Supporting Information

Chantratita et al. 10.1073/pnas.1111020108

SI Text

Culture and Microscopy of Clinical Isolates. The six initial and variant strain pairs of *B. pseudomallei* were frozen at −80 °C in tryptone soya broth (TSB) with 15% glycerol at the time of isolation from diagnostic samples, where they remained until used in this study. These samples were isolated from patients presenting to a single hospital in northeast Thailand in the years 2007 (two cases), 2006, 1993, 1990, and 1988 (one case each). Identification of the six primary *B. pseudomallei* isolates from admission cultures was performed as described previously (1), together with a highly specific latex agglutination test based on a monoclonal antibody to exopolysaccharide (2), because the growth defect precluded the use of standard methodology that depends on bacterial growth. In brief, bacterial cells picked from a colony on ASH were emulsified in 30 μL of latex suspension on a glass slide, which was rocked gently for 1 min and observed for evidence of agglutination. This was compared with a positive and negative control using *B. pseudomallei* K96243 and *B. thailandensis* E264, respectively.

Growth of the six strain pairs on routine laboratory agar or in liquid culture was tested as follows. Growth of the 12 strains on ASH was confirmed by streaking frozen stock onto an ASH plate, incubating at 37 °C in air and examining daily for 7 d. Gram stain and light microscopy was performed on day 2 colonies. Several colonies were picked from day 2 ASH plates to make a suspension of *B. pseudomallei* in sterile normal saline. This was streak inoculated onto one agar plate per strain for each of the following: tryptone soya agar, Columbia agar, *B. cepacia* agar, Mueller Hinton agar, blood agar (all from Oxoid, Ltd.), and LB agar (Becton Dickinson). All plates were incubated at 37 °C in air and examined daily for 7 d for the presence of visible colonies. The bacterial suspension was also used to inoculate tryptone soya broth, Mueller Hinton broth, and BacT/Alert FA blood culture bottles (AB bioMèrieux) to achieve a final bacterial concentration of 1×10^5 cfu/mL. Liquid cultures were incubated by shaking at 200 rpm at 37 °C in air in a shaking incubator (New Brunswick Scientific Co. Inc), and examined daily for 7 d for evidence of cloudiness. A Gram stain was performed on the pellet of a 50-μL aliquot of each liquid culture at 24, 48, and 72 h, and day 7. A 50-μL aliquot of each liquid culture was streak inoculated onto ASH at these time points, incubated at 37 °C in air, and examined daily for 7 d.

Real-time microscopy was performed on colonies picked from ASH after incubating at 37 °C in air for 48 h. Bacteria were suspended in sterile saline and 10 μL placed onto a glass slide (75 × 25 mm) and covered with a glass coverslip (22 × 22 mm). The samples were observed and images recorded using a real-time microscope (RTM-3; Richardson Technologies, Inc.) at 1,000× magnification.

Bacterial Growth Curves. Bacterial growth was monitored using a Synergy HT Multi-Mode Microplate Reader (BioTek). Briefly, 100 μL of overnight culture grown in LB + 4% glycerol was washed once in 1 mL saline and resuspended in 50 μL saline (glycerol was essential for growth of ΔBPSS1219 mutants.). A 5-μL aliquot of this cell suspension was added to 195 μL of LB medium in each well of a 96-well flat-bottom plate. Plates were incubated at 37 °C with constant shaking at 200 rpm, and the optical density at 600 nm was read every 30 min for 48 h.

In Vitro Induction and Reversion of Bacterial Filamentation. The six primary ceftazidime-susceptible *B. pseudomallei* isolates from study patients (415a, 699c, 1142a, 3351c, 4236a, and 4241a) were tested for in vitro induction of filamentation following exposure to ceftazidime. A pilot experiment was performed to confirm the presence of filament formation after exposure to ceftazidime at a concentration around the MIC for each of the six initial isolates. 5×10^4 cfu of a log-phase culture was mixed with ceftazidime at a final concentration of 1, 2, or 4 μg/mL in a total volume of 100 μL within a microtiter plate well. This mixture was incubated static at 37 °C in air for 16 h, after which the bacteria were spun and the pellet examined by Gram stain and light microscopy. This procedure confirmed the presence of bacterial filaments at all three ceftazidime concentrations, and 2 μg/mL was used for the remaining experiments. For each of the six initial isolates, two 10-mL log-phase cultures of *B. pseudomallei* in MHB were grown to ∼1×10^6 cfu/mL. One culture was exposed to a final ceftazidime concentration of 2 μg/mL, and the second (control) was sham treated with the same volume of diluent but no drug. Cultures were incubated shaking at 37 °C in air for 16 h, after which 2 mL was removed, spun, and the bacterial pellet examined by Gram stain and light microscopy. The remaining culture was washed three times in sterile normal saline, resuspended in antibiotic-free MHB to a concentration of 1×10^6 cfu/mL, and an aliquot removed for MIC determination using the standard E test on MHA. The remaining washed culture was incubated shaking at 37 °C in air for 24 h, after which Gram stain and MICs were performed as before.

High-Resolution Comparative Genomic Hybridization Array (aCGH). For probe preparation, genomic DNAs from parental and variant *B. pseudomallei* strains were sonicated (3× of 30% amplitude for 40 s with 1-min interval each, on ice) to generate DNA fragments of 100–500 bp. Fragmented samples were labeled with a Kreatech BAC array labeling kit using 2 μg of DNA per array. Hybridizations were performed at 50 °C for 18 h using the MAUI hybridization system (BioMicro Systems, Inc.), then washed with 0.2× SSC/0.2% SDS at 65 °C for 1 min, followed by a half minute each in nonstringent wash buffer (6× SSPE, 0.01% Tween-20), then in 0.2× SSC, 0.05× SSC, and in milliQ water at room temperature. Slides were dried and then scanned on an Axon 4000B scanner (Molecular Devices). Signal intensity output files were generated by NimbleScan version 2.2 (NimbleGen) and normalized using the NimbleScan RMA function.

Sequence Analysis of the BPSS0946 *penA* Gene in Initial and Variant *B. pseudomallei* Strains. PCR amplifications were performed using a MJ Research DNA engine Dyad Peltier thermocycler (Bio-Rad Laboratories) in a total reaction volume of 100 μL [50 ng of genomic DNA, 10 μL of 10× PCR buffer (Invitrogen), 2 mM $MgCl_2$, 10% (vol/vol) DMSO, 200 μM dNTPs, 200 pmol of each primer, and 5 U of Platinum Taq DNA polymerase (Invitrogen)]. Cycling was conducted under the following parameters: denaturation at 94 °C for 4 min, followed by 34 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and a final step of 72 °C for 10 min. PCR products were sequenced by a commercial vendor (1st BASE), and sequences were aligned and analyzed using Vector NTI suite 10 software (Invitrogen). Primers were as follows: primer 90L (For): ATTCGCACGCACTCCTGT (position: 110 upstream of BPSS0946 *penA*); Primer 1256R (Rev): GTGAGCACGCGGATGAGC (position: +168 nt downstream of BPSS0946 *penA*). Though there were genetic differences between some of the strains and the BpK96243 reference sequence, these alterations were seen in both the initial and variant

strains and are unlikely to explain the ceftazidime resistance of the variant strains.

Bioinformatic Analysis. *B. pseudomallei* K96243 was used as a reference sequence (accession nos. BX571965 and BX571966). The deleted regions were mapped and visualized using Artemis software (3). The annotation was curated using updated data, as described previously for the original annotation (4). Comparison of the genome sequences was facilitated using the Artemis Comparison Tool (ACT) (5), which enabled the visualization of BLASTN and TBLASTX comparisons (6) between the genomes. Orthologous proteins were identified as reciprocal best matches using FASTA (7) with subsequent manual curation. Pairwise Needleman-Wunsch (8) global alignments of proteins were performed using needle program from the EMBOSS suite of tools (9).

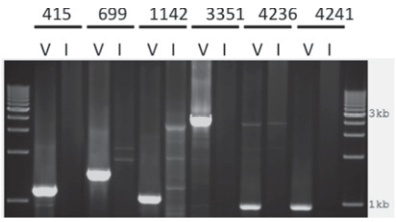
Bacterial Mutagenesis. ***Culture Media.*** LB Lennox (LB; MO BIO Laboratories) broth or agar was used for routine growth of all *E. coli* or *B. pseudomallei* strains during genetic construct experiments. This medium was supplemented with 400 μg/mL diaminopimelic acid (DAP; LL-, DD-, and meso-isomers; Sigma) for *E. coli* RHO3 (10). MHB and MHA were prepared according to manufacturer's specifications (BD). YT agar [10 g/L yeast extract (Difco) and 10 g/L tryptone (Fisher Scientific)] with 15% sucrose was used for *sacB*-mediated *B. pseudomallei* merodiploid resolution. ASH consisted of 10 g/L tryptone soya broth, 15 g/L agar, 5 mg/L crystal violet, 50 mg/L neutral red, 4% glycerol, and 4 mg/L gentamicin (11). Antibiotics used with the *E. coli* cloning DH5α (12) and mobilizer strain RHO3 (10) were 100 μg/mL ampicillin (Ap), 15 μg/mL zeocin (Zeo), and 35 μg/mL kanamycin (Km). For *B. pseudomallei*, we used 1,000 μg/mL Km or 2,000 μg/mL Zeo. Antibiotics were purchased from Sigma (Ap, Km) and Invitrogen (Zeo). For screening of pEXKm5-carrying *B. pseudomallei* merodiploids, media were supplemented with 50 μg/mL 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (Xgluc; Gold Biotechnology). Where required, media were supplemented with 4% (vol/vol) glycerol. Strains were routinely grown at 37 °C, except for manipulations such as merodiploid resolution on YT-sucrose medium and propagation of cells harboring temperature-sensitive plasmids, when bacteria were grown at 30 °C.

Construction and Integration of a BPSS1219 Expression Construct. The BPSS1219 gene was cloned into the mini-Tn*7* delivery vector pPS2736. This vector was derived in two steps. First, pPS2735 was created by replacing the 16-bp SmaI-SacI fragment from the MCS of pUC18T-mini-Tn*7*T-Km-*FRT* with a *s12* promoter-containing fragment, which was obtained by linker tailing using primers S12-UP and 2154. Second, pPS2735 was digested with SfiI and EcoRI, and the intervening sequences were replaced with a 147-bp SfiI-MfeI fragment from pUC57-*loxP*-MCS, which introduces a MCS (SacI-NruI-AgeI-KpnI-SacII-XhoI-EcoRI-ApoI-NotI-SmaI-HindIII) flanked by Cre recombinase target (*loxP*) sites. Next, primers 2027 and 2028 and *Pfu* polymerase were used to PCR amplify a 2,149-bp fragment containing BPSS1219 from 1026b (13) chromosomal DNA. The PCR fragment was adenylated with Taq polymerase, gel purified, and ligated into pCR2.1 (Invitrogen) to yield pPS2619. The 2,165-bp EcoRI fragment from pPS2619 was subsequently ligated into pPS2736 to create pPS2752. In this construct, expression of BPSS1219 is driven by the *B. thailandensis s12* ribosomal promoter. The mini-Tn*7* element contained on pPS2752 was inserted into the *B. pseudomallei* genome either by coelectroporation (1026b) or coconjugation (other clinical isolates) with helper plasmid pTNS3 (14) and mobilizer strain RHO3 (10). In coelectroporation experiments, 100 ng each of pPS2752 and pTNS3 DNA were electroporated into electrocompetent 1026b cells using previously described procedures (14). Transformation mixtures were plated on LB medium with 1,000 μg/mL Km and 4% glycerol (LBKG), and plates were incubated at 37 °C. For coconjugation experiments, 100 μL of the respective *B. pseudomallei* recipient strain (415a, 415e, 699c, 699d, 1142a, or 1142b), 100 μL of RHO3/pTNS3, and 100 μL of RHO3/pPS2752 were mixed and processed as previously described (10). After an overnight conjugation, conjugation mixtures were plated on LBKG plates, and plates were incubated at 37 °C. Colonies were purified and checked for Tn*7* insertion at the *glmS*-associated attachment sites using primer sets of Tn*7*L and BPGLMS1, BPGLMS2, or BPGLMS3, as previously described (14). For consistency, we used strains with a single mini-Tn*7* insertion at the *glmS2*-associated Tn*7* attachment site. An exception was strain Bp562, which contains two mini-Tn*7* insertions, one at *glmS2* and the second at *glmS3*.

Deletion of BPSS1239 and BPSS1240. Using the ceftazidime-susceptible strain K96243 (4) chromosomal DNA and Taq polymerase, PCR fragments were amplified and used for engineering gene deletion constructs as follows. For BPSS1239, its coding sequence was amplified using primers 1879 and 1882. The resulting 1,381-bp DNA fragment was gel purified and ligated into pCR2.1 to create pPS2568. A 1,397-bp EcoRI fragment from pPS2568 was then cloned into the EcoRI site of pEXKm5 to form pPS2569. Finally, pPS2569 DNA was digested with DraIII to delete 279 bp of the BPSS1239 coding sequence to create pPS2584. For BPSS1240, a 1,573-bp PCR amplicon obtained with primers 1884 and 1887 was ligated into pCR2.1 to create pPS2586. DNA of this plasmid was digested with HincII, which deleted 293 bp from the BPSS1240 coding sequence, creating pPS2587. A 1,296-bp EcoRI fragment from pPS2587 was then ligated with EcoRI-digested pEXKm5 to yield pPS2589. After transformation of pPS2584 (BPSS1239) and pPS2589 (BPSS1240) into *E. coli* mobilizer strain RHO3, the respective plasmid-borne deletions were transferred to the 1026b genome using a previously described method (10). Using this method, only ΔBPSS1239 (Bp276) and ΔBPSS1240 (Bp307) mutants could be obtained. The presence of the correct deletions in strains Bp276 and Bp307 was checked by colony PCR using primers 1986 and 1993 (BPSS1239) or 1987 and 1988 (BPSS1240). The ΔBPSS1239 ΔBPSS1240 double-mutant Bp308 was obtained by transferring the BPSS1240 deletion allele contained on pPS2589 to the genome of Bp276.

Deletion of BPSS1219. Because BPSS1219 could not be deleted from 1026b using the above-described conventional gene replacement procedure, a rescue copy of BPSS1219 expressed from *Ps12* was introduced into chromosome 1 of 1026b, and pFLPe2 (14) was used to remove the Km^r marker from this strain to create the unmarked Bp483. A 656-bp PCR fragment where the entire 1,785-bp BPSS1219 coding region plus 93 bp of the upstream and 277 bp of the downstream intergenic regions were deleted was generated using splicing-by-overlap-extension (SOEing) PCR, which assembled two fragments of 351 bp (primer sets 2268 and 2279) and 326 bp (primer sets 2265 and 2280). This fragment was cloned into pGEM-T Easy (Promega), and the ΔBPSS1219-containing region was excised on a 676-bp EcoRI fragment that was cloned into the gene replacement vector pEXKm5 to form pPS2828. This plasmid was conjugally transferred to Bp483, and merodiploids selected on LB medium with 1,000 μg/mL Km and 50 μg/mL XGluc, which were then resolved on YT-sucrose-XGluc medium as previously described (10). White colonies were checked for the presence of the correct ΔcBPSS1219 allele using primers 2265 and 1979 and presence of the intact Tn*7*-harbored copy of BPSS1219 using primers 2058 and 2205. Next, the Cre recombinase expression plasmid pCRE5 (14) was transformed into one retained Bp483 ΔcBPSS1219 mutant (Bp561) and plated on LB medium with 1,000 μg/mL Km and 0.2% rhamnose at 30 °C. Small colonies were picked and purified on the same medium at 30 °C. Excision of the *loxP*::BPSS1219 cassette was then verified using primers 2058 and 2205. Finally, mutants were grown at 37 °C on LB plates supplemented with 4% glycerol to cure pCRE5, which resulted in strain Bp560.

1. Dance DA, Wuthiekanun V, Naigowit P, White NJ (1989) Identification of *Pseudomonas pseudomallei* in clinical practice: Use of simple screening tests and API 20NE. *J Clin Pathol* 42:645–648.
2. Wuthiekanun V, Anuntagool N, White NJ, Sirisinha S (2002) Short report: A rapid method for the differentiation of *Burkholderia pseudomallei* and *Burkholderia thailandensis*. *Am J Trop Med Hyg* 66:759–761.
3. Rutherford K, et al. (2000) Artemis: Sequence visualization and annotation. *Bioinformatics* 16:944–945.
4. Holden MT, et al. (2004) Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei*. *Proc Natl Acad Sci USA* 101:14240–14245.
5. Carver TJ, et al. (2005) ACT: The Artemis Comparison Tool. *Bioinformatics* 21: 3422–3423.
6. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410.
7. Pearson WR, Lipman DJ (1988) Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA* 85:2444–2448.
8. Needleman SB, Wunsch CD (1970) A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J Mol Biol* 48: 443–453.
9. Rice P, Longden I, Bleasby A (2000) EMBOSS: The European Molecular Biology Open Software Suite. *Trends Genet* 16:276–277.
10. López CM, Rholl DA, Trunck LA, Schweizer HP (2009) Versatile dual-technology system for markerless allele replacement in *Burkholderia pseudomallei*. *Appl Environ Microbiol* 75:6496–6503.
11. Ashdown LR (1979) An improved screening technique for isolation of *Pseudomonas pseudomallei* from clinical specimens. *Pathology* 11:293–297.
12. Liss L (1987) New M13 host: DH5αF′ competent cells. *Focus* 9:13.
13. DeShazer D, Brett PJ, Carlyon R, Woods DE (1997) Mutagenesis of *Burkholderia pseudomallei* with Tn*5*-OT182: Isolation of motility mutants and molecular characterization of the flagellin structural gene. *J Bacteriol* 179:2116–2125.
14. Choi KH, et al. (2008) Genetic tools for select-agent-compliant manipulation of *Burkholderia pseudomallei*. *Appl Environ Microbiol* 74:1064–1075.



Strain	Region of genomic deletion		Size of genomic deletion (kb)
	aCGH estimate	Actual loss defined by PCR and sequencing	
415	1466086…1716130	1465823…1716237	250.4 kb
699	1531183…1840758	1531113…1840924	309.8 kb
1142	1577881…1850326	1577786…1850350	272.6 kb
3351	1477390…1746859	1476713…1747554	270.8 kb
4236	1499023…1716931	1498895…1716991	218.1 kb
4241	1644136…1789203	1644220…1789681	145.5 kb

Fig. S1. PCR confirmation of flanking breakpoint and summary table confirming regions of deletion in variant *B. pseudomallei* strains. PCR primers were designed to amplify across the predicted breakpoints in each variant strain. Successful PCR products of the expected sizes were obtained in all variant strains (V) but not initial/parental (I) strains. Lanes 1 and 14: 1-kb molecular weight ladder. Strains are labeled as variant/initial in the following order: 415, 699, 1142, 3351, 4236, and 4241, respectively. PCR products were directly sequenced to confirm the specific region of deletion.

Table S1. Minimal common region of deletion comprising 49 genes in six ceftazidime-resistant *B. pseudomallei* strains

Gene	Putative function
BPSS1217	Conserved hypothetical protein
BPSS1218	Putative methyl-accepting chemotaxis protein
BPSS1219	Putative penicillin-binding protein
BPSS1220	Putative L-arabinose-binding periplasmic protein precursor
BPSS1221	Putative short-chain dehydrogenase/oxidoreductase
BPSS1222	Putative cationic exchange protein
BPSS1223	Putative metal-dependent hydrolase
BPSS1224	Exported protein
BPSS1225	Membrane protein
BPSS1226	Hypothetical protein
BPSS1227	Putative 3-demethylubiquinone-9 3-methyltransferase
BPSS1228	Cora-like Mg^{2+} transporter protein
BPSS1229	Deor family regulatory protein
BPSS1230	Major Facilitator Superfamily protein
BPSS1231	Exported protein
BPSS1232	ABC transporter periplasmic binding protein
BPSS1233	ABC transporter permease protein
BPSS1234	ABC transporter permease protein
BPSS1235	Putative zinc-binding xylitol/sorbitol dehydrogenase
BPSS1236	Sugar transporter ATP-binding protein
BPSS1237	AraC family transcriptional activator protein
BPSS1238	Putative xylulokinase
BPSS1239	Putative penicillin-binding protein
BPSS1240	Putative penicillin-binding protein
BPSS1241	Putative assimilatory nitrate reductase
BPSS1242	Putative nitrite reductase [NAD(P)H], small subunit
BPSS1243	Putative nitrite reductase [NAD(P)H], large subunit
BPSS1244	Putative nitrate transporter
BPSS1245	Putative uroporphyrin-III c-methyltransferase
BPSS1246	Putative response regulator transcriptional regulator protein
BPSS1247	Putative nitrate transporter component
BPSS1248	GTP cyclohydrolase I
BPSS1249	Lipoprotein
BPSS1250	Putative acetylpolyamine aminohydrolase
BPSS1251	Putative *N*-carbamoyl-L-amino acid aminohydrolase
BPSS1252	Major Facilitator Superfamily protein
BPSS1253	LysR family transcriptional regulator
BPSS1254	Hypothetical protein (pseudogene)
BPSS1255	LysR family transcriptional regulator
BPSS1256	Short-chain dehydrogenase
BPSS1257	Putative CoA transferase
BPSS1258	Putative zinc-binding dehydrogenase
BPSS1259	Putative iron-containing alcohol dehydrogenase
BPSS1260	Exported metallopeptidase
BPSS1261	Conserved hypothetical protein
BPSS1262	Exported protein
BPSS1263	Membrane protein
BPSS1264	OmpA family membrane protein
BPSS1265	Hypothetical protein

Table S2. Primers, bacterial strains and plasmids used in this study

Primer, strain, or plasmid name	Relevant properties	Source
Genetic constructs for BPSS1219 deletion and expression; verification of Tn*7* insertions		
S12-UP	5′-GAGCTG*TTGACT*CGCTTGGGATTTTCG *GAATATCAT*GCCGGGTGGGCC*	(1)
2154	5′-GGCCCACCCGGC*ATGATATT*CCGAAA ATCCCAAGCG*AGTCAA*CAGCTCAGCT*	This study
1879	5′-CGGCGATTTGCGCTTCG	This study
1882	5′-CGCGATCGGATCGCTGTC	This study
1884	5′-TGCTCGCGCTCCTCTGC	This study
1887	5′-ATGATCTCCGCCCATGCG	This study
2280	5′-**CATTCGTCAGATCTTTCAACG**ATATCGAA GCCCGCCGACGCGC[†]	This study
2279	5′-**CGTTGAAAGATCTGACGAATG**	This study
1986	5′-AGGCACGCATGTATCTGACG	This study
1993	5′-CAACATCGACAACGACAACGC	This study
1987	5′-CCTGTACCTGAAGCGCTGGAT	This study
1988	5′-GTATAGACGTCCGCGAGTTGC	This study
1979	5′-CGCTCGCCGTTTCACGCC	This study
2265	5′-GAGCGTGAAGTGCTTGTC	This study
2268	5′GTGGCGCGTAGAGTGACC	This study
Tn*7*L	5′-ATTAGCTTACGACGCTACACCC	(2)
BPGLMS1	5′-GAGGAGTGGGCGTCGATCAAC	(1)
BPGLMS2	5′-ACACGACGCAAGAGCGGAATC	(1)
BPGLMS3	5′-CGGACAGGTTCGCGCCATGC	(1)
2058	5′-CTGGGTGTAGCGTCGTAAGC	This study
2205	5′-GGGCTGCAGGAATTGATAAC	This study
2027	5′-CAGCCAATCCAGACGAAATC	This study
2028	5′-ATTGCGAGGGGTTCCTAAAG	This study
Confirmation of MCR deletion (1-kb region between *BPSS1245* and *BPSS1246*)		
Forward	5′-ATGAACACGATGGGCAAAGT	This study
Reverse	5′-GATCGGCTTGTCGGTGTC	This study
Confirmation of breakpoint regions in variant strains		
415-F	5′-GTATTCCGTCGCGGCAAG	This study
415-R	5′-CATCGAGCAGCAGGTGTG	This study
699-F	5′-AGTGCGAGGTGAACGAACA	This study
699-R	5′-AACGAAGGACGCATCACAC	This study
1142-F	5′-CGTATAGAGGCCGATCTTGC	This study
1142-R	5′-CCGCAGATGATGTAGTCGAA	This study
3351-F	5′-AGCCGCCCGAAGAAGAAC	This study
3351-R	5′-CGTCCGAAAGCAGTCACAG	This study
4236-F	5′-GCAGAACGAAGGCTACCG	This study
4236-R	5′-GACGAGCAGGTGAAAGTGC	This study
4241-F	5′-GACCCGCAAGTTTGTATTGG	This study
4241-R	5′-GCGCAGATCCTTCAGTTGAT	This study
BPSS0946/*penA* sequencing		
90L	5′-ATTCGCACGCACTCCTGT (−110 bp upstream of *BPSS0946*/*penA*)	This study
1256R	5′-GTGAGCACGCGGATGAGC (+168 bp downstream of *BPSS0946*/*penA*)	This study
Strain *B. pseudomallei*		
1026b	Clinical isolate and common laboratory strain	(3)
Bp276	1026b Δ*BPSS1239*	This study
Bp307	1026b Δ*BPSS1240*	This study
Bp308	1026b Δ*BPSS1239* Δ*BPSS1240*	This study
Bp478	Km^r; 415e::mini-Tn*7*T-*FRT-nptII-FRT-Ps12-loxP-BPSS1219-loxP* from pPS2752[‡]	This study
Bp480	Km^r; 699d::mini-Tn*7*T-*FRT-nptII-FRT-Ps12-loxP-BPSS1219-loxP* from pPS2752	This study
Bp483	1026b::mini-Tn*7*T-*FRT-Ps12-loxP-BPSS1219-loxP* from pPS2752	This study
Bp484	Km^r; 1142b::mini-Tn*7*T-*FRT-nptII-FRT-Ps12-loxP-BPSS1219-loxP* from pPS2752	This study
Bp504	Km^r; 415a::mini-Tn*7*T-*FRT-nptII-FRT-Ps12-loxP-BPSS1219-loxP* from pPS2752	This study

Table S2. Cont.

Primer, strain, or plasmid name	Relevant properties	Source
Bp506	Km^r; 699c::mini-Tn*7T-FRT-nptII-Ps12-loxP-BPSS1219-loxP* from pPS2752	This study
Bp508	Km^r; 1142a::mini-Tn*7T-FRT-nptII-FRT-Ps12-loxP-BPSS1219-loxP* from pPS2752	This study
Bp561	1026b::mini-Tn*7T-FRT-Ps12-loxP-BPSS1219-loxP* Δc*BPSS1219*[§]	This study
Bp560	1026b::mini-Tn*7T-FRT-Ps12-loxP*; Δc*BPSS1219*	This study
Bp562	Bp560::mini-Tn*7T-FRT-nptII-FRT-Ps12-loxP-BPSS1219-loxP*[¶]	This study
Plasmids		
pEXKm5	Km^r; gene replacement vector	(4)
pUC57-*loxP*-MCS	Ap^r; contains a multiple cloning site (MCS) flanked by *loxP* sites (synthesized by GenScript)	This study
pUC18T-mini-Tn*7T*-Km-*FRT*	Ap^r Km^r; mini-Tn*7* delivery vector	(1)
pPS2735	Ap^r Km^r; pUC18T-mini-Tn*7T*-Km-*FRT* with *Ps12*	This study
pPS2736	Ap^r Km^r; pUC18T-mini-Tn*7T*-Km-*FRT-Ps12-loxP*-MCS-*loxP*	This study
pPS2619	Ap^r Km^r; pCR2.1 (Invitrogen) with 2,149-bp *BPSS1219* PCR fragment	This study
pPS2752	Ap^r Km^r; pPS2736 with 2,165-bp EcoRI fragment from pPS2619	This study
pPS2568	Ap^r Km^r; pCR2.1 with 1,381-bp *BPSS1239* PCR fragment	This study
pPS2569	Km^r; pEXKm5 with 1,397-bp EcoRI fragment from pPS2568	This study
pPS2584	Km^r; pPS2569 with 279-bp DraIII fragment deleted from *BPSS1239*	This study
pPS2586	Ap^r Km^r; pCR2.1 with 1,573-bp *BPSS1240* PCR fragment	This study
pPS2587	Ap^r Km^r; pPS2586 with 293-bp HincII fragment deleted from *BPSS1240*	This study
pPS2589	Km^r; pEXKm5 with 1,296-bp EcoRI fragment from pPS2587	This study
pPS2828	Km^r; pEXKm5 with 676-bp EcoRI fragment Δ*BPSS1219*	This study

Ap, ampicillin; Km, kanamycin; *Ps12*, *B. thailandensis* ribosomal *s12* gene promoter; Zeo, zeocin.

*Italicized letters indicate *B. thailandensis* ribosomal *s12* gene promoter sequences.

[†]The underlined sequence represents an EcoRV restriction site, and the bold sequences signify overlap of primers 2279 and 2280 for SOEing PCR.

[‡]Unless otherwise stated, all mini-Tn*7* elements were integrated at the *glmS2*-associated Tn*7* attachment site.

[§]c*BPSS1219* denotes the chromosomal BPSS1219 gene.

[¶]The mini-Tn*7T-FRT-nptII-FRT-Ps12-loxP-BPSS1219-loxP* was integrated at the *glmS3*-associated Tn*7* attachment site.

1. Choi KH, et al. (2008) Genetic tools for select-agent-compliant manipulation of *Burkholderia pseudomallei*. *Appl Environ Microbiol* 74:1064–1075.
2. Choi KH, et al. (2005) A Tn*7*-based broad-range bacterial cloning and expression system. *Nat Methods* 2:443–448.
3. DeShazer D, Brett PJ, Carlyon R, Woods DE (1997) Mutagenesis of *Burkholderia pseudomallei* with Tn*5*-OT182: Isolation of motility mutants and molecular characterization of the flagellin structural gene. *J Bacteriol* 179:2116–2125.
4. López CM, Rholl DA, Trunck LA, Schweizer HP (2009) Versatile dual-technology system for markerless allele replacement in *Burkholderia pseudomallei*. *Appl Environ Microbiol* 75: 6496–6503.