

1 **SUPPLEMENTAL MATERIAL**

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3 Culturing and characterization of the gut symbiont *Burkholderia* from the Southern chinch bug,  
4 *Blissus insularis* (Hemiptera: Blissidae)

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25 **TABLE S1** List of primers used to PCR-amplify the *COI* genes of *Blissus insularis* and bacterial genes of crypt-associated symbionts.

Gene	Forward (F) and reverse (R) primer sequences (5' to 3')	Amplicon size (bp)	Thermal cycling profile	References
<i>COI</i>	TonyaF: GAAGTTTATATTTTAATTTTACCGGG HobbesR: AAATGTTGNGGRAAAAATGTTA	565	35 cycles: 94 °C, 40 sec 51 °C, 40 sec 72 °C, 1 min	(1)
Universal 16S rRNA	10F: AGTTTGATCATGGCTCAGATTG 1507R: TACCTTGTTACGACTTCACCCCAG	1500	30 cycles: 94 °C, 30 sec 55 °C, 30 sec 68 °C, 1 min	(2)
<i>Burkholderia</i> -specific 16S rRNA	degBurk16SF: TTTTGGACAATGGGSGMAA Burk16SR: GCTCTTGCGTAGCAACTAAG	750	30 cycles: 94 °C, 30 sec 60 °C, 30 sec 68 °C, 1 min	degBurk16SF: this study; Burk16SR: (3)
<i>Burkholderia atpD</i>	atpD_F: ATGAGTACTRCTGCTTTGGTAGAAGG atpD_R: CGTGAAACGGTAGATGTTGTCG	756		
<i>Burkholderia lepA</i>	lepA_F: CTSATCATCGAYTCSTGGTTTCG lepA_R: CGRTATTCCTTGAACCTCGTARTCC	975	30 cycles: 96 °C, 1 min 58 °C, 1 min 72 °C, 2 min	(4)
<i>Burkholderia recA</i>	recA_F: AGGACGATTCATGGAAGAWAGC recA_R: GACGCACYGAYGMRTAGAACTT	704		

26 **TABLE S2** The single-nucleotide polymorphisms (SNPs) detected in the universal 16S rRNA  
 27 gene partial sequences (~1.4 kb) and the BOX-PCR fingerprinting of crypt-associated and  
 28 cultured counterpart bacteria isolated from *Blissus insularis*.

Clade <sup>a</sup>	Identity	16S rRNA ribotyping			BOX-PCR % similarity <sup>e</sup>
		SNPs <sup>b</sup> (bp)	% SNPs <sup>c</sup>	Hypervariable regions <sup>d</sup>	
SBE	Bi18MC_R	0	0	N/A	85
	Bi22MC_R	0	0	N/A	89
	Bi23MC_R	0	0	N/A	92
	Bi12MC_S	0	0	N/A	88
	Bi14MC_S	14	1.0	V2-4	18
PBE	Bi20MC_R	0	0	N/A	71
	Bi21MC_R	0	0	N/A	94
	Bi16MC_S	0	0	N/A	87
	Bi19MC_S	0	0	N/A	80
BCC	Bi17MC_R	0	0	N/A	88
	Bi19MC_R	63	4.5	V1-4, V6-8	28
	Bi24MC_R	0	0	N/A	96
	Bi25MC_R	0	0	N/A	77
	Bi20MC_S	0	0	N/A	98
	Bi21MC_S	0	0	N/A	88
	Bi26MC_S	67	4.8	V1-8	12
	Bi27MC_S	0	0	N/A	91
Bi28MC_S	0	0	N/A	84	

29 <sup>a</sup> Clades were defined by the universal 16S rRNA gene sequences, see details in Fig. 3.

30 <sup>b</sup> The SNPs detected using pairwise alignments of the universal 16S rRNA gene sequences of  
 31 crypt-associated and cultured counterpart bacteria isolated from each individual *B. insularis*.

32 <sup>c</sup> Percentage of the SNPs in the respective ~1.4-kb universal 16S rRNA gene sequences.

33 <sup>d</sup> The hypervariable regions where the SNPs are detected in the 16S rRNA gene sequence (see  
 34 region definition in Fig. S1). N/A, not available.

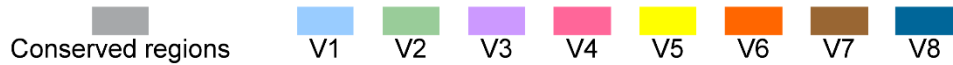
35 <sup>e</sup> The similarity between the BOX-PCR patterns of crypt-associated and cultured counterpart  
 36 bacteria isolated from each individual *B. insularis*.

37 **TABLE S3** The nucleotide sequence accession numbers of MLST (*atpD*, *recA*, *lepA*) and 16S  
 38 rRNA gene sequences generated from eight cultured *Burkholderia* isolates and four dissected  
 39 crypt preparations of *Blissus insularis*.

Identity	<i>atpD</i>	<i>recA</i>	<i>lepA</i>	16S rRNA
Bi12MC_S_vitro	KU242611	KU247540	KU247552	KU242599
Bi19MC_S_vitro	KU242612	KU247541	KU247553	KU242602
Bi20MC_S_vitro	KU242613	KU247542	KU247554	KU242603
Bi21MC_S_vitro	KU242614	KU247543	KU247555	KU242604
Bi16MC_R_vitro	KU242615	KU247544	KU247556	KU242589
Bi17MC_R_vitro	KU242616	KU247545	KU247557	KU242590
Bi18MC_R_vitro	KU242617	KU247546	KU247558	KU242591
Bi20MC_R_vitro	KU242618	KU247547	KU247559	KU242593
Bi01MC_R	KU242619	KU247548	KU247560	KP683095
Bi02MC_R	KU242620	KU247549	KU247561	KP683096
Bi07MC_S	KU242621	KU247550	KU247562	KP683112
Bi08MC_S	KU242622	KU247551	KU247563	KP683113

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TGCAAGTCGAACGGCAGCACGGGTGCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGTAATACATC  
 60 80 100 120  
 GGAACATGTCCTGTAGTGGGGGATAGCCCGGCCGAAAGCCGGATTAATACCGCATACGATCTACGGATG  
 140 160 180  
AAAGCGGGGACCTTCGGGCCTCGCGCTATAGGGTTGGCCGATGGCTGATTAGCTAGTTGGTGGGGT  
 200 220 240 260  
 AAAGGCCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGAC  
 280 300 320  
ACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGC  
 340 360 380  
 AATGCCGCGTGTGTGAAGAAGGCCCTTCGGGTTGTAAGCACTTTTGTCCGAAAGAAATCCTGAGGGC  
 400 420 440 460  
TAATATCCTTCGGGGATGACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGT  
 480 500 520  
AATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGCGGTTTGTTAAGAC  
 540 560 580  
CGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTGGTGACTGGCAAGCTAGAGTATGGCAGAG  
 600 620 640 660  
GGGGGTAGAATTCCACGTGTAGCAGTGAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCA  
 680 700 720  
 GCCCCCTGGGCCAATACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG  
 740 760 780 800  
TAGTCCACGCCCTAAACGATGTCAACTAGTTGTTGGGGATTCAATTCCTTAGTAACGTAGCTAACCGGT  
 820 840 860  
GAAGTTGACCGCCTGGGGAGTAGGGTCGCAAGATTAAACTCAAAGGAATTGACGGGGACCCGCACAA  
 880 900 920  
GCGGTGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGGTTCGGAAC  
 940 960 980 1000  
CTTGAGAGATCTGAGGGTGCTCGAAAGAGAACCGATACACAGGTGCTGCATGGCTGTCGTCAGCTCG  
 1020 1040 1060  
TGTCGTGAGATGTTGGTTAAGTCCCGCAACGAGCGCAACCCTGTCCTTAGTTGCTACGCAAGAGCA  
 1080 1100 1120 1140  
CTCTAGGGAGACTGCCGGTGACAAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTATGGCCCTTA  
 1160 1180 1200  
 TGGGTAGGGCTTACACGTCATAACAATGGTCCGGAACAGAGGGTCGCCAACCCGCGAGGGGGAGCTAA  
 1220 1240 1260  
TCCAGAAAAACCGATCGTAGTCCGGATTGCACTCTGCAACTCGAGTGCATGAAGCTGGAATCGCTAGT  
 1280 1300 1320 1340  
 AATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCGTCACACCATGG  
 1360 1380 1400  
 GAGTGGGTTTTACCAGAAGTGGCTAGTCTAACC GCAAGGAGGACGGTCAACCAGC  
 1420 1440 1460



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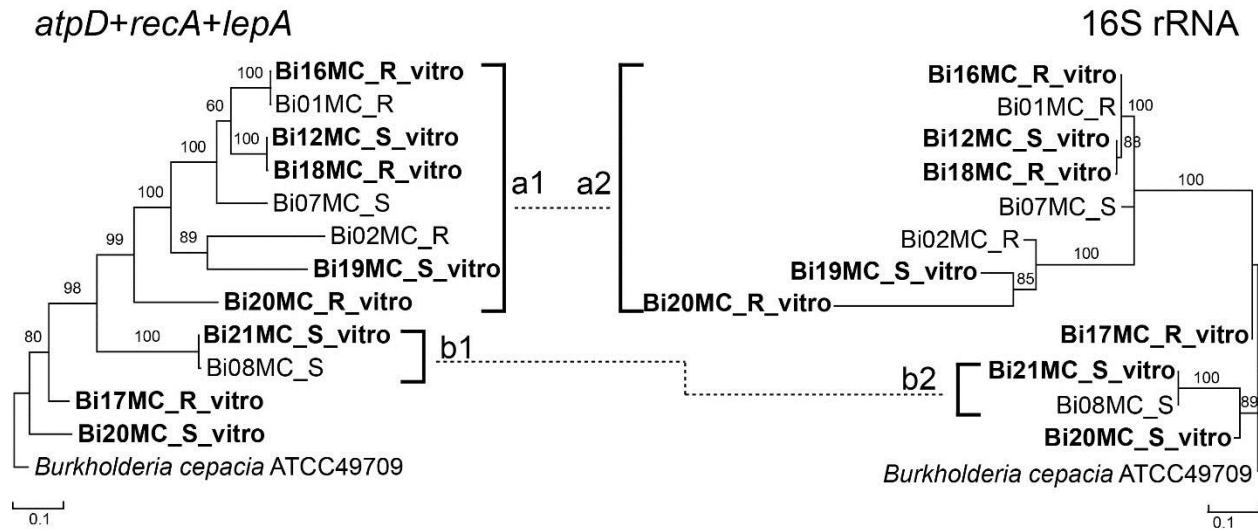
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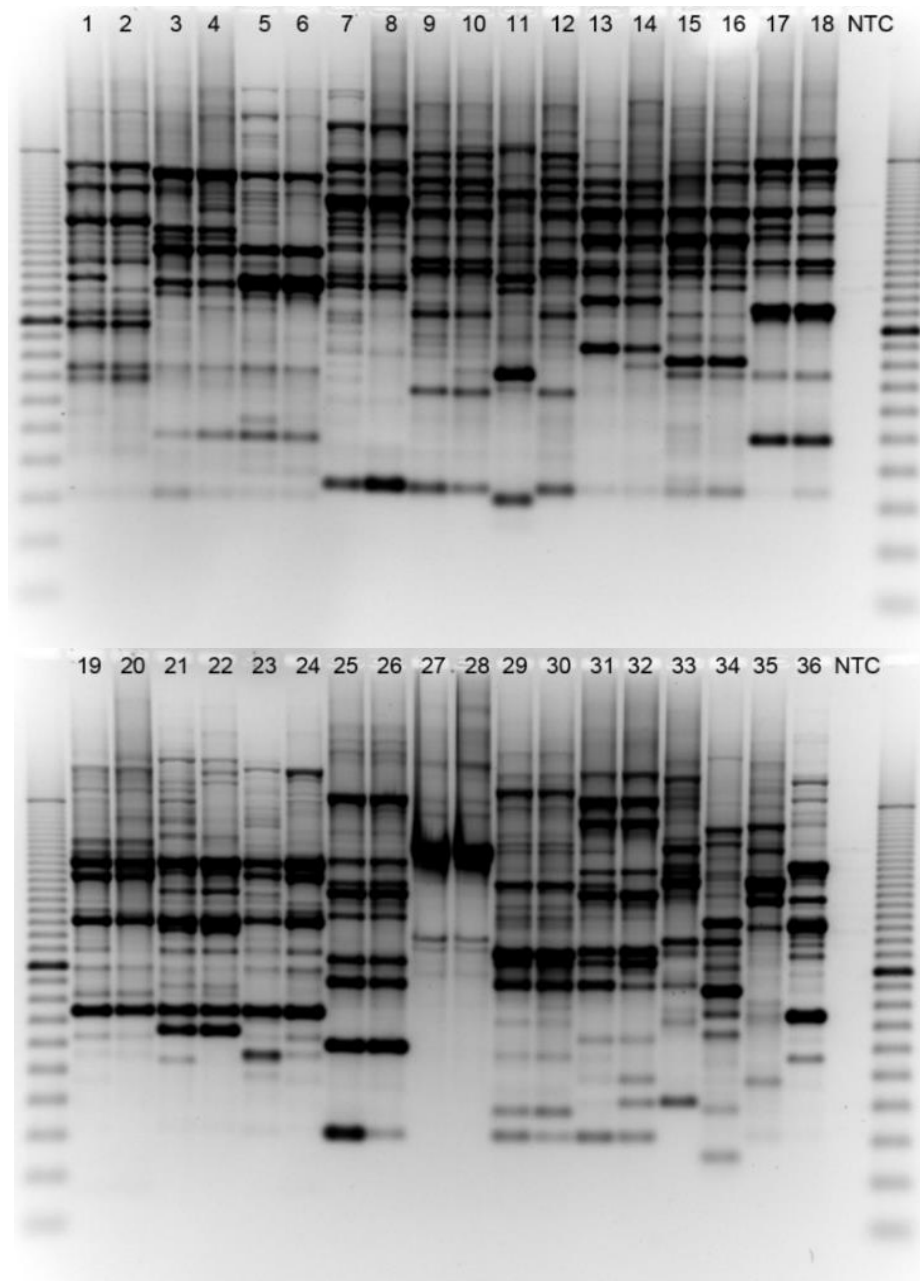
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**FIG S1** The schematic overview of the 16S rRNA gene sequence of the crypt-associated *Burkholderia* in *Blissus insularis*. The underlined nucleotides indicate the genus-specific *Burkholderia* 16S rRNA gene sequence. The conserved region (5) is shown in gray and hypervariable (V1-V8) (6) regions are shown in different colors. Numbers below the nucleotides indicate the positions of 16S rRNA gene sequences, labelled according to the relative positions in *E. coli* 16S rRNA gene (5).



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50 **Fig S2** Comparison of phylogenetic trees of four crypt-associated bacteria *in vivo*  
 51 (*Bi01MC\_R*, *Bi02MC\_R*, *Bi07MC\_S*, *Bi08MC\_S*) obtained from *Blissus insularis* crypts and  
 52 eight representative cultured bacteria isolates (denoted *vitro*, in bold) on the basis of the  
 53 concatenated 1,233-bp MLST gene sequences (left, *atpD+recA+lepA*) and the 1,326-bp universal  
 54 16S rRNA gene sequences (right, 16S rRNA). Numbers at the tree nodes represent the  
 55 maximum-likelihood bootstrap values obtained after 100 repetitions; only values over 50 are  
 56 shown. Corresponding clades are indicated by connecting dashed lines. The 16S rRNA gene  
 57 nucleotide sequence accession number of outgroup *Burkholderia cepacia* ATCC49709 is  
 58 AY741349. See Table S3 for the generated nucleotide sequence accession numbers of samples in  
 59 the current study.



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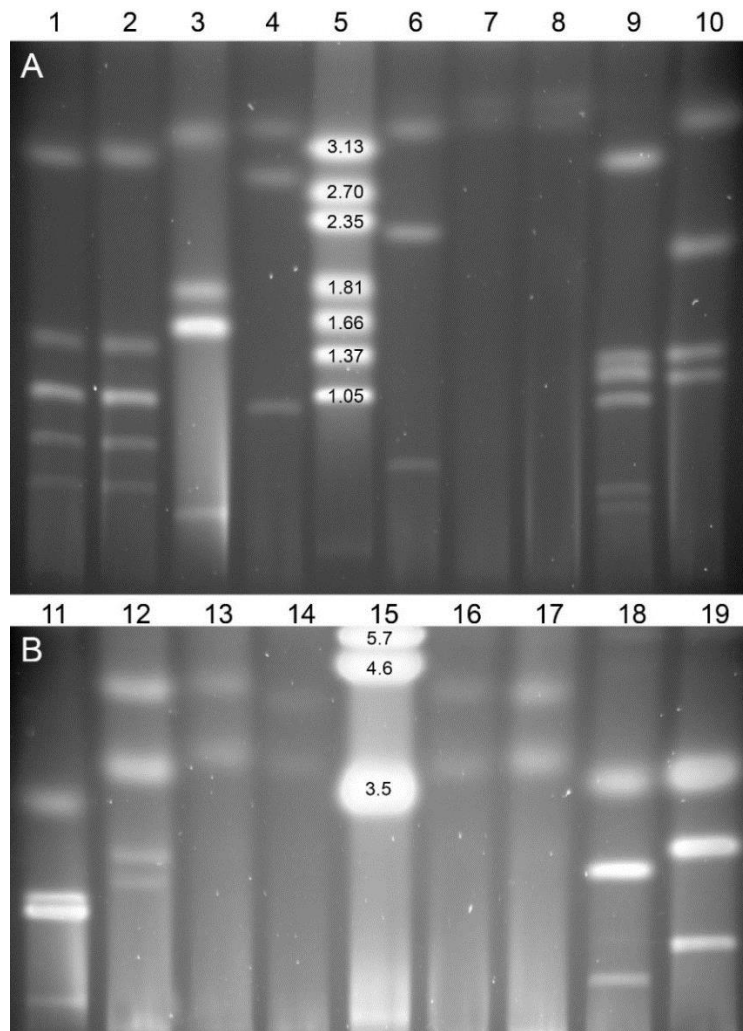
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62 **FIG S3** The representative BOX-PCR gel of crypt-associated bacteria *in vivo* (denoted  
 63 *vivo*) and according cultured *Burkholderia* isolates (denoted *vitro*). Lanes 1 = Bi16MC\_S\_ vivo,  
 64 2 = Bi16MC\_S\_ vitro, 3 = Bi19MC\_S\_ vivo, 4 = Bi19MC\_S\_ vitro, 5 = Bi21MC\_R\_ vivo, 6 =  
 65 Bi21MC\_R\_ vitro, 7 = Bi20MC\_R\_ vivo, 8 = Bi20MC\_R\_ vitro, 9 = Bi12MC\_S\_ vivo, 10 =  
 66 Bi12MC\_S\_ vitro, 11 = Bi14MC\_S\_ vivo, 12 = Bi14MC\_S\_ vitro, 13 = Bi18MC\_R\_ vivo, 14 =

67 Bi18MC\_R\_vitro, 15 = Bi22MC\_R\_vivo, 16 = Bi22MC\_R\_vitro, 17 = Bi23MC\_R\_vivo, 18 =  
68 Bi23MC\_R\_vitro, 19 = Bi21MC\_S\_vivo, 20 = Bi21MC\_S\_vitro, 21 = Bi24MC\_R\_vivo, 22 =  
69 Bi24MC\_R\_vitro, 23 = Bi25MC\_R\_vivo, 24 = Bi25MC\_R\_vitro, 25 = Bi17MC\_R\_vivo, 26 =  
70 Bi17MC\_R\_vitro, 27 = Bi20MC\_S\_vivo, 28 = Bi20MC\_S\_vitro, 29 = Bi27MC\_S\_vivo, 30 =  
71 Bi27MC\_S\_vitro, 31 = Bi28MC\_S\_vivo, 32 = Bi28MC\_S\_vitro, 33 = Bi19MC\_R\_vivo, 34 =  
72 Bi19MC\_R\_vitro, 35 = Bi26MC\_S\_vivo, 36 = Bi26MC\_S\_vitro. NTC = non-template negative  
73 control. The standard makers are 100 bp PCR molecular rulers (Bio-Rad).

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76 **FIG S4** The representative PFGE patterns of 17 cultured *Burkholderia* isolates generated

77 from *Blissus insularis* midgut crypts using different standard markers (lanes 5 and 15) under

78 different electrophoresis conditions (A and B) (see details in Materials and Methods). Lanes 1 =

79 Bi12MC\_S\_vitro, 2 = Bi14MC\_S\_vitro, 3 = Bi16MC\_R\_vitro, 4 = Bi17MC\_R\_vitro, 6 =

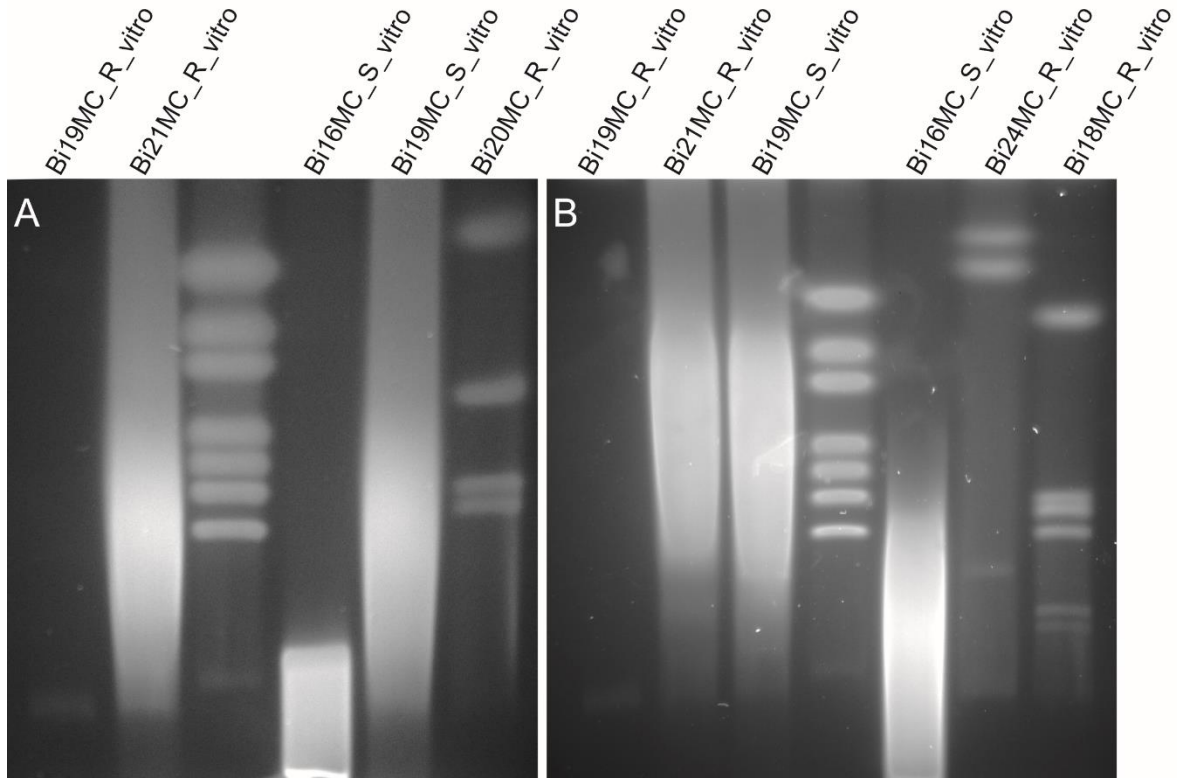
80 Bi20MC\_S\_vitro, 7 = Bi21MC\_S\_vitro, 8 = Bi22MC\_S\_vitro, 9 = Bi18MC\_R\_vitro, 10 =

81 Bi20MC\_R\_vitro, 11 = Bi16MC\_R\_vitro, 12 = Bi24MC\_R\_vitro, 13 = Bi25MC\_R\_vitro, 14 =

82 Bi21MC\_S\_vitro, 16 = Bi22MC\_S\_vitro, 17 = Bi26MC\_S\_vitro, 18 = Bi20MC\_S\_vitro, 19 =

83 Bi28MC\_S\_vitro. Standard marker present in lanes 5 and 15 are *H. wingei* and *S. pombe*,

84 respectively.



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**FIG S5** The PFGE patterns of representative cultured *Burkholderia* isolates obtained by using gel electrophoresis buffer without (A) and with 50  $\mu$ M thiourea (B). Standard markers are *H. wingei*. Four isolates (Bi19MC\_R\_vitro, Bi21MC\_R\_vitro, Bi19MC\_S\_vitro, and Bi16MC\_S\_vitro) had DNA degradation (smear patterns) during the PFGE process, whereas three normal isolates (Bi20MC\_R\_vitro, Bi24MC\_R\_vitro, and Bi18MC\_R\_vitro) were used as positive controls.

93 **REFERENCES**

- 94 1. **Rand D, Heath A, Suderman T, Pierce N.** 2000. Phylogeny and life history evolution of  
95 the genus *Chrysoritis* within the Aphnaeini (Lepidoptera: Lycaenidae), inferred from  
96 mitochondrial *cytochrome oxidase I* sequences. *Mol Phylogenet Evol* **17**:85–96.
- 97 2. **Sandström JP, Russell JA, White JP, Moran NA.** 2001. Independent origins and  
98 horizontal transfer of bacterial symbionts of aphids. *Mol Ecol* **10**:217–228.
- 99 3. **Kikuchi Y, Meng XY, Fukatsu T.** 2005. Gut symbiotic bacteria of the genus  
100 *Burkholderia* in the broad-headed bugs *Riptortus clavatus* and *Leptocorisa chinensis*  
101 (Heteroptera: Alydidae). *Appl Environ Microbiol* **71**:4035–4043.
- 102 4. **Spilker T, Baldwin A, Bumford A, Dowson CG, Mahenthiralingam E, LiPuma JJ.**  
103 2009. Expanded multilocus sequence typing for *Burkholderia* species. *J Clin Microbiol*  
104 **47**:2607–2610.
- 105 5. **Wang Y, Qian PY.** 2009. Conservative fragments in bacterial 16S rRNA genes and  
106 primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One*  
107 **4**:e7401.
- 108 6. **Chakravorty S, Helb D, Burday M, Connell N, Alland D.** 2007. A detailed analysis of  
109 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *J Microbiol*  
110 *Methods* **69**:330–339.
- 111