

Distinct colicin M-like bacteriocin-immunity pairs in *Burkholderia*

Supplementary information

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Fig. S1. Multiple sequence alignment of BurM proteins. Abbreviations for species names: Bamb, *Burkholderia ambifaria*; Bcen, *Burkholderia cenocepacia*; Bcep, *Burkholderia cepacia*; Bcon, *Burkholderia contaminans*; Bglu, *Burkholderia glumae*; Bokl, *Burkholderia oklahomensis*; Bpyr, *Burkholderia pyrrocinia*; Bubo, *Burkholderia ubonensis*; Burk, *Burkholderia* sp. Different shading indicates the degree of conservation. Predicted Sec signal sequences are boxed in red and Tat signal sequences in orange, the conserved motif at the amino-terminus in purple (see Sequence alignment and logo in Fig. 2), the ColM domain in yellow, conserved residues in the catalytic domain in green. The aspartate residue that was mutated in BurM2 is marked with an asterisk.

Burmese Kingfisher (Burk A1) - MGNS ND NNKFK IR RSLIGAIA SAPALG LSGMSK S LA - IQPE ITLAFI IIV GSGNV I - TSSPADLPR - MGH PAL PP ELMT
 Bamb AMMD (BurM2) - MRKRGSS SDHVA EQ TORH KR KREMLVIAAS P YPILAKLQKOS ISLA - QTMVLEEL SVNA PRL - PSL - DGFS - NKVGGG GAMS EA
 Bcep GG4 - MRKSNSGL ERAAHAEQOTQRHKR REILVTG AVSPFLY ALQKRSICLA - QANVLEEL SVNA PRL - PSL - DGFS - NKVGGG GAMS EA
 Burk A9 - MRKSNSGL ERAAHAEQOTQRHKR REILVTG AVSPFLY ALQKRSICLA - QANVLEEL SVNA PRL - PSL - DGFS - NKVGGG GAMS EA
 Bubo Bu - MIKRD TLVSA NVMDNNDKROHQNRFL ASTALP FGS LKHATAMA - QNMVL P IITTPR P - PDFNFGDFK - INS NE IPGANI
 Bpyr CH-67 - MTRD SSAPENT ERIAQT RHRERRFL ASSTALP FGS LKHATAMA - QSNMVL P IITTSRP - PDFNFGDFG - VNING I PGANP G
 Bamb MC40-6 - MTRNP WKOTS PTS EGKQRLS LVRRLL ASTALP FGS LKHATAMA - QSNMVL P IITTSRP - PDFNFGDFT - IKANE I PGAN
 Bcep ATCC 25416 - MKRNPLKTNHAS EEKQOPLMRRLAAS TMLPF GLT LKHATAMA - QSNMVL P IITTSRP - PDFNFGDFG - IKVNEIPGANP G
 Bcon LMG 23361 - MTRQNP LKHVTRPS EENQOSLS MRRLAAS TMLPF GLT LKHATAMA - DINTAPL IIVNGQTNCSNFSNDCLQFP FGMDPLAQDLRV P IALP - LTD
 Blgu PG1 - MINFH EEKPN SRLST TRRAFLK NGSLP LLLI ALNPK SWG - APPDMC P IISVTTGPP - TDMY PGSGTLLGTGLPPSRIPS LAN
 Bamb MEX-5 (BurM1) - MIKKLSKYERVVDTNTRRLVLRASCAVPLL GLLSS SKS SLA - MKRHF LGA STAL - LASSP IRALA - QGHGPADLMEP ISVIA PTG - LSLP IA TP N - GPAGGY HWAING
 Bcep DWS 37UF10B-2 - MKRNFLGASTAF - LVSNPVRSLA - QGHGPADLMEP ISVIA PTG - LALPIA TP N - GPAGGY HWAING
 Bcen CEIB S5-1 - MKRNFLGASTAF - LVSNPVRSLA - QGHGPADLMEP ISVIA PTG - LALPIA TP N - GPAGGY HWAING
 Bcep DWS 16B-4 - MKRNFLGASTAF - LVSNPVRSLA - QGHGPADLMEP ISVIA PTG - LALPIA TP N - GPAGGY HWAING
 Bokl C6786 - MEEEEKFRHS IS NRDRVMH VS KRS LIR TSIAFP AAM LPSV IWA QTKGHGP A - DELEMISVTA PRE - TNLPVPSV G - GPVGGYSWTAG F
 Bokl EO147 - MEEEEKFRHS IS NRDRVMH VS KRS LIR TSIAFP AAM LPSV IWA QTKGHGP A - DELEMISVTA PRE - TNLPVPSV G - GPVGGYSWTAG F

Burmese Kingfisher (Burk A1) - SG TTP ILLLGKVYLINGNLI LGQA S QCDT IGT L TAL A QGL - VNAS DMVLR A QL G IGE GFKNIA GAT PYGLT - LNQS N I LTSI FGV YAAQ Y
 Bamb AMMD (BurM2) - LG YPK LLC FGRY - CSA TEMFNNADHG DMISVTE EFKFL - FEYRNE I FWRNOLI I LVGEET I WLARG - GYONFP GANSYLNVALQHG DVS I LFG I YANQLN
 Bcep GG4 - LG YPK LLC FGRY - CSA TEMFNNADHG DMISVTE EFKFL - FEYRNE I FWRNOLI I LVGEET I WLARG - GYONFP GANSYLNVALQHG DVS I LFG I YANQLN
 Burk A9 - LG YPK LLC FGRY - CSA TEMFNNADHG DMISVTE EFKFL - FEYRNE I FWRNOLI I LVGEET I WLARG - GYONFP GANSYLNVALQHG DVS I LFG I YANQLN
 Bubo Bu - GIPPPSVC FNQY - CASTNYMEL FANTGNMVRATEM FFRYLP I GRNDNTFWRNOLIVLVGGEET I WLARG - GYKLP GANOQYGFNFAPGH YANISALFG I YVQLD
 Bpyr CH-67 - LG YPK LLC FGRY - CSA TEI FNADYGDMMIATTE EFKFL - FESRNL FWRNOLIVLVGGEET I WLARG - GYKHP GANSYLNVALQHG DVS I LFG I YANQLN
 Bamb MC40-6 - GIPAP TVC FNQY - CTS NFMLH FANTGNMVRATEMELKHL LEI FGRNDNTFWRNOLIVLVGGEET I WLARG - GYKLP GANOQYGFNFEPGQFANLT I FG I YQTK
 Bcep ATCC 25416 - GMPAPSIC FNQY - CTS NFMLH FANTGNMVRATEMELKHL LEI FGRNDNTFWRNOLIVLVGGEET I WLARG - GYKLP GANOQYGFNFEPGQFANLT I FG I YQTK
 Bcon LMG 23361 - GIPAP TVC FNQY - CTS NFMLH FANTGNMVRATEMELKHL LEI FGRNDNTFWRNOLIVLVGGEET I WLARG - GYKLP GANOQYGFNFEPGQFANLT I FG I YQTK
 Blgu PG1 - GITAPL LLQVNLNWL VNGAKILLTAAMGDP IQLVLAFA EGL - I QARDGAIRSOAMIYGTET I WLARG - GYKTV DGDYPG YPLT - ASNLGNTLKL FGLY I GY TKW
 Bamb MEX-5 (BurM1) - NISGPVLRVGEI YLVNGKKILLEAIE RCGDS NGTILVE FA TGL - EHATESAVRA QAVYGLV T I WLATG - GYINOP GANOYA FT - RVGSMSTI FGV FSTWY F
 Bcep DWS 37UF10B-2 - NI RGG I FQIKFRLVNGKKILLEAIAAGRSRDVLS EFG YGM - ELASKQVVOQOIS TYALET S WIADM - SWOGIS GATOYGLS - DTF SGL LTIA FGL FSDYY F
 Bcen CEIB S5-1 - NI RGG I FQIKFRLVNGKKILLEAIAAGRSRDVLS EFG YGM - ELASKQVVOQOIS TYALET S WIADM - SWODIT GATOYGLS - DTF SGL LTIA FGL FSDYY F
 Bcep DWS 16B-4 - NI RGG I FQIKFRLVNGKKILLEAIAAGRSRDVLS EFG YGM - ELASKQVVOQOIS TYALET S WIADM - SWODIT GATOYGLS - DTF SGL LTIA FGL FSDYY F
 Bokl C6786 - NI RGG I FQIKFRLVNGKKILLEAIAAGRSRDVLS EFG YGM - ELASNNLVKAQTA TYGLE I WANN - SWOS IV GADQYGLS - NTQPSGL LTIA FGL FSDYY F
 Bokl EO147 - NI RGG I FQIKFNLVNGKKILLEAIAAGRSRDVLS EFG YGM - ELASNNLVKAQTA TYGLE I WANN - SWOS IV GADQYGLS - NTQPSGL LTIA FGL FSDYY F

Burmese Kingfisher (Burk A1) - GMFLQQPSIN FQ FYGTALLTTSIAI YWLYGDGS MRSMN I S MNL Q MG IADFD PI K RVALD L A N G P G T Y H D A Q F DT N L F SHG DKD I W V A G I L G R V T G R F G D L V
 Bamb AMMD (BurM2) - -- IR P VSE FQ FYGNPMFMLGAV Y Y W F GNG ERS IN L E S M N L R M S A D F K L I Q D S I D N P G Y G P G T Y S I D G P F S T N I F H N G A H D F W S A T T V G R V S G H V R G L T I M
 Bcep GG4 - -- IR PASEFQ FYGNPMFMLGAV Y Y W F GNG ERS IN L E S M N L R M G V S D F E L I R S S I D N P G Y G P G T Y P I D G P F S T N V F S H G A Q D F W S A T T V G R V S G H V R G L T I M
 Burk A9 - -- IR PTSEFQ FYGNPMFMLGAV Y Y W F GNG ERS IN L E S M N L R M G V S D F E L I R S S I D N P G Y G P G T Y P I D G P F S T N V F S H G A Q D F W S A T T V G R V S G H V R G L T I M
 Bubo Bu - -- TP PTSK F D FYGNPMFMLF LEAVNWH F GNG V Q R S I N L E S M N L R M N V S D F G E L R A J E N P G Y G P G T Y P I N S F T N H G O D F W S A T T V G R V S G H V R G L T I M
 Bpyr CH-67 - -- IR PISEFQ LYGS P FMF L GAV Y Y W F GNG ERS I N L E S M N L R M G V S D F E L I R S S I D N P G Y G P G T Y P I D G P F S T N V F S H G A Q D F W S A T T V G R V S G H V R G L T I M
 Bamb MC40-6 - -- LP PTSMFAS YGNPLM F V E A I N H W I H G D G F O R T V N I E S L N L K M S A T D F N E I A K A I E N P G Y G P G T Y S F D T A F S T N I F S H G T K D L W S A S T F G R V S G R I R G T L M
 Bcep ATCC 25416 - -- IP PTRE F AS YGNPLM F V E A I N H W I H G D G F O R T V N I E S L N L K M S A T D F N E I A K A I E N P G Y G P G T Y S F D T A F S T N I F S H G T K D L W S A S T F G R V S G R I R G T L M
 Bcon LMG 23361 - -- IP PTSF E S YGNPLM F V E A I N H W I H G D G F O R T V N I E S L N L K M G V A D F E L I T K A I E N P G Y G P G V Y S F D T A F S T N I F S H G T O D L W S A S T F G R V S G R I R G T L M
 Blgu PG1 - QG F Y L P V S E F K Y G S P F T L S S I Y W F G N G A T T I M N L N A M N L D V N I D E F G V P F E I G N P D M G P G T Y N I D A E F S F N L F N L A S N L T A L V N G R V S G H V E T L M
 Bamb MEX-5 (BurM1) - NLPSPRASE F A F Y G S P F M S I A Y Y W M D R D G S E RTMNIOSL A M S I L S D F A Q I N R A V E P A Y S H I CTY F D S E F S T N L F D H H T K D L W A G V I G R V S G R V T G I S L E
 Bcep DWS 37UF10B-2 - HLPAPPIES F K F Y A T P F T L S A Y Y N W I R G N G D P K S V D L K S L R L G I G A Q Q S I P R D I V N D A M P G T Y R I D A Q F S T N L L S I - D Q E L I V G S A L G R V S G H I Q L E F L
 Bcen CEIB S5-1 - HLPAPPIES F K F Y A T P F T L S A Y Y N W I R G N G D P K S V D L K S L R L G I G V Q O Q S I P R D I V N D A M P G T Y R I D A Q F S T N L L S I - D Q E L I V G S A L G R V S G H I Q L E F L
 Bcep DWS 16B-4 - HLPAPPIES F K F Y A T P F T L S A Y Y N W I R G N G D P K S V D L K S L R L G I G V Q O Q S I P R D I V N D A M P G T Y R I D A Q F S T N L L S I - D Q E L I V G S A L G R V S G H I Q L E F L
 Bokl C6786 - HLPPTPPISE F Q F Y A T P F T L A A Y D Y W I R G N G S P R A V D L K S L R L G I G A N E I G P I R S I V N D M G M G P G A Y P I D A E F S T N L L S I - D K E Y I V G S A L G R V S G H V G Q L V
 Bokl EO147 - HLPPTPPISE F Q F Y A T P F T L A A Y D Y W I R G N G S P R A V D L K S L R L G I G A N E I G P I R S I V N D M G M G P G A Y P I D A E F S T N L L S I - D K E Y I V G S A L G R V S G H V G Q L V

Burmese Kingfisher (Burk A1) - T T T Y C F N G W T L Y D R Y A Y P S T - R E G Y Q E V I N N E L M N L G A K S H N D Y D I T F I G N K V T I S G O R P N - K E Q A V S - P T G Q N T R P S G F G G L P N P V V I D
 Bamb AMMD (BurM2) - Q D N Y R P G V E T L Y N P K F A D S N - R P F L O E W M V L R E I G S M L G H A D Y O I Y T G E K V S G O R T I - Q N G Q T R - P P Q O A V R P S F G G L M P R P Q
 Bcep GG4 - E D N Y R P G V E T L Y N P D K F A D R S N - R P F L O E W M V L R E I G S T L G H T D Y Q I Y T G E K E I S F S G Q R P V - R N A E T R - P P Q O A V R P S F G G L M P R P L W
 Burk A9 - E D N Y R P G V E T L Y N P D K F A D R S N - R P F L O E A A I F L A K L G S V L G H T D Y Q I Y T G E K I S F S G Q R P V - R N A E I R - P P E A V R P S F G G L M P R P G F
 Bubo Bu - E D N Y R P G V E T L Y N P D K F A D R S N - R P F L O E W M V L R E I G S V L G H T D Y Q I Y E D G E K I S F S G Q R P V - R N A E T R - P P Q O A V R P S F G G L M P R P L R
 Bpyr CH-67 - E D G Y P D S Y S L N P D R F A D P S N - R P F L O E A A I F L A K L G A I L G H D Y Q I N E F E K G E K N L S F S G Q R P V - K N A D I R - P P Q O A V R P S F G G L M P R P
 Bamb MC40-6 - E D D Y P R F D S Y S L N P D R F A D P S N - R P F L O E A A I F L A K L G A I L G H D Y Q I N E F E K G E K N L S F S G Q R P V - K N A D I R - P P Q O A V R P S F G G L M P R P
 Bcep ATCC 25416 - E D D Y P R F D S Y S L N P D R F A D P S N - R P F L O E A A I F L A K L G A I L G H D Y Q I N E F E K G E K N L S F S G Q R P V - K N A D I R - P P Q O A V R P S F G G L M P R P
 Bcon LMG 23361 - G D E S Y A F N G E W T L N P D R Y A Y P S N - R T F O E V L I T S F L S R I G S L - G H V D Y D I L F T G S K E V M L N S G P R S - K F F A S - P G A - - R R S T G G I F N R N V N Y
 Blgu PG1 - D K G D Y F A S F T L N P D K F A D S R S R E S I O A L I T F V R K I G E F T D H K D F M I Y E T G S Q P L N I N G T R A S I K A T N P D G T P A A V H T A R P G G F G R P
 Bamb MEX-5 (BurM1) - S D D S F E R G E Y T L N P D K F A D S R S R E S I O A L I T F V R K I G E F T D H K D F M I Y E T G S Q P L N I N G T R A S I K A T N P D G T P A A V H T A R P G G F G R P
 Bcep DWS 37UF10B-2 - S D D S F E R G E Y T L N P D K F A D S R S R E S I O A L I T F V R K I G E F T D H K D F M I Y E T G S Q P L N I N G T R A S I K A T N P D G T P A A V H T A R P G G F G R P
 Bcen CEIB S5-1 - A D G G F S E C T L N P D K F A D S R S R E S I O A L I T F V R K I G E F T D H K D F M I Y E T G S Q P L N I S R N I K A M D E - G I V H R P S M G G L P H L G M R D I I N G N V S G L
 Bcep DWS 16B-4 - A D G G F S E C T L N P D K F A D S R S R E S I O A L I T F V R K I G E F T D H K D F M I Y E T G S Q P L N I S R N I K A M D E - G I V H R P S M G G L P H L G M R D I I N G N V S G L
 Bokl C6786 - Bokl EO147

2

Fig. S2. Purification of BurM proteins. SDS PAGE electrophoresis of purified BurM1 (lane 2), BurM2 (lane 3) and BurM2(D297A) (lane 4). Kaleidoscope ladder with size marks (kDa) is visible in line 1.

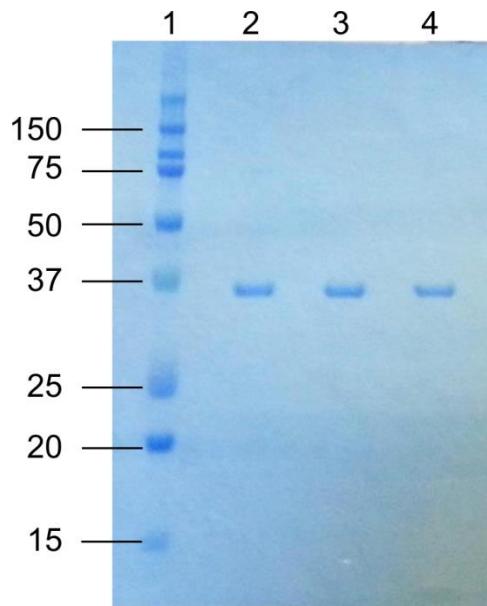
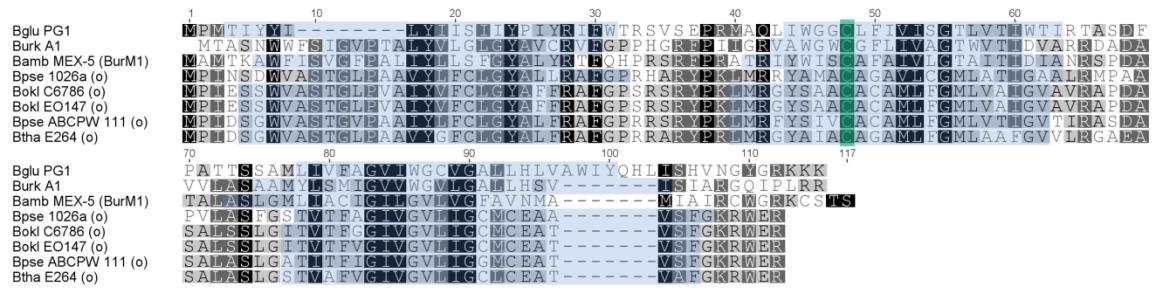


Fig. S3. Homology and phylogeny of BmiA proteins. Multiple sequence alignment (A) and unrooted maximum likelihood phylogenetic tree (B) of BmiA proteins. Encoded proteins without associated toxin (orphans) are marked with (o). Abbreviations for species names: Bpse, *Burkholderia pseudomallei*; Btha, *Burkholderia thailandensis*; other abbreviations as in Fig. S1. (A) Predicted transmembrane regions are boxed in blue and the conserved cysteine residue is colored green. Different grey shading indicates the degree of conservation. (B) Scale bar represents 0.3 substitutions per site. Bootstrap values (percentages of 1000 replicates) are shown at the branches.

A



B

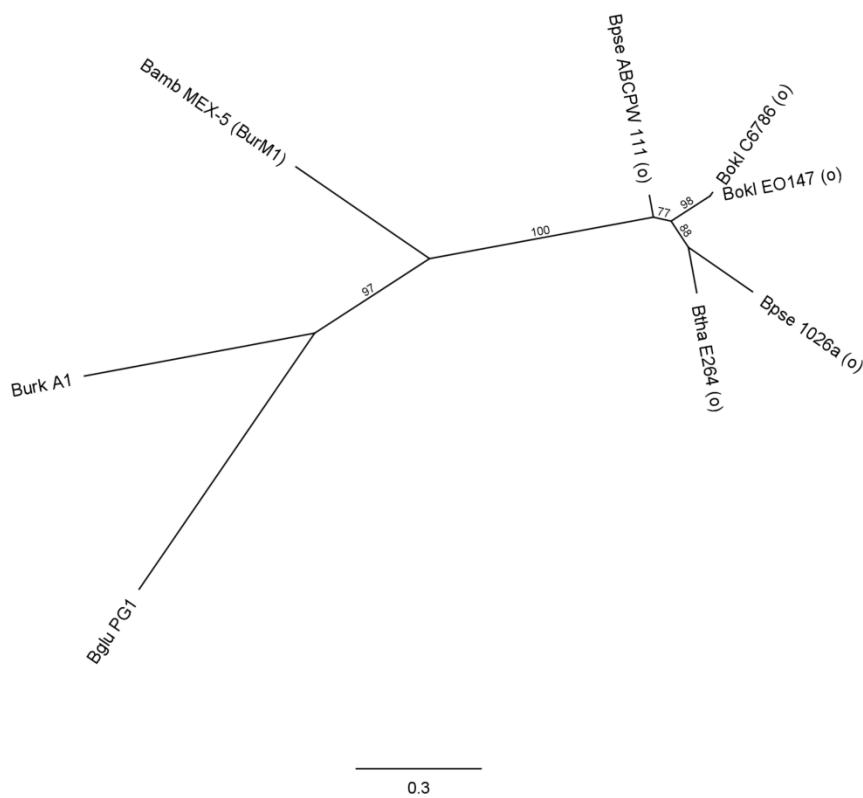
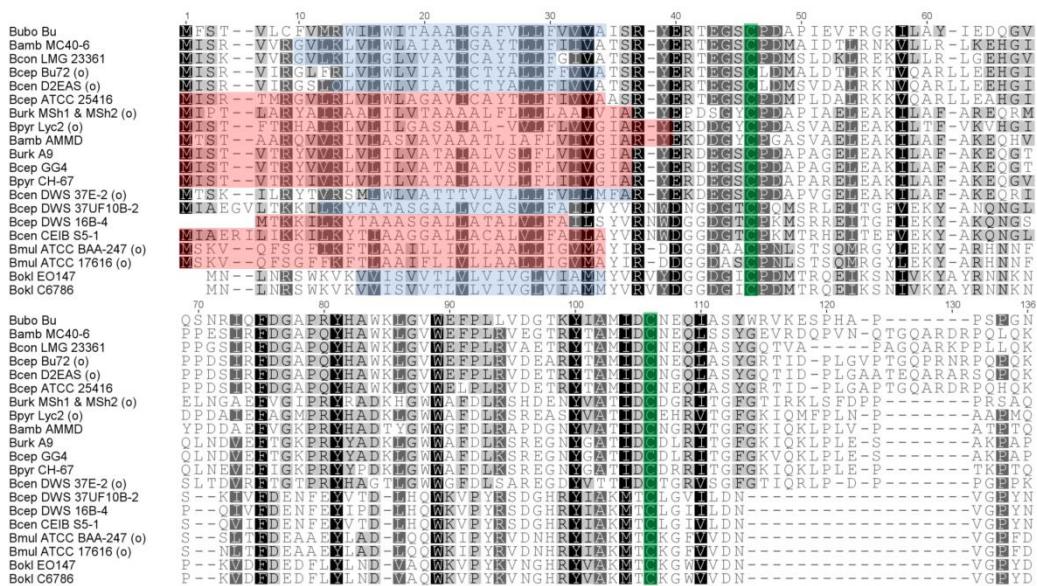


Fig. S4. Homology and phylogeny of BmiB proteins. Multiple sequence alignment (A) and unrooted maximum likelihood phylogenetic tree (B) of BmiB proteins. Encoded proteins without associated toxin (orphans) are marked with (o). Abbreviations for species names: Bmul, *Burkholderia multivorans*; other abbreviations as in Fig. S1. (A) Predicted transmembrane regions are boxed in blue, and predicted Sec-dependent signal peptide sequences in red. The conserved cysteine residues are highlighted in green boxes. Different shading indicates a different degree of conservation. (B) Scale bar represents 0.4 substitutions per site. Bootstrap values (percentages of 1000 replicates) are shown at the branches.

A



B

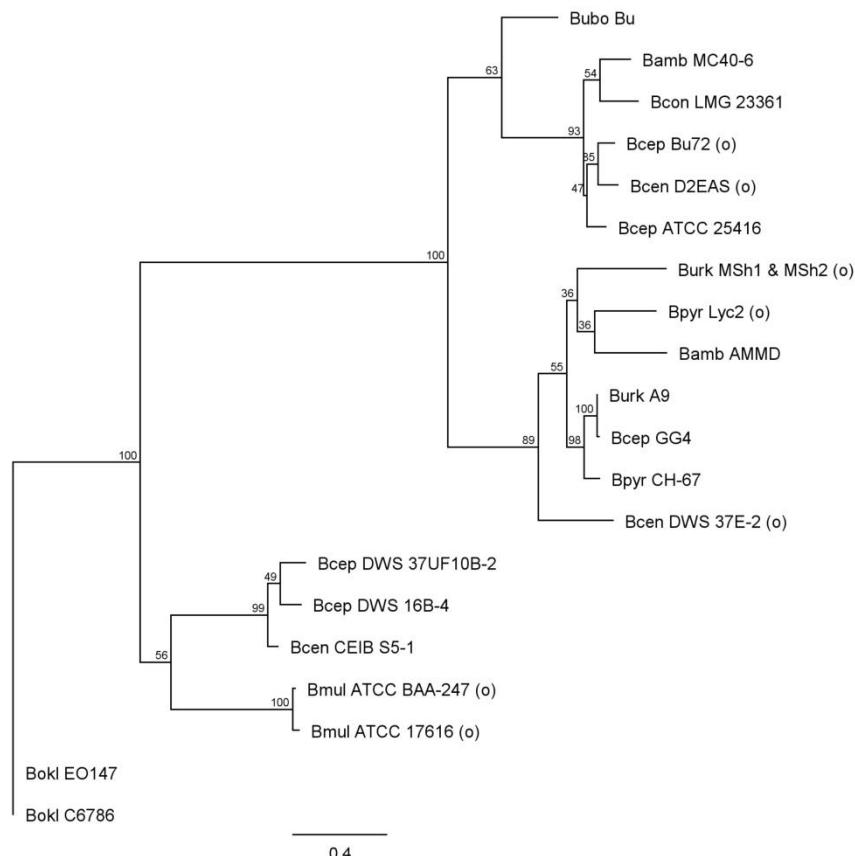


Table S1. Susceptibility patterns of *Burkholderia* strains to BurM1 and BurM2. +, clear halo; T, turbid halo: reduced cell density compared to cell lawn; -, no growth inhibition of the indicator strain; /, not informative.

Species	Strain number	BurM1	BurM2
<i>B. ambifaria</i>	LMG 17828	-	-
	LMG 17829	-	-
	LMG 19182 (or AMMD)	-	/
	LMG 19466	-	-
	LMG 19467	-	-
	LMG 26702 (or MEX-5)	/	-
<i>B. anthina</i>	LMG 20980	-	-
	LMG 20983	-	-
<i>B. arboris</i>	LMG 24066	-	-
<i>B. cenocepacia</i>	LMG 6986	-	-
	LMG 16656	-	-
	LMG 16659	+	-
	LMG 18826	-	-
	LMG 18827	-	-
	LMG 18828	-	-
	LMG 18829	+	+
	LMG 18830	+	-
	LMG 18863	-	-
	LMG 19230	-	-
	LMG 21461	+	-
	LMG 21462	+	-
	LMG 24506	-	-
<i>B. cepacia</i>	LMG 1222	-	-
<i>B. contaminans</i>	LMG 18821	-	-
	LMG 16227	+	-
<i>B. diffusa</i>	LMG 24065	-	-
<i>B. dolosa</i>	LMG 24266	-	-
	LMG 18941	+	T
<i>B. lata</i>	LMG 18943	+	T
	LMG 6992	-	-
<i>B. latens</i>	LMG 24064	-	-
<i>B. metallica</i>	LMG 24068	T	-
<i>B. multivorans</i>	LMG 13010	-	-
	LMG 18825	-	-
<i>B. pyrrhociniae</i>	LMG 14191	T	-
	LMG 21824	-	-
<i>B. seminalis</i>	LMG 24067	+	-
	LMG 24272	-	-
<i>B. stabilis</i>	LMG 14086	-	-
	LMG 14294	-	-
<i>B. ubonensis</i>	LMG 20358	-	-
	LMG 24263	-	-
<i>B. vietnamensis</i>	LMG 10929	-	-
	LMG 18835	-	-

Table S2. List of primers and plasmids used in this study.

Primer/plasmid	Sequence ^a /characteristics	Purpose of use/origin
PGPRB-8001	AAGTTGGGTAACGCCAGGGT	Sequencing of inserts in pUC18
PGPRB-8002	GCACCCCAGGCTTACACTT	Sequencing of inserts in pUC18
PGPRB-8461	GCTCACTCATAGGCACCC	Sequencing of inserts in pJB3Tc20
PGPRB-10029	TGGCAGCAGCCAACTCAGCTT	Sequencing of inserts in pET28(a)
PGPRB-10030	TATAGGCGCCAGCAACCGCA	Sequencing of inserts in pET28(a)
PGPRB-10070	TGGCTACTCGAGTGTCCCCTAGGACGCGGAC	Cloning of <i>burM1</i> in pET28(a)
PGPRB-10072	TGGCTACCAGGGACCTCCTGATCCTATGTTGCCCGT	Cloning of <i>burM1</i> in pET28(a)
PGPRB-10077	TGGCTACTCGAGCTGGGGTCCGGGGCGATAA	Cloning of <i>burM2</i> in pET28(a)
PGPRB-10080	TGGCTACCAGGGAAACCAATGTGTTGCCGGAAATTTC	Cloning of <i>burM2</i> in pET28(a)
PGPRB-10117	GAGATGTTCAATAACGCTGACCACGGCGACATGATCTC CACACCGGA	Removal of internal NcoI site in <i>burM2</i>
PGPRB-10118	TCCGTGGTGGAGATCATGTCGCCGTGGTCAGCGTTATTG AACATCTC	Removal of internal NcoI site in <i>burM2</i>
PGPRB-10141	TGGCTAGCATGCCAGTAGACTGCGATCAGTGTC	Cloning of <i>bmiB</i> in pJB3Tc20
PGPRB-10142	TGGCTAGGATCCCCCTGTGAGCGGAGTCATT	Cloning of <i>bmiB</i> in pJB3Tc20
PGPRB-10167	TGGCTACTGCAGTATTGACAGCGAAGGAGATGCAAAA TGA	Cloning of <i>burM2</i> in pUC18
PGPRB-10168	TGGCTATCTAGAACAGGGCCAATGTGACACTGA	Cloning of <i>burM2</i> in pUC18
PGPRB-10179	TCCTACACGTTGAATCCAGCAAATTGACGCCGATAGC	Construction of a D297A mutant in <i>burM2</i>
PGPRB-10180	GCTATCGCGTCGAATTGGCTGGATTCAACGTGTAGGA	Construction of a D297A mutant in <i>burM2</i>
PGPRB-10214	TGGCTACTGCAGATGGATATCGATCCCCAGGGGA	Cloning of <i>bmiA</i> in pJB3Tc20
PGPRB-10215	TGGCTATCTAGATTGGCTGGACGTCGTAGAGGAA	Cloning of <i>bmiA</i> in pJB3Tc20
pET28(a)	pBR322 origin, His-tag/thrombin/T7 tag ; Km ^R	Novagen
pJB3Tc20	Broad-host-range cloning vector; Ap ^R , Tc ^R	1
pUC18	ColE1 replicon, lacZ, cloning vector; Ap ^R	2
pCMPG6228	pET28(a) with 1013-bp PCR-amplified fragment containing BamMEX5DRAFT_6664 from <i>B. ambifaria</i> MEX-5 cloned in NcoI/XhoI	This study
pCMPG6229	pET28(a) with 983-bp PCR-amplified fragment containing BAMB_RS01670 from <i>B. ambifaria</i> AMMD cloned in NcoI/XhoI	This study
pCMPG6230	Mutant construct of pCMPG6229; D297A of the encoded BmiB	This study
pCMPG6231	pJB3Tc20 with 411-bp PCR-amplified fragment containing BamMEX5DRAFT_6663 from <i>B. ambifaria</i> MEX-5 cloned in PstI/XbaI	This study
pCMPG6232	pJB3Tc20 with 447-bp PCR-amplified fragment containing BAMB_RS01665 from <i>B. ambifaria</i> AMMD cloned in SphI/BamHI	This study

^a Restriction sites incorporated in the primers are shown in bold: GGATCC, BamHI; CCATGG, NcoI; CTGCAG, PstI; GCATGC, SphI; TCTAGA, XbaI; CTCGAG, XhoI.

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