Whole-Genome Assemblies of 56 Burkholderia Species

H. E. Daligault, a K. W. Davenport, a T. D. Minogue, b K. A. Bishop-Lilly, c,d S. M. Broomall, a D. C. Bruce, a P. S. Chain, a S. R. Coyne, b K. G. Frey, c,d H. S. Gibbons, a J. Jaisisse, a G. I. Koroleva, f J. T. Ladner, a C.-C. Lo, a C. Munk, a G. F. Palacios, f C. L. Redden, c,d C. N. Rosenzweig, a M. B. Scholz, f,h S. L. Johnson a

Los Alamos National Laboratory (LANL), Los Alamos, New Mexico, USAa; Diagnostic Systems Division (DSD), United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland, USAa; Naval Medical Research Center (NMRC)-Frederick, Fort Detrick, Maryland, USAa; Henry M. Jackson Foundation, Bethesda, Maryland, USAc; United States Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, Aberdeen, Maryland, USAa; Center for Genome Sciences (CGS), USAMRIID, Fort Detrick, Maryland, USAa

* Present address: M. B. Scholz, Michigan State University, East Lansing, Michigan, USA.

Burkholderia is a genus of betaproteobacteria that includes three notable human pathogens: B. cepacia, B. pseudomallei, and B. mallei. While B. pseudomallei and B. mallei are considered potential biowarfare agents, B. cepacia infections are largely limited to cystic fibrosis patients. Here, we present 56 Burkholderia genomes from 8 distinct species.

Received 22 September 2014 Accepted 10 October 2014 Published 20 November 2014

Citation Daligault HE, Davenport KW, Minogue TD, Bishop-Lilly KA, Broomall SM, Bruce DC, Chain PS, Coyne SR, Frey KG, Gibbons HS, Jaisisse J, Koroleva GI, Ladner JT, Lo C-C, Munk C, Palacios GF, Redden CL, Rosenzweig CN, Scholz MB, Johnson SL. 2014. Whole-genome assemblies of 56 Burkholderia species. Genome Announc. 2(6):e01106-14. doi: 10.1128/genomeA.01106-14.

Copyright © 2014 Daligault et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to S. L. Johnson, shannonj@lanl.gov.

Burkholderia is a diverse genus of Gram-negative aerobic bacilli that was previously considered part of the genus Pseudomonas (1). Of the 82 recognized Burkholderia species, three are notable human pathogens: B. pseudomallei, B. mallei, and B. cepacia (an opportunistic lung infection pathogen in cystic fibrosis patients).

B. pseudomallei, the causative agent of melioidosis, is an environmental bacterium commonly found in southeastern Asia and northern Australia, where it causes multiple annual outbreaks. B. mallei is an obligate pathogen that primarily causes disease in horses, mules, and donkeys (called engrailed) but is also the causative agent of glanders in humans, which can be either an acute or chronic infection (1). Both B. pseudomallei and B. mallei are on the CDC category B list due to their low infectious dose and high mortality rates, even with antibiotic therapy (2). While no evidence exists for the weaponization of B. pseudomallei, B. mallei has been developed as a weapon (2), and with their high transmission and mortality rates, both are considered potential biowarfare agents (3–5).

We sequenced the genomes of two Burkholderia cenocepacia, four B. cepacia, one Burkholderia gladioli, eight B. mallei, two Burkholderia multivorans, one Burkholderia oklahomensis, 34 B. pseudomallei, two Burkholderia thailandensis, one Burkholderia xenovorans, and one strain not identified to species level. These additions will increase the publicly available scaffolded and completed genomes by 11 to 100% for each species (46% overall species).

High-quality genomic DNA was extracted from 100-ml bacterial cultures of purified isolates for each strain using the Qiagen Genomic tip-500, per the manufacturer’s recommendations, with one minor variation. For biosafety level 3 (BSL3) Burkholderia organisms, all cultures were lysed overnight to ensure the sterility of the resulting extracted material. If sterility was not achieved, the nucleic acid was passed through a 0.45-μM filter and rechecked for viable organisms before removal from the BSL3 suite.

The sequence data for each draft genome include at least two data types: Illumina (6), 454 (7), and for some, PacBio (8) technologies. The draft genome coverages for each data type are included in the NCBI submission records; however, the Illumina (either unpaired or short-insert) coverages ranged from 114- to 1,067-fold, and the 454-based long-insert (insert size range, 5.2 to 12.7 kb) coverages were generally <50-fold. The combined draft data had genome coverages between 134- and 1,186-fold. The 454 and Illumina data were assembled together using Newbler and Velvet. All draft data were assembled together with PathFinder (9), and if the PacBio data were available and at ≥100X coverage, they were assembled using HGAP (10). The consensus sequences from all assemblers were computationally shredded and assembled with a subset of read pairs from the long-insert library using Phrap (11, 12). The resulting assemblies were manually and computationally improved using Consed (13) and in-house scripts.

The annotations were completed using the Ergatis workflow manager (14) and in-house scripts. The genomes are available in NCBI, and the raw data can be provided upon request. In-depth comparative analyses of these and other genomes will be published in subsequent reports.

Nucleotide sequence accession numbers. The genome accession numbers to public databases are listed in Table 1.

ACKNOWLEDGMENTS

Funding for this effort was provided by the Defense Threat Reduction Agency’s Joint Science and Technology Office (DTRA J9-CB/JSTO).

This paper is approved by the LANL for unlimited release (LA-UR-14-25908).

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or positions of the Department of the Navy, Department of Defense, or the U.S. Government.

REFERENCES

1. Asche V, Haase A. 2002. Burkholderia mallei and Burkholderia pseudomallei, p 961–972. In Sussman M (ed), Molecular medical microbiology; volumes 1–3.
2. National Institute of Allergy and Infectious Diseases (NIAID). 2003. NIAID biodefense research agenda for category B and C priority pathogens. National Institute of Allergy and Infectious Diseases, Bethesda, MD. http://www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/Documents/categorybandc.pdf.

3. Nierman WC, DeShazer D, Kim HS, Tettelin H, Nelson KE, Feldblum T, Ulrich RL, Ronning CM, Brinkac LM, Daughtery SC, Davidson TD, Deboy RT, Dimitrov G, Dodson RJ, Durkin AS, Gwinn ML, Haft DH, Khouri H, Kolonay JF, Madupu R, Mohammad Y, Nelson WC, Radune D, Romero CM, Sarria S, Selengut J, Shalmin C, Sullivan SA, White O, Yu Y, Zafar N, Zhou L, Fraser CM. 2004. Structural flexibility in the *Burkholderia mallei* genome. Proc. Natl. Acad. Sci. U. S. A. 101: 14246–14251. http://dx.doi.org/10.1073/pnas.0403061101.

4. Sharrer GT. 1995. The great glanders epidemic, 1861–1866: a civil war legacy. Agric. Hist. 69:79–97.

5. Christopher LW, Cieslak IJ, Pavlin JA, Itzen EM, Jr. 1997. Biological warfare: a historical perspective. JAMA 278:412–417. http://dx.doi.org/10.1001/jama.1997.03550050074036.

6. Bennett S. 2004. Solesa Ltd. Pharmacogenomics 5:433–438. http://dx.doi.org/10.1517/14622416.5.4.433.

7. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Izyk GP, Jando SC, Leeniker ML, Jarvie TP, Jirage KB, Kim J-F, Knight JR, Lanza JR, Lehman JH, Lefkowitz CM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McBride KE, McNamara MP, Myers EW, Nickerson E, Nobile JF, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380. http://dx.doi.org/10.1038/nature03959.

---

### TABLE 1 Accession numbers and basic assembly statistics for each assembled *Burkholderia* genome

| Strain          | Alternate ID | Source                  | Accession no. (no. of contigs) | Size (bp)/G+C (%) | No. of CDS |
|-----------------|--------------|-------------------------|--------------------------------|------------------|-----------|
| **Burkholderia congoensis** |              |                         |                                |                  |           |
| D7S 22E-1       | BHS          | Australia, aerosol, 2005| CP007782-CP007784              | 8,045,250/67.0    | 7,088     |
| DWS 37E-2       | BHT          | Australia, soil, 2007   | CP007779-CP007781              | 6,612,421/66.5    | 5,775     |

| **Burkholderia cepacia** |              |                         |                                |                  |           |
| ATCC 25416       | BGF          | United States, plant, 1948| CP007745-CP007747              | 8,567,011/66.6    | 7,739     |
| D7S 7H-2        | BHR          | Australia, aerosol, 2005| CP007785-CP007787              | 8,147,114/67.1    | 7,337     |
| DWS 16B-4       | BXH          | Australia, soil, 2007   | JPGA000000000 (4)              | 8,112,163/67.1    | 7,289     |

| **Burkholderia gladioli** |              |                         |                                |                  |           |
| ATCC 25417       | ICPB PM 2    | Plant                   | JPPG000000000 (18)             | 9,311,425/67.4    | 8,044     |

| **Burkholderia mallei** |              |                         |                                |                  |           |
| 092700E          | NCTC 10247   | Turkey, 1960            | CP007801 and CP007802          | 5,827,656/68.5    | 5,001     |
| ATCC 23344       | China 7, BFM | Burma, human, 1944      | CP008704 and CP008705          | 5,625,292/68.5    | 4,883     |
| BMK             | ATCC 15310   | Hungary, horse, 1961    | CP008731 and CP008732          | 5,872,022/68.5    | 5,069     |
| BMQ             | 106          | India, horse, 1936      | CP008722 and CP008723          | 5,630,231/68.5    | 4,892     |
| BMY             | 6            | Turkey, human, 1950     | CP008710 and CP008711          | 5,647,769/68.5    | 4,872     |
| BMZ             | ATCC 10399   | China, horse, 1956      | JPNX000000000 (3)             | 5,856,639/68.5    | 5,031     |
| FMH             | BGL          | Burma, human, 1944      | CP009197 to CP009198           | 5,835,541/68.5    | 5,026     |
| SR092700I       | BMP          | NA$^c$                  | JNLV000000000 (246)           | 5,675,037/68.5    | 5,236     |

| **Burkholderia multivorans** |              |                         |                                |                  |           |
| D5S 15A-1       | BHQ          | Australia, aerosol, 2005| CP008729 and CP008730          | 7,281,867/66.6    | 6,529     |
| DWS 42B-1       | BTH          | Thailand, soil, 2007    | JNLW000000000 (6)             | 6,505,001/67.3    | 5,773     |

| **Burkholderia oklahomensis** |              |                         |                                |                  |           |
| BDU             | E0147        | United States, human    | CP008726 and CP008727          | 7,313,673/66.9    | 6,312     |

| **Burkholderia pseudomallei** |              |                         |                                |                  |           |
| BDD             | DSTO T18     | Australia, human, 1996  | JNOW000000000 (80)            | 7,361,146/68.0    | 6,206     |
| BDE             | DSTO T21     | Australia, human, 1997  | JNPW000000000 (118)           | 7,253,846/68.1    | 6,144     |
| BDI             | DSTO T27     | Australia, human, 1998  | JNPJ000000000 (76)            | 7,268,791/68.1    | 6,106     |

(Continued on following page)
| Strain     | Alternate ID  | Source             | Accession no. (no. of contigs) | Size (bp)/G+C (%) | No. of CDS
|------------|---------------|--------------------|-------------------------------|-------------------|-------------
| BDM        | DSTO T30      | Australia, human, 1998 | JPNV00000000 (102)           | 7,495,075/67.9    | 6,411       |
| BDT        | DSTO T43      | Australia, human, 1999 | JOT500000000 (33)            | 7,358,678/67.9    | 6,143       |
| BDZ        | DSTO T43      | Australia, human, 1996 | JPN000000000 (397)           | 7,296,307/68.0    | 6,519       |
| BEB        | DSTO T82      | Australia, human, 2000 | JPNP000000000 (142)          | 7,310,901/68.1    | 6,367       |
| BCD        | DSTO T87      | Australia, human, 2000 | JOTX000000000 (248)          | 7,533,026/68.0    | 6,797       |
| BED        | DSTO T2       | Australia, human, 1996 | JPNO000000000 (254)          | 7,244,575/68.0    | 6,418       |
| BEF        | DSTO T14      | Australia, human, 1996 | JPNR000000000 (232)          | 7,297,941/68.0    | 6,473       |
| BEG        | DSTO T17      | Australia, human, 1996 | JOTY000000000 (245)          | 7,445,118/67.9    | 6,604       |
| BEH        | DSTO T106     | Australia, human, 2001 | JOTZ000000000 (187)          | 7,362,104/67.9    | 6,413       |
| BEJ        | PHLS 112      | Thailand            | JPN000000000 (4)             | 7,198,519/68.2    | 5,884       |
| BEK        | 9             | Pakistan            | CP008754 and CP008755        | 7,228,737/68.1    | 5,978       |
| BEM        | Pasteur 52237 | Vietnam             | JPNT000000000 (9)            | 7,358,604/68.0    | 6,090       |
| BEO        | 1106a         | Thailand, human, 1993 | CP008758 to CP008759         | 7,086,433/68.3    | 5,758       |
| BES        | DSTO T75      | Australia, human, 2000 | JPHA0000000 (305)            | 7,720,797/67.6    | 7,070       |
| BEX        | MSHR576A      | Thailand            | CP008777 and CP008778        | 7,266,604/68.0    | 5,944       |
| BEZ        | MSHR1655      | NA                  | CP008779 and CP008780        | 7,027,950/68.0    | 5,798       |
| BFB        | MSHR346       | NA                  | CP008763 and CP008764        | 7,354,216/67.9    | 6,044       |
| BFD        | DSTO T9       | Australia, human, 1996 | JOTT000000000 (67)          | 7,343,224/67.9    | 6,201       |
| BGF        | DSTO T88      | Australia, human, 2000 | JOTU000000000 (15)          | 7,506,190/67.9    | 6,269       |
| BGQ        | 576a          | Thailand            | JOTV000000000 (79)          | 7,245,828/67.9    | 6,128       |
| BGR        | 1026b         | NA                  | CP008834 and CP008835        | 7,231,385/68.2    | 5,960       |
| BGS        | 1106b         | Thailand            | JOTW000000000 (52)          | 7,077,890/68.2    | 5,853       |
| BGV        | DSTO T6       | Australia, human, 1996 | JPHB000000000 (23)          | 7,451,876/67.9    | 6,204       |
| BSR        | 406e          | NA                  | CP009127 and CP009128        | 7,272,702/68.1    | 5,941       |
| HBPUB10134a| BHN           | Thailand, human, 2010 | CP008911 and CP008912        | 7,218,403/68.1    | 5,858       |
| HBPUB10303a| BHO           | Thailand, human, 2011 | CP008893 and CP008894        | 7,178,167/68.2    | 5,834       |
| Mahidol-1106a| BGI          | Thailand            | CP008781 and CP008782        | 7,085,397/68.3    | 5,748       |
| MSHR305    | BDP           | Human, 1994         | CP009029 and CP009210        | 7,442,161/67.9    | 6,144       |
| MSHR5848   | BHL           | Australia, human, 2011 | CP008909 and CP008910        | 7,290,434/68.1    | 5,989       |
| MSHR5855   | BHK           | Australia, human, 2011 | CP008783 and CP008784        | 7,297,804/68.0    | 6,001       |
| MSHR5858   | BHM           | Australia, human, 2011 | CP008891 and CP008892        | 7,070,528/68.3    | 5,781       |

**Burkholderia thailandensis**

| Strain     | Accession no. (no. of contigs) | Size (bp)/G+C (%) | No. of CDS
|------------|-------------------------------|-------------------|-------------
| BDK        | 2003015869                    | 6,728,580/67.7    | 5,709       |
| BLY        | E264                          | 6,722,099/67.6    | 5,655       |

**Burkholderia xenovorans**

| Strain     | Accession no. (no. of contigs) | Size (bp)/G+C (%) | No. of CDS
|------------|-------------------------------|-------------------|-------------
| BXA        | LB400, BXA                    | 9,702,951/62.6    | 8,684       |

**Burkholderia sp.**

| Strain     | Accession no. (no. of contigs) | Size (bp)/G+C (%) | No. of CDS
|------------|-------------------------------|-------------------|-------------
| BGI        | 1710a                         | 5,472,690/67.9    | 5,983       |
| BGK        | 1710b                         | 7,304,000/68.0    | 5,962       |

---

a alternate ID, b source accession no. (no. of contigs), c size (bp)/G+C (%)