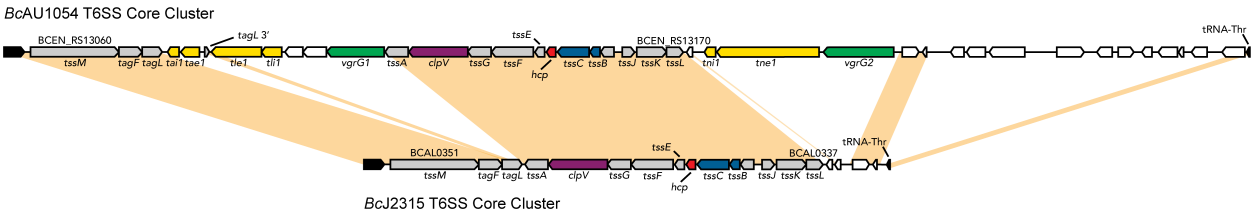

Figure S1. The *Bc*AU1054 T6SS core cluster is homologous to the *Bc*J2315 T6SS core cluster, though contains insertions; Related to Figure 1.

The genomic regions encoding the T6SSs of *Bc*AU1054 and *Bc*J2315 were aligned to each other using Mauve. Orange shading represents regions of homology (≥90% nucleotide identity). All the structural protein-encoding genes are homologous (≥90% nucleotide identity) between the two strains, though the *Bc*AU1054 core cluster has multiple insertions – regions spanning *tae1-tai1*, *vgrG1-tle1*, *vgrG2-tni2*, and genes downstream of the core cluster predicted to encode proteins involved in phage and mobile genetic elements (see **Figure 1**).

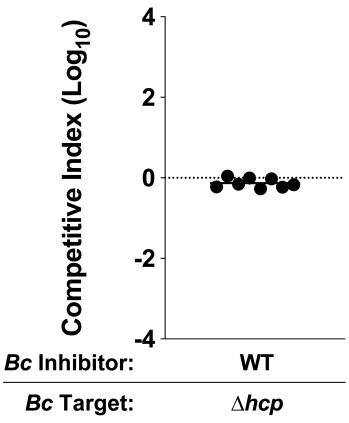

Figure S2. *Bc*AU1054 Δ*hcp* does not have a growth defect; Related to Figure 1.

5 h cocultures between *Bc*AU1054 WT and Δ*hcp* strains. Circles represent individual cocultures from two biological replicates, each with four technical replicates. Solid horizontal line represents mean $\log_{10}$ C.I. Dotted horizontal line ($\log_{10}$ C.I. = 0) indicates no competitive advantage for either inhibitor or target.

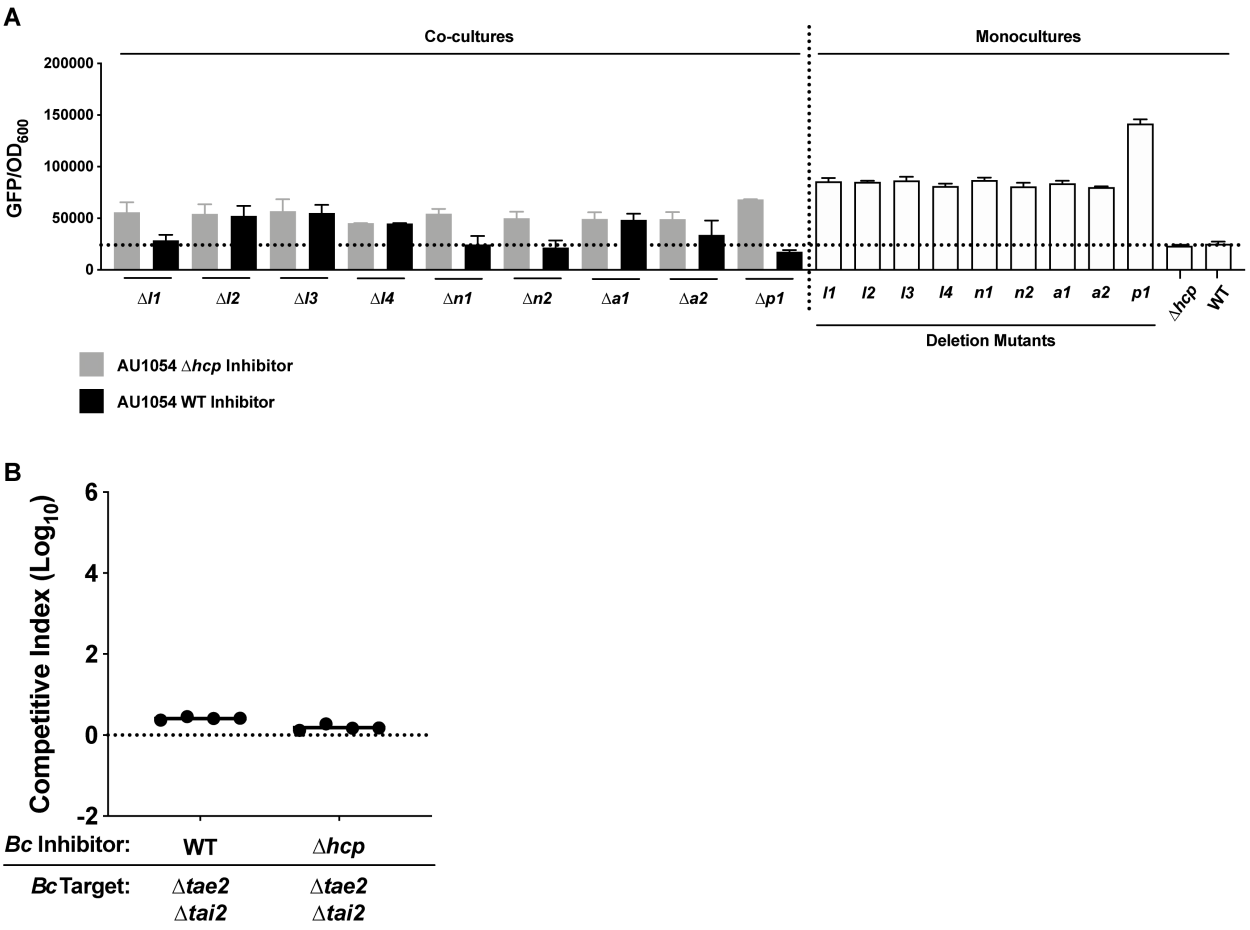

Figure S3. Screening approach to identify probable *Bc*AU1054 T6SS E-I pairs; Related to Figure 2.

(A) GFP/OD_{600} values for ~20 h cocultures between WT and Δ*hcp Bc*AU1054 inhibitor strains and *Bc*AU1054 E-I deletion mutants constitutively producing GFP, as well as GFP/OD_{600} values for ~20 h monocultures of each strain. GFP measured at 485 nm excitation and 530 nm emission. Data resulting from at least two biological replicates, with height of bar representing mean GFP/OD_{600} value and error bars representing one standard deviation. Dotted horizontal line represents baseline GFP/OD_{600} value, taken from non-GFP-producing WT and Δ*hcp Bc*AU1054 monocultures. See **Figure 1** and **Table S2** for location of E-I-encoding gene pairs and predicted effector activity, respectively. (B) 5 h competition experiment between WT and Δ*hcp Bc*AU1054 inhibitor strains and *Bc*AU1054 Δ*tae2*Δ*tai2* target strain. *Bc*AU1054 Δ*tae2*Δ*tai2* is not outcompeted in a T6SS-dependent manner and thus *tae2-tai2* likely do not encode a T6SS E-I pair. Circles represent individual cocultures (four technical replicates for each competition). Solid horizontal lines represent mean $\log_{10}$ C.I. values. Dotted horizontal line ($\log_{10}$ C.I. = 0) indicates no competitive advantage for either inhibitor or target.

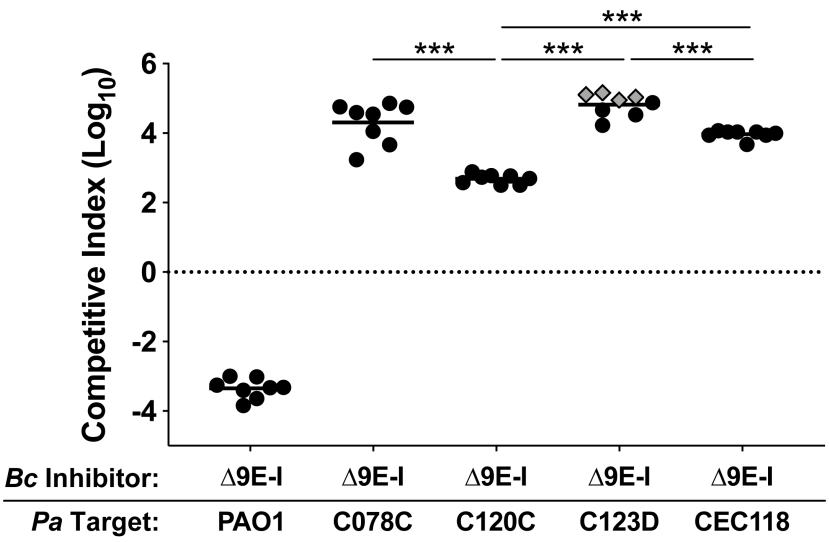

Figure S4. *Bc*AU1054 T6SS effectors exhibit target strain-specific variability in toxicity; Related to Figure 3.

Interbacterial competition experiments between *Bc*AU1054 ∆9E-I inhibitor strain and PAO1, C078C, C120C, C123D, and CEC118 *P. aeruginosa* target strains. Circles/diamonds represent individual cocultures from two biological replicates, each with four technical replicates. Grey-filled diamonds represent competitions from which no target cells were recovered. Solid horizontal lines represent mean $\log_{10}$ C.I. values. Dotted horizontal line ($\log_{10}$ C.I. = 0) indicates no competitive advantage for either inhibitor or target. ***P<0.0005, Mann-Whitney test.

Table S2. Bioinformatically-predicted *Bc*AU1054 T6SS E-I pairs and predicted effector enzymatic activities; Related to Figures 1 and 2.

E-I Pair	Locus Tag	Associated *vgrG*	Predicted Effector Activity	Notes
tle1-til1	BCEN_RS13090-BCEN_RS13095	*vgrG1*	Phospholipase	
tle2-tli2	BCEN_RS06980-BCEN_RS06975	*vgrG3*	Phospholipase	Referred to as *tle5-tli5* in (Russell *et al.*, 2013)
tle3-tli3	BCEN_RS25350-BCEN_RS25355	*vgrG7*	Phospholipase	
tle4-tli4	BCEN_RS23700-BCEN_RS23705	None	Phospholipase	*tle4* has an RHS domain; *tli4* has DUF3304, which otherwise is present in the *Bc*AU1054 genome only in *tli1* and *tli3*
tne1-tni1	BCEN_RS13180-(not annotated)	*vgrG2*	Nuclease	See **Table S1** for *tni1* sequence
tne2-tni2	BCEN_RS16675-Bcen_3345 (old locus tag)	*vgrG5*	Nuclease	*tni2* not annotated with new locus tags (see **Table S1** for *tni2* sequence)
tae1-tai1	BCEN_RS13080-BCEN_RS13075	None	Amidase	May have inserted into T6SS core cluster via transposon (see duplication of 3' end of *tagL* in **Figure 1**)
tae2-tai2	BCEN_RS10100-BCEN_RS10105-BCEN-RS10110	*vgrG4*	Amidase	Two annotated ORFs (10100 and 10105) have glycosylhydrolase domains and may encode an amidase effector, sequencing errors may have split into two ORFs
tpe1-tpi1	BCEN_RS22905-BCEN_RS22900	*vgrG6*	Pore-forming toxin	Tpe1 has homology to *V. cholerae* VasX (Miyata *et al.*, 2013) and *B. thailandensis* BTH_I2691 (Russell *et al.*, 2012); *tpi1* is misannotated – full-length gene is 969 bps (126 bps missing from 5' end in annotation, see **Table S1**)

Table S3. Bacterial strains used in this study; Related to STAR Methods.

Strain Name	Source	WGS Identifier
Burkholderia cepacia complex strains		
*Bc*AU1054	BcRLR*	GCA_000014085.1
*Bc*AU1054 *att*Tn7::*lacZ*	This paper	
*Bc*AU1054 Δ*hcp*	This paper	
*Bc*AU1054 Δ*hcp att*Tn7::*hcp*	This paper	
*Bc*AU1054 Δ*hcp att*Tn7::*lacZ*	This paper	
*Bc*AU1054 Δ*tle1*Δ*tli1*	This paper	
*Bc*AU1054 Δ*tle1*Δ*tli1 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tle1*Δ*tli1 att*Tn7::*tli1*	This paper	
*Bc*AU1054 Δ*tle2*Δ*tli2*	This paper	
*Bc*AU1054 Δ*tle2*Δ*tli2 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tle3*Δ*tli3*	This paper	
*Bc*AU1054 Δ*tle3*Δ*tli3 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tle4*Δ*tli4*	This paper	
*Bc*AU1054 Δ*tle4*Δ*tli4 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tne1*Δ*tni1*	This paper	
*Bc*AU1054 Δ*tne1*Δ*tni1 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tne1*Δ*tni1 att*Tn7::*tni1*	This paper	
*Bc*AU1054 Δ*tne2*Δ*tni2*	This paper	
*Bc*AU1054 Δ*tne2*Δ*tni2 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tne2*Δ*tni2 att*Tn7::*tni2*	This paper	
*Bc*AU1054 Δ*tae1*Δ*tai1*	This paper	
*Bc*AU1054 Δ*tae1*Δ*tai1 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tae2*Δ*tai2*	This paper	
*Bc*AU1054 Δ*tae2*Δ*tai2 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tpe1*Δ*tpi1*	This paper	
*Bc*AU1054 Δ*tpe1*Δ*tpi1 att*Tn7::*gfp*	This paper	
*Bc*AU1054 Δ*tpe1*Δ*tpi1 att*Tn7::*tpi1*	This paper	
*Bc*AU1054 Δ9E-I	This paper	
*Bm*CGD2M	BcRLR*	GCA_000182295.1
*Bm*CGD2M *tssC1*::pAP82	This paper	
*Bd*AU0158	BcRLR*	GCA_000959505.1
*Bd*AU0158 *tssC1*::pAP83	This paper	
*Bc*AU4392	BcRLR*	
*Bc*AU5161	BcRLR*	
*Bc*AU7523	BcRLR*	
*Bc*AU10618	BcRLR*	
*Bc*AU19695	BcRLR*	
*Bc*AU22760	BcRLR*	
*Bc*AU23782	BcRLR*	
*Bc*AU29704	BcRLR*	
Pseudomonas aeruginosa strains		
PAO1	(Holloway, 1955) Wolfgang Lab collection	GCA_000006765.1
PAO1 *vipA1*::Tn (PAO1 *vipA1*::IS*phoA*/*hah*)	(Held *et al*., 2012)	
CEC32	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC36	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994

CEC42	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC44	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC66	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC73	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC83	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC87	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC112	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
C078C	(Radlinski *et al*., 2017) (referred to as BC236)	BioProject PRJNA609958
C078C *att*Tn7::*pppA*	This paper	
C120C	(Radlinski *et al*., 2017) (referred to as BC238)	BioProject PRJNA609958
C123D	(Radlinski *et al*., 2017) (referred to as BC239)	BioProject PRJNA609958
CEC118	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC118 *att*Tn7::*fha1*	This paper	
CEC119	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC120	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC121	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC122	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
CEC116	(Burns *et al*., 2001; Rosenfeld *et al*., 2001)	BioProject PRJNA607994
*Pa*AU4391	BcRLR*	
*Pa*AU5159	BcRLR*	
*Pa*AU7618	BcRLR*	
*Pa*AU10617	BcRLR*	
*Pa*AU19694	BcRLR*	
*Pa*AU22775	BcRLR*	
*Pa*AU23781	BcRLR*	
*Pa*AU29744	BcRLR*	
Escherichia coli strains		
DH5α	Cotter Lab collection	
RHO3	(López *et al*., 2009) Cotter Lab collection	

**Burkholderia cepacia* Research Laboratory and Repository, University of Michigan, Ann Arbor, MI USA.

Table S4. Plasmids used in this study; Related to STAR Methods.

Plasmid Name	Source	Identifier
pEXKm5	(López et al., 2009)	N/A
pAP17 (pEXKm5-Δ*hcp*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP65 (pEXKm5-Δ*tle1*Δ*tli1*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP63 (pEXKm5-Δ*tle2*Δ*tli2*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP66 (pEXKm5-Δ*tle3*Δ*tli3*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP73 (pEXKm5-Δ*tle4*Δ*tli4*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP64 (pEXKm5-Δ*tne1*Δ*tni1*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP25 (pEXKm5-Δ*tne2*Δ*tni2*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP67 (pEXKm5-Δ*tae1*Δ*tai1*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP70 (pEXKm5-Δ*tae2*Δ*tai2*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP72 (pEXKm5-Δ*tpe1*Δ*tpi1*$_{Bc\text{AU1054}}$)	This paper	N/A
pUCS12Km	(Anderson et al., 2012)	N/A
pAP53 (pUCS12Km-*hcp*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP68 (pUCS12Km-*tli1*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP69 (pUCS12Km-*tni1*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP26 (pUCS12Km-*tni2*$_{Bc\text{AU1054}}$)	This paper	N/A
pAP78 (pUCS12Km-*tpi1*$_{Bc\text{AU1054}}$)	This paper	N/A
pUC18T-mini-Tn7T-Tet	(Anderson et al., 2012)	N/A
pAP85 (pUCS12Tet-*pppA*$_{\text{PAO1}}$)	This paper	N/A
pAP85 (pUCS12Tet-*fha1*$_{\text{PAO1}}$)	This paper	N/A
pJN105	(Newman and Fuqua, 1999)	N/A
pJN-*rsmZ*	(Intile et al., 2014)	N/A
pUC18T-mini-Tn7T-Km	(Choi et al., 2005)	N/A
pAP82 (pUC18T-mini-Tn7T-Km-*tssC1*$_{Bm\text{CGD2M}}$)	This paper	N/A
pAP83 (pUC18T-mini-Tn7T-Km-*tssC1*$_{Bd\text{AU0158}}$)	This paper	N/A
pUC18T-mini-Tn7T-Tp-P$_{S12}$-*mCherry*	(LeRoux et al., 2012)	N/A
pUC18T-mini-Tn7T-Tp	This paper	N/A
pECG10 (pUCS12Km-*lacZ*)	(Anderson et al., 2012)	N/A
pUC18-mini-Tn7-*kan-gfp*	(Norris et al., 2010)	N/A
pTNS3	(Choi et al., 2008)	N/A