

Table 1. Bacterial strains used in this study.

Strain	Genotype	Source or reference
<i>B. dolosa</i>		
BdAU0158		BcRLR*
$\Delta bcp-1$		(1)
$\Delta bcp-2$		(1)
$\Delta bcp-3$		This study
$\Delta\Delta$	<i>BdAU0158</i> $\Delta bcp-1\Delta bcp-2\Delta bcp-3$	This study
<i>BdAU0158</i>	<i>BdAU0158 attTn7::Km</i>	This study
<i>BdAU0158</i>	<i>BdAU0158 attTn7::Tet</i>	This study
$\Delta bcp-1$	<i>BdAU0158</i> $\Delta bcp-1 attTn7::Km$	This study
$\Delta bcp-1$	<i>BdAU0158</i> $\Delta bcp-1 attTn7::Tet$	This study
$\Delta bcp-1 attTn7::bcpI-1$	<i>BdAU0158</i> $\Delta bcp-1 attTn7::bcpI-1-Km$	This study
$\Delta bcp-1 attTn7::bcpI-2$	<i>BdAU0158</i> $\Delta bcp-1 attTn7::bcpI-2-Km$	This study
$\Delta bcp-2$	<i>BdAU0158</i> $\Delta bcp-2 attTn7::Km$	This study
$\Delta bcp-2 attTn7::bcpI-2$	<i>BdAU0158</i> $\Delta bcp-2 attTn7::bcpI-2-Km$	This study
$\Delta bcp-2 attTn7::bcpI-1$	<i>BdAU0158</i> $\Delta bcp-2 attTn7::bcpI-1-Km$	This study
$\Delta bcp-3$	<i>BdAU0158</i> $\Delta bcp-3 attTn7::Km$	This study
$\Delta bcp-3 attTn7::bcpI-3$	<i>BdAU0158</i> $\Delta bcp-3 attTn7::bcpI-3-Km$	This study
<i>bcp-3^C</i>	<i>BdAU0158</i> $\Omega pAP29$	This study
$\Delta bcp-3$	<i>BdAU0158</i> $\Delta bcp-3 attTn7::Tet$	This study
$\Delta bcp-3 attTn7::bcpI-3$	<i>BdAU0158</i> $\Delta bcp-3 attTn7::bcpI-3-Tet$	This study
<i>P_{bcp-1}-lacZ</i>	<i>BdAU0158 attTn7::P_{bcp-1}-lacZ</i>	This study
<i>P_{bcp-2}-lacZ</i>	<i>BdAU0158 attTn7::P_{bcp-2}-lacZ</i>	This study
<i>P_{bcp-3}-lacZ</i>	<i>BdAU0158 attTn7::P_{bcp-3}-lacZ</i>	This study
<i>P_{S12}-lacZ</i>	<i>BdAU0158 attTn7::P_{S12}-lacZ</i>	This study
<i>lacZ</i>	<i>BdAU0158 attTn7::lacZ</i>	This study
<i>P_{bcpI-3}-lacZ</i>	<i>BdAU0158 attTn7::P_{bcpI-3}-lacZ</i>	This study
$\Delta bcp-1 attTn7::bcpI_{E264}$	<i>BdAU0158</i> $\Delta bcp-1 attTn7::bcpI_{E264}-Tet$	This study
$\Delta bcpO-1$	<i>BdAU0158</i> $\Delta bcpO-1 attTn7::Km$	This study
$\Delta bcpO-2$	<i>BdAU0158</i> $\Delta bcpO-2 attTn7::Tet$	This study
<i>bcp-3^C</i> $\Delta bcpO-3$	<i>BdAU0158</i> $\Omega pAP29$ $\Delta bcpO-3$	This study
$\Delta bcpO-3$	<i>BdAU0158</i> $\Delta bcpO-3 attTn7::Tet$	This study
<i>B. thailandensis</i>		
<i>BtE264</i>		(2)
$\Delta bcpAIOB$		(3)
<i>BtE264</i>	<i>BtE264 attTn7::Cm</i>	(3)
$\Delta bcpAIOB$	<i>BtE264</i> $\Delta bcpAIOB attTn7::Km$	(3)

<i>ΔbcpAIOB attTn7::bcpI_{AU0158-1}</i>	<i>BtE264 ΔbcpAIOB attTn7::bcpI_{AU0158-1}-Km</i>	This study
<u><i>E. coli</i></u>		
BL21(DE3) pAP30	pET-28(a):: <i>bcpA-1-CT</i>	This study
BL21(DE3) pAP31	pET-28(a):: <i>bcpA-1-CT—bcpI-1</i>	This study
BL21(DE3) pAP46	pET-28(a):: <i>bcpI-1</i>	This study
BL21(DE3) pAP32	pET-28(a):: <i>bcpA-2-CT</i>	This study
BL21(DE3) pAP33	pET-28(a):: <i>bcpA-2-CT—bcpI-2</i>	This study
BL21(DE3) pAP47	pET-28(a):: <i>bcpI-2</i>	This study
BL21(DE3) pAP34	pET-28(a):: <i>bcpA-3-CT</i>	This study
BL21(DE3) pAP35	pET-28(a):: <i>bcpA-3-CT—bcpI-3</i>	This study
BL21(DE3) pAP48	pET-28(a):: <i>bcpI-3</i>	This study

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Table 2. Plasmids used in this study.

Plasmid	Backbone	Description	Antibiotic Resistance	Source or Reference
pEXKm5	--	Allelic exchange vector	Km	(4)
pAP10	pEXKm5	To delete <i>BdAU0158 bcp-3</i>	Km	This study
pAP49	pEXKm5	To delete <i>BdAU0158 bcpO-1</i>	Km	This study
pAP50	pEXKm5	To delete <i>BdAU0158 bcpO-2</i>	Km	This study
pAP51	pEXKm5	To delete <i>BdAU0158 bcpO-3</i>	Km	This study
pUC18Tmini-Tn7T-Km	--	To deliver Km ^R cassette to <i>attTn7</i> site	Amp, Km	(5)
pUC18Tmini-Tn7T-Tet	pUC18Tmini-Tn7T-Km	To deliver Tet ^R cassette to <i>attTn7</i> site	Amp, Tet	(3)
pTNS3	--	Helper plasmid to deliver cassettes to <i>attTn7</i> site	Amp	(6)
pUCS12Km	pUC18Tmini-Tn7T-Km	To deliver P _{S12} -driving cassettes to the <i>attTn7</i> site	Amp, Km	(3)
pAP3	pUCS12Km	To deliver <i>BdAU0158 bcpI-1</i> to <i>attTn7</i> site	Amp, Km	This study
pAP5	pUCS12Km	To deliver <i>BdAU0158 bcpI-2</i> to <i>attTn7</i> site	Amp, Km	This study
pAP42	pUC18Tmini-Tn7T-Tet	To deliver <i>BdAU0158 bcpI-3</i> (constitutively expressed) to <i>attTn7</i> site	Amp, Tet	This study
pAP29	pUCS12Km	1 st 501 bp of <i>BdAU0158 bcpA-3</i> , used to generate <i>BdAU0158 bcp-3^C</i>	Amp, Km	This study
pMA43	pUC18Tmini-Tn7T-Tet	To deliver <i>BtE264 bcpI</i> (constitutively expressed) to <i>attTn7</i> site	Amp, Tet	(3)
pUClacZ	pUC18Tmini-Tn7T-Km	To deliver promoterless <i>lacZ</i> to <i>attTn7</i> site	Amp, Km	(3)
pECG10	pUClacZ	To deliver P _{S12} - <i>lacZ</i> to <i>attTn7</i> site	Amp, Km	(3)
pAP23	pUClacZ	500 bp upstream of <i>BdAU1058 bcp-1</i> , to generate <i>BdAU0158 P_{bcp-1}-lacZ</i> reporter	Amp, Km	This study
pAP22	pUClacZ	300 bp upstream of <i>BdAU1058 bcp-2</i> , to generate <i>BdAU0158 P_{bcp-2}-lacZ</i> reporter	Amp, Km	This study
pAP24	pUClacZ	500 bp upstream of <i>BdAU1058 bcp-3</i> , to generate <i>BdAU0158 P_{bcp-3}-lacZ</i> reporter	Amp, Km	This study

pAP61	pUClacZ	Last 500 bp of <i>BdAU1058 bcpA-3</i> , to generate <i>BdAU0158 P_{bcpI-3}-lacZ</i> reporter	Amp, Km	This study
pET-28(a)	--	IPTG-inducible expression vector	Km	Novagen
pAP30	pET-28(a)	<i>BdAU0158 bcpA-1-CT</i>	Km	This study
pAP31	pET-28(a)	<i>BdAU0158 bcpA-1-CT—bcpI-1</i>	Km	This study
pAP46	pET-28(a)	<i>BdAU0158 bcpI-1</i>	Km	This study
pAP32	pET-28(a)	<i>BdAU0158 bcpA-2-CT</i>	Km	This study
pAP33	pET-28(a)	<i>BdAU0158 bcpA-2-CT—bcpI-2</i>	Km	This study
pAP47	pET-28(a)	<i>BdAU0158 bcpI-2</i>	Km	This study
pAP34	pET-28(a)	<i>BdAU0158 bcpA-3-CT</i>	Km	This study
pAP35	pET-28(a)	<i>BdAU0158 bcpA-3-CT—bcpI-3</i>	Km	This study
pAP48	pET-28(a)	<i>BdAU0158 bcpI-3</i>	Km	This study

References

1. **Garcia EC, Perault AI, Marlatt SA, Cotter PA.** 2016. Interbacterial signaling via *Burkholderia* contact-dependent growth inhibition system proteins. *Proc Natl Acad Sci USA* **113**:8296–8301.
2. **Brett PJ, DeShazer D, Woods DE.** 1998. *Burkholderia thailandensis* sp. nov., a *Burkholderia pseudomallei*-like species. *Int J Syst Bacteriol* **48 Pt 1**:317–320.
3. **Anderson MS, Garcia EC, Cotter PA.** 2012. The *Burkholderia* *bcpA*IOB genes define unique classes of two-partner secretion and contact dependent growth inhibition systems. *PLoS Genet* **8**:e1002877.
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.
5. **Choi K-H, Mima T, Casart Y, Rholl D, Kumar A, Beacham IR, Schweizer HP.** 2008. Genetic tools for select-agent-compliant manipulation of *Burkholderia pseudomallei*. *Appl Environ Microbiol* **74**:1064–1075.
6. **Choi K-H, Gaynor JB, White KG, Lopez C, Bosio CM, Karkhoff-Schweizer RR, Schweizer HP.** 2005. A Tn7-based broad-range bacterial cloning and expression system. *Nature Methods* **2**:443–448.

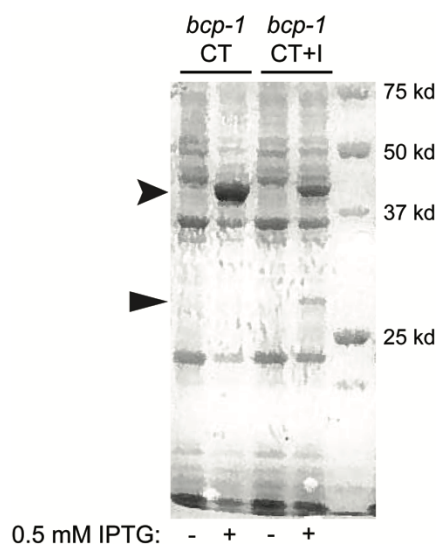


Figure S1. Production of *Bd*AU0158 BcpA-1-CT and BcpI-1 by *E. coli* BL21(DE3) as visualized by Coomassie staining of whole cell lysates separated on a 12% SDS-PAGE gel. Cells were harvested after 6 h growth (4 h post IPTG-induction). The accumulation of BcpA-1-CT by the induced strains harboring the plasmid containing *bcpA-1-CT* (left two lanes) and the plasmid containing *bcpA-1-CT* and *bcpI-1* (right two lanes) is indicated by the arrowhead. The wedge indicates production of BcpI-1, which only occurs in the induced strain harboring the plasmid containing *bcpA-1-CT* and *bcpI-1*. The sizes of the two indicated proteins match the predicted sizes of BcpA-1-CT and BcpI-1.