and NZ\_ABBO00000000, respectively; for *B. oklahomensis* strains C6786 and E0147, NZ\_ABBG00000000 and NZ\_ABBF00000000; for *B. thailandensis* strains Bt4, MSMB43, and CDC3015869, NZ\_ABBH00000000, NZ\_ABBM00000000, and NZ\_ABBD00000000, respectively; and for *B. ubonensis* strain Bu, NZ\_ABBE00000000.

We thank Patricia Wilkins, Alex Hoffmaster, David Ussery, and Peter Hallin for their help with this study.

This work was funded by grant XX0013\_06\_NM\_B from the Defense Threat Reduction Agency to T.D.R. Some of the authors are employees of the U.S. Government, and this work was prepared as part of their official duties.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the U.S. Department of the Navy, U.S. Department of Defense, U.S. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, or U.S. Government.

#### REFERENCES

- Deshazer, D., and D. Waag. 2004. Glanders: new insights into an old disease, p. 209–237. In F. Lindler, F. Lebeda, and G. W. Korch (ed.), *Biological weapons defense: infectious diseases and counter bioterrorism*. Humana Press, Totowa, NJ.
- Druar, C., F. Yu, J. L. Barnes, R. T. Okinaka, N. Chantrattita, S. Beg, C. W. Stratilo, A. J. Olive, G. Soltes, M. L. Russell, D. Limmathurotsakul, R. E. Norton, S. X. Ni, W. D. Picking, P. J. Jackson, D. I. Stewart, V. Tsvetnitsky, W. L. Picking, J. W. Cherwonogrodzky, N. Ketheesan, S. J. Peacock, and E. J. Wiersma. 2008. Evaluating *Burkholderia pseudomallei* Bip proteins as vaccines and Bip antibodies as detection agents. *FEMS Immunol. Med. Microbiol.* 52:78–87.
- Gee, J. E., M. B. Glass, R. T. Novak, D. Gal, M. J. Mayo, A. G. Steigerwalt, P. P. Wilkins, and B. J. Currie. 2008. Recovery of a *Burkholderia thailandensis*-like isolate from an Australian water source. *BMC Microbiol.* 8:54.
- Glass, M. B., C. A. Beesley, P. P. Wilkins, and A. R. Hoffmaster. 2009. Comparison of four selective media for the isolation of *Burkholderia mallei* and *Burkholderia pseudomallei*. *Am. J. Trop. Med. Hyg.* 80:1023–1028.
- Glass, M. B., J. E. Gee, A. G. Steigerwalt, D. Cavuoti, T. Barton, R. D. Hardy, D. Godoy, B. G. Spratt, T. A. Clark, and P. P. Wilkins. 2006. Pneumonia and septicemia caused by *Burkholderia thailandensis* in the United States. *J. Clin. Microbiol.* 44:4601–4604.
- Glass, M. B., and T. Popovic. 2005. Preliminary evaluation of the API 20NE and RapID NF Plus systems for rapid identification of *Burkholderia pseudomallei* and *B. mallei*. *J. Clin. Microbiol.* 43:479–483.
- Glass, M. B., A. G. Steigerwalt, J. G. Jordan, P. P. Wilkins, and J. E. Gee. 2006. *Burkholderia oklahomensis* sp. nov., a *Burkholderia pseudomallei*-like species formerly known as the Oklahoma strain of *Pseudomonas pseudomallei*. *Int. J. Syst. Evol. Microbiol.* 56:2171–2176.
- Howard, K., and T. J. Inglis. 2003. Novel selective medium for isolation of *Burkholderia pseudomallei*. *J. Clin. Microbiol.* 41:3312–3316.
- Inglis, T. J., S. C. Garrow, C. Adams, M. Henderson, and M. Mayo. 1998. Dry-season outbreak of melioidosis in Western Australia. *Lancet* 352:1600.
- Inglis, T. J., S. C. Garrow, C. Adams, M. Henderson, M. Mayo, and B. J. Currie. 1999. Acute melioidosis outbreak in Western Australia. *Epidemiol. Infect.* 123:437–443.
- Inglis, T. J., S. C. Garrow, M. Henderson, A. Clair, J. Sampson, L. O'Reilly, and B. Cameron. 2000. *Burkholderia pseudomallei* traced to water treatment plant in Australia. *Emerg. Infect. Dis.* 6:56–59.
- Levy, A., A. J. Merritt, M. Aravena-Roman, M. M. Hodge, and T. J. Inglis. 2008. Expanded range of *Burkholderia* species in Australia. *Am. J. Trop. Med. Hyg.* 78:599–604.
- Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y. J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Irzyk, S. C. Jando, M. L. Alenquer, T. P. Jarvie, K. B. Jirage, J. B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley, and J. M. Rothberg. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380.
- McCormick, J. B., R. E. Weaver, P. S. Hayes, J. M. Boyce, and R. A. Feldman. 1977. Wound infection by an indigenous *Pseudomonas pseudomallei*-like organism isolated from the soil: case report and epidemiologic study. *J. Infect. Dis.* 135:103–107.
- Nussbaum, J. J., D. S. Hull, and M. J. Carter. 1980. *Pseudomonas pseudomallei* in an anophthalmic orbit. *Arch. Ophthalmol.* 98:1224–1225.
- Pearson, T., P. Giffard, S. Beckstrom-Sternberg, R. Auerbach, H. Hornstra, A. Tuanyok, E. P. Price, M. B. Glass, B. Leadem, J. S. Beckstrom-Sternberg, G. J. Allan, J. T. Foster, D. M. Wagner, R. T. Okinaka, S. H. Sim, O. Pearson, Z. Wu, J. Chang, R. Kaul, A. R. Hoffmaster, T. S. Brettin, R. A. Robison, M. Mayo, J. E. Gee, P. Tan, B. J. Currie, and P. Keim. 2009. Phylogeographic reconstruction of a bacterial species with high levels of lateral gene transfer. *BMC Biol.* 7:78.
- Rolim, D. B., D. C. Vilar, A. Q. Sousa, I. S. Miralles, D. C. de Oliveira, G. Harnett, L. O'Reilly, K. Howard, I. Sampson, and T. J. Inglis. 2005. Melioidosis, northeastern Brazil. *Emerg. Infect. Dis.* 11:1458–1460.
- Rotz, L. D., A. S. Khan, S. R. Lillibridge, S. M. Ostroff, and J. M. Hughes. 2002. Public health assessment of potential biological terrorism agents. *Emerg. Infect. Dis.* 8:225–230.
- U'Ren, J. M., J. M. Schupp, T. Pearson, H. Hornstra, C. L. Friedman, K. L. Smith, R. R. Daugherty, S. D. Rhoton, B. Leadem, S. Georgia, M. Cardon, L. Y. Huynh, D. DeShazer, S. P. Harvey, R. Robison, D. Gal, M. J. Mayo, D. Wagner, B. J. Currie, and P. Keim. 2007. Tandem repeat regions within the *Burkholderia pseudomallei* genome and their application for high resolution genotyping. *BMC Microbiol.* 7:23.
- Ussery, D. W., K. Kil, K. Lagesen, T. Sicheritz-Ponten, J. Bohlin, and T. M. Wassenaar. 2009. The genus *Burkholderia*: analysis of 56 genomic sequences. *Genome Dyn.* 6:140–157.