Supplemental material

Table S1: Bacterial Strains and plasmids used in this study

Bacterial strain	Genotype/description	Source/reference
***B. cepacia complex* strains**		
B .cenocepacia H111	CF isolate, prototroph.	[1, 2]
B .cenocepacia H111Δc3	*B .cenocepacia* H111, pC3 deletion mutant.	[3]
B .cenocepacia H111-hfe3	*B .cenocepacia* H111 bearing *lacI* and *aacI* on pC3 and *dhfrII* under the control of LacI-repressed promoter pMT on chromosome 1.	This study
B. cenocepacia K56-2	CF isolate, prototroph, $BCESM^{+}$ $cblA^{+}$.	[4]
B. cenocepacia K56-2ΔpC3/pBBRatoxA	*B. cenocepacia* K56-2, pC3 deletion mutant containing pBBRatoxA.	This study
B. multivorans LMG18825	CF isolate, prototroph.	BCCM/LMG Bacteria Collection
B. multivorans LMG18825ΔpC3	*B. multivorans* LMG18825, pC3 deletion mutant.	This study
B. contaminans LMG23361	Isolate from milk of sheep with mastitis, prototroph.	BCCM/LMG Bacteria Collection
B. contaminans LMG23361ΔpC3	*B. contaminans* LMG23361, pC3 deletion mutant.	This study
B. diffusa LMG24065	CF isolate, prototroph.	BCCM/LMG Bacteria Collection
B. diffusa LMG24065ΔpC3	*B. diffusa* LMG24065, pC3 deletion mutant.	This study
B. latens LMG24064	CF isolate, prototroph.	BCCM/LMG Bacteria Collection
B. latens LMG24064ΔpC3	*B. latens* LMG24064, pC3 deletion mutant.	This study
B. metallica LMG24068	CF isolate, prototroph.	BCCM/LMG Bacteria Collection
B. metallica LMG24068ΔpC3	*B. metallica* LMG24068, pC3 deletion mutant.	This study
B. seminalis LMG24067	CF isolate, prototroph.	BCCM/LMG Bacteria Collection
B. seminalis LMG24067ΔpC3	*B. seminalis* LMG24067, pC3 deletion mutant.	This study
B. arboris 24066	Soil isolate, prototroph.	BCCM/LMG Bacteria Collection
B. arboris 24066ΔpC3	*B. arboris* 24066, pC3 deletion mutant.	This study
B. stabilis LMG7000	Blood isolate, prototroph.	BCCM/LMG Bacteria Collection
B. stabilis LMG7000 ΔpC3	*B. stabilis* LMG7000, pC3 deletion mutant.	This study
B. dolosa LMG18943	CF isolate, prototroph.	BCCM/LMG Bacteria Collection
B. dolosa LMG18943 ΔpC3	*B. dolosa* LMG18943, pC3 deletion mutant.	This study

***E. coli* strains**		
CC118(λ*pir*)	Δ*(ara, leu)*$_{7697}$ *araD*139 Δ*lacX74 galE galK phoA20 thi-1 rpsE rpoB(*Rf^R*) argE(am) recA1 λpir*$^+$	[5]
MC1061	*hsdR araD139* Δ(*ara-leu*)7697 Δ*lacX74 galU galK rpsL* (Sm^R)	[6]
XL1-blue	*endA1 gyrA96*(nal^R) *thi-1 recA1 relA1 lac glnV44 F'[::Tn10 proAB*$^+$ *lacI*^{q} *Δ(lacZ)M15] hsdR17(*r_K^- m_K^+*)*	Stratagene
Plasmids		
pMinic3	Mobilisable vector consisting of the *B. cenocepacia* H111 c3 replicatory region and pEX18Tp.	[3]
pSHAFT2	Broad-host-range suicide plasmid (Cm^R), mobilisable for conjugation.	S. Shastri and M.S.Thomas, manuscript in preparation
pEX18Tp	pEX18 containing *dhfrII* (Tp^R) between *Eco*RV and *Aat*II sites.	[3]
pEX18Gm	*sacB*$^+$, mobilisable gene replacement vector with MCS from pUC18, Gm^R.	[7]
pBBR1MCS	Mobilizable BHR cloning vector; IncP and ColE1 compatible (Cm^r).	[8]
pSHAFT2-gabD	pSHAFT2 bearing an amplified fragment of H111 pC3.	This study
pBBR1MCS-atox2	pBBR1MCS bearing the *atox2a, atox2b* and *tox2* TAS. Intermediate vector for pBBR1MCS::atoxA construction.	This study
pBBR1MCS-atox2-atox1	pBBR1MCS bearing the *atox2a, atox2b* and *tox2* TAS and the *atox1* antitoxin gene. Intermediate vector for pBBR1MCS::atoxA construction.	This study
pBBR1MCS::atoxA	pBBR1MCS bearing the *atox2a, atox2b* and *tox2* TAS, and the *atox1* and *atox3* antitoxin genes	This study
pBBR1MCS-pMT-Tp	pBBR1MCS bearing *dhfrII* under the control of the LacI-controlled pMT promoter. Intermediate vector for pEX18Gm-pMT-Tp-queF construction.	This study
pEX18Gm-pMT-Tp-queF	pEX18 bearing *dhfrII* under the control of the LacI-controlled pMT promoter	This study
pBS-pJ23109-lacI	pBLUESCRIPTIIKS bearing *lacI* under the control of promoter *pJ23109*. Intermediate vector for pSHAFT2-noncon-pJ23109-lacI-aacI construction.	This study
pSHAFT2-noncon-pJ23109-lacI-aacI	pSHAFT2 bearing *lacI* under the control of promoter *pJ23109*, flanked by regions amplified from pC3.	This study
pBluescriptIIKS	High copy number cloning vector (Ap^R).	Stratagene
pRK2013	Helper plasmid for conjugation (Km^R), ColE1 replicon.	[9]

Abbreviations used within this table: Gm^R, gentamycin resistance; Rf^R, rifampicin resistance; Sm^R, streptomycin resistance; Tp^R, trimethoprim resistance; Cm^R, chloramphenicol resistance; Km^R, kanamycin resistance; Ap^R, ampicillin resistance.

TABLE S2 Oligonucleotides used in this study

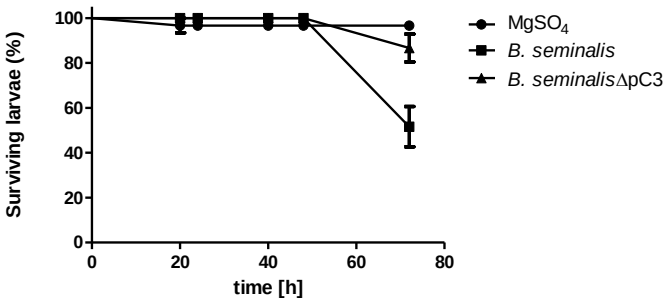
Primer name	Sequence (5'-3')	Reference/ notes
For diagnosing pC3 presence		
repAFor	AACAGCAACAACACCAAGTA	This study
repARev	TTGCTTGCCTTCTTCTTCG	This study
oriCFor1	ACCCATTTATGGGAGCACAG	[3]
oriCRev1	TGCTCCAGCATCACTTTCAC	[3]
dolpC3repAFor	CGGGCTGAACAGTAACAATA	This study
dolpC3repARev	GCTTGCTCGCTT TCT TTTTC	This study
For cloning		
queFXhofor	GCGCCTCGAGGTTCAAGCTGTATCTCGGCT	This study
queFKpnRev	GCGCGGTACCATCGGCTGGTTGTAGTTGGT	This study
queFdownxbaFor 2	GCGCTCTAGATTACTTCGCCGGCGCCTTGT	This study
queFdownPstRev2	GCGCCTGCAGGCAACAACCAGATGGACCGC	This study
MikeTFor	GTACCTTTATCAAAAAGAGTGTTGACTTGTGAGCGGATAACAAT GATACTTAGATTCAATTGTGAGCGGATAACAATTTCACACAGC	[10] Oligonucleotides annealed to form double stranded insert.
MikeTRev	TCGAGCTGTGTGAAATTGTTATCCGCTCACAATTGAATCTAAGT ATCATTGTTATCCGCTCACAAGTCAACACTCTTTTTGATAAAG	
dhfrFor	GCGCAAGCTTACAAGAAGGATTCGACATGG	This study
dhfrRev	GCGCGGATCCTTAGGCCACACGTTCAAGTG	This study
dsJ23109For2	CAGCTAGCTCAGTCCTAGGGACTGTGCTAGCA	Registry of Standard Biological Parts. Oligonucleotides annealed to form double stranded insert.
dsJ23109Rev2	AGCTTGCTAGCACAGTCCCTAGGACTGAGCTAGCTGGTAC	
GmSmaFor	GCGCCCCGGGAAACTGTAATGCAAGTAGCG	This study
GmSacIRev	GCGCGAGCTCCGAATTGTTAGGTGGCGGTA	This study
lacIHindFor	GCGCAAGCTTACGGGTAGCAAAACAGATCG	This study
lacIPstRev	GCGCCTGCAGTATCCGCTCACAATTCCACA	This study
gabDXbaFor	GCGCTCTAGAGGCGACGTTGAAGAAATTG	This study
gabDBglRev	GCGCAGATCTGTGTAGAAATAGGCGGCGA	This study
Atox1KpnI_F	GCGCGGTACCGATGTTCGGAAAGATGTCG	This study
Atox1ClaI_R	GCGCATCGATTCGTCTTGAGCGAAATAGC	This study
Atox2PstI_F	GCGCCTGCAGCTGATCCCGACTATCTTCC	This study
Atox2BamHI_R	GCGCGGATCCCACATTGGTGGAAACGCTA	This study
Atox3SacI_R	GCGCGAGCTCGTTCCGGCATACGTTCGTTA	This study
Atox3XbaI_F	GCGCTCTAGACCATTCGCCCAGATAACTA	This study
For species identification by *recA* sequencing		
BUR1	GATCGARAAGCAGTTCGGCAA	[11]
BUR2	TTGTCCTTGCCCTGRCCGAT	
For differentiating between H111 and K56-2 cells		
R33For R33Rev		Anneal to the H111, and not to the K56-2, genome
cblCFor	GATTCACATAAGCCAGCGTC	Anneal to the *cblC* gene, present in K56-2
cblCRev	ACGCATCAACGTCAATTTTC	

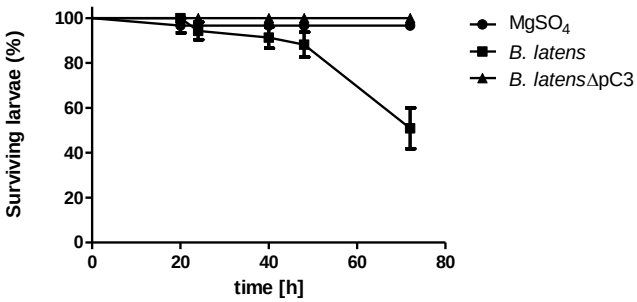
TABLE S3 Laboratory collection strains screened for pC3 presence

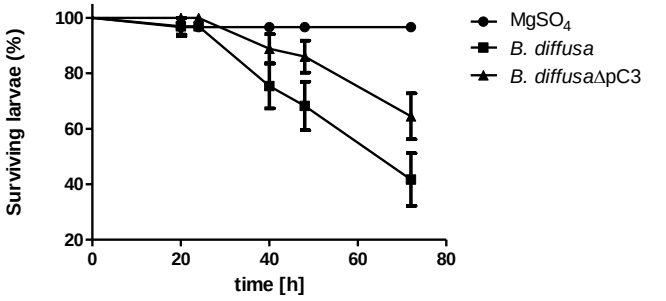
Species/ Strain ID	Characteristics	Source/ Reference
Burkholderia ambifaria		
LMG17828	Isolated from corn roots	[12]
LMG19182	Rhizosphere organism, effective in stimulating plant growth and suppressing soil borne plant pathogens, AMMD	[12]
LMG19467	Isolated from CF patient, Australia	[13]
Burkholderia anthina		
LMG20980	Type strain; soil rhizosphere	[13]
LMG20983	Isolated from CF patient, UK	[13]
LMG21821	Isolated from CF patient, USA	BCCM/LMG collection
Burkholderia arboris		
LMG24066	Type strain; isolated from soil in Philadelphia, USA	[14]
Burkholderia cenocepacia		
LMG18826	BC7, R-3220, type strain, GV IIIA (ET-12); CF isolate, Canada	BCCM/LMG collection
LMG12615	GV IIIA (ET-12), Cbl+, BCESM+; CF isolate, UK	[15]
LMG16659	GV IIIB, Cbl^-, $BCESM^+$; CF isolate, UK	[15]
LMG14271	GV IIIB; isolated from CF patient, Belgium	[15]
LMG6981	GV IIIA; bronchial washing isolate, USA	[15]
LMG16655	GV III	Laboratory strain collection
LMG16657	GV III	Laboratory strain collection
R6108	GV III	Laboratory strain collection
R651	GV III	Laboratory strain collection
LMG24308	MCO-3; isolated from a research field, maize rhizosphere, USA	BCCM/LMG collection
LMG24506	GV IIIB, PHDC lineage, CF isolate, USA	BCCM/LMG collection
LMG24507	HI2424, soil isolate, GV IIIB, PHDC lineage, New York, USA	BCCM/LMG collection
H147	GV III, R-6274	Laboratory strain collection
Burkholderia cepacia		
LMG1222	Type strain, isolated from onion, USA	[16]
IST408	CF isolate, Portugal	Laboratory strain collection
H125	Isolated from CF patient, Germany	Laboratory strain collection
H194	Isolated from CF patient, Germany	Laboratory strain collection
LMG18821	GV I, R–1464	BCCM/LMG collection
LMG6963	GV I	[15]
LMG14087	GV I	Laboratory strain collection
S3.1	Isolated from a painting facility	Laboratory strain collection
RA231	Isolated from a painting facility	Laboratory strain collection
HH156.3	Isolated from a painting facility	Laboratory strain collection
BASF	Isolated from a painting facility	Laboratory strain collection
speaky5	Isolated from a painting facility	Laboratory strain collection
speaky6	Isolated from a painting facility	Laboratory strain collection
speaky8	Isolated from a painting facility	Laboratory strain collection
W220	Isolated from a painting facility	Laboratory strain collection
ATCC25416	LMG1222, type strain, GV I	ATCC strain collection
BC2A	Clinical isolate	Laboratory strain collection
Burkholderia contaminans		
LMG23361	Isolated from sheep with mastitis (milk), Spain	BCCM/LMG collection
Burkholderia diffusa		
LMG24065	Isolated from CF patient, USA	BCCM/LMG collection
Burkholderia dolosa		
LMG21819	Isolated from CF patient, USA	BCCM/LMG collection
LMG18941	Isolated from CF patient, USA	BCCM/LMG collection
LMG18942	Isolated from CF patient, USA	BCCM/LMG collection
LMG18943	Isolated from CF patient, USA	BCCM/LMG collection

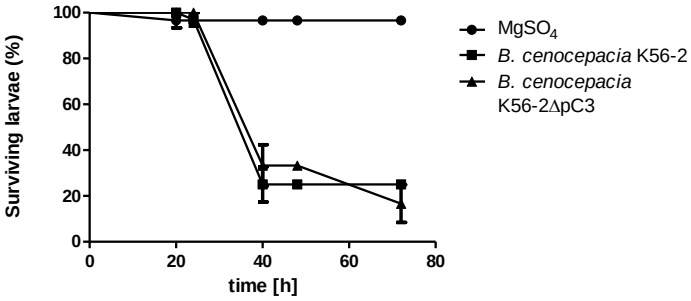
LMG18944	Isolated from CF patient, USA	BCCM/LMG collection
LMG18946	Isolated from CF patient, USA	BCCM/LMG collection
LMG21443	Isolated from *Alysicarpus glumaceus* (root nodule), Senegal	BCCM/LMG collection
LMG21820	Isolated from CF patient, UK	BCCM/LMG collection
LMG18943	Type strain; isolated from CF patient, USA	[17]
Burkholderia lata		
LMG6993	DSMZ 50180; Isolated from soil in Trinidad	[18]
LMG22485	Type strain; Isolated from forest soil in Trinidad and Tobago	BCCM/LMG collection
Burkholderia latens		
LMG24064	Type strain; isolated from CF patient, Italy	BCCM/LMG collection
Burkholderia metallica		
LMG24068	Type strain; isolated from CF patient, USA	BCCM/LMG collection
Burkholderia multivorans		
LMG18825	Isolated from CF patient, UK	BCCM/LMG collection
LMG17588	Soil enriched with anthranilate	BCCM/LMG collection
LMG16665	Isolated from brain abscess, UK	BCCM/LMG collection
LMG18945	Isolated from CF patient, UK	BCCM/LMG collection
R139	GV II	Laboratory strain collection
R654	GV II	Laboratory strain collection
H59	GV II	Laboratory strain collection
H107	GV II	Laboratory strain collection
H115	GV II, R-6269	Laboratory strain collection
H131	GV II	Laboratory strain collection
H132	GV II	Laboratory strain collection
H133	GV II	Laboratory strain collection
H158	GV II, R-6275	Laboratory strain collection
H174	GV II, R-6278	Laboratory strain collection
H179	GV II	Laboratory strain collection
H191	GV II, R-6284	Laboratory strain collection
LMG16660	Isolated from CF patient, UK	BCCM/LMG collection
LMG18822	Isolated from CF patient, Canada	BCCM/LMG collection
LMG18824	Isolated from a chronic granulomatous disease patient	BCCM/LMG collection
R1914	GV II	Laboratory strain collection
Burkholderia pyrrocinia		
LMG14191	Type strain; isolated from soil	BCCM/LMG collection
LMG21822	Isolated from cornfield soil	BCCM/LMG collection
LMG21823	Isolated from water	BCCM/LMG collection
Burkholderia seminalis		
LMG24067	Type strain; isolated from CF patient, USA	[14]
Burkholderia stabilis		
LMG7000	Isolated from blood, Sweden	BCCM/LMG collection
LMG14291	isolated from CF patient, Belgium	BCCM/LMG collection
R6270	GV IV, H118	Laboratory strain collection

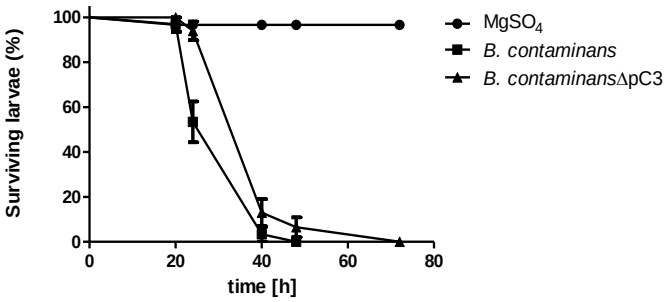
H134	GV IV	Laboratory strain collection
H145	GV IV, R-6273	Laboratory strain collection
H162	GV IV, R-6276	Laboratory strain collection
H173	GV IV, R-6277	Laboratory strain collection
H175	GV IV, R-10033	Laboratory strain collection
H177	GV IV, R-6279	Laboratory strain collection
H193	GV IV, R6280	Laboratory strain collection
H236	GV IV	Laboratory strain collection
H177 Epi	GV IV, R-6281	Laboratory strain collection
LMG6997	Isolated from ear infection, Sweden	BCCM/LMG collection
LMG18138	isolated from CF patient, Belgium	BCCM/LMG collection
LMG14294	Type strain, isolated from CF patient, Belgium	BCCM/LMG collection
R3338	GV VI	[19]
Burkholderia ubonensis		
LMG20358	Type strain; isolated from surface soil, Thailand	[16]
LMG24263	Isolated from a nosocomially infected patient, Thailand	BCCM/LMG collection
Burkholderia vietnamiensis		
LMG18835	isolated from CF patient, USA	BCCM/LMG collection
LMG18836	Isolated from patient suffering from chronic granulomatous disease, Canada	BCCM/LMG collection
LMG6998	Isolated from blood, Sweden	BCCM/LMG collection
LMG10929	Type strain; isolated from rhizosphere of rice	BCCM/LMG collection
LMG16232	isolated from CF patient, Sweden	BCCM/LMG collection
LMG 6999	Isolated from a neck abscess, Chile	BCCM/LMG collection
R921	Type strain, GV V	Laboratory strain collection
R128	Type strain, GV V	[2]
R706	Type strain, GV V	Laboratory strain collection
R723	Type strain, GV V	Laboratory strain collection
R1904	Type strain, GV V	
LMG18835	isolated from CF patient, USA	BCCM/LMG collection
G4		Laboratory strain collection
Strains included as negative controls		
Burkholderia gladioli		
H129		Laboratory strain collection
Burkholderia phymatum		
LMG21445	Type strain; isolated from root nodule, French Guiana	BCCM/LMG collection
Burkholderia tuberum		
LMG21444	Type strain; isolated from root nodule, South Africa	BCCM/LMG collection

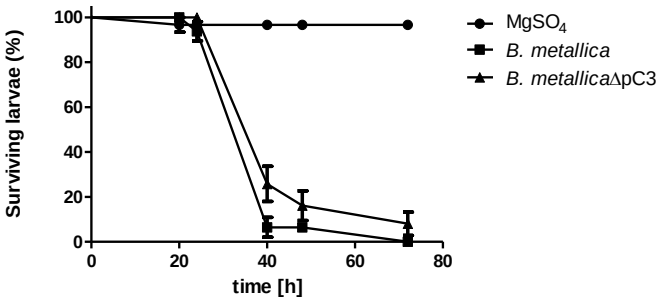

Surviving larvae (%)
time [h]
MgSO4
B. seminalis
B. seminalisΔpC3

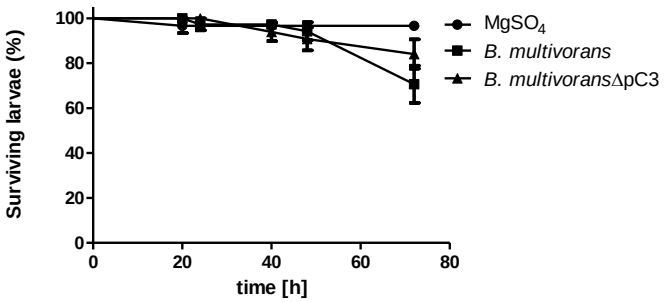

Surviving larvae (%)
time [h]
MgSO4
B. latens
B. latensΔpC3

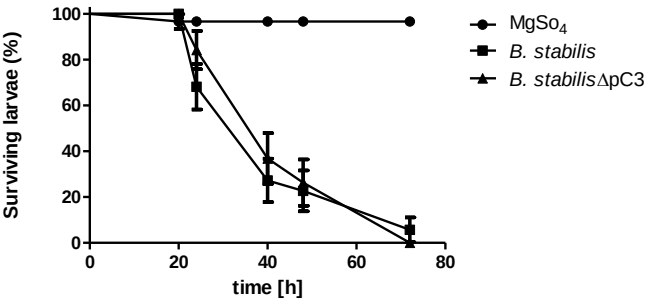

Surviving larvae (%)
time [h]
MgSO4
B. diffusa
B. diffusaΔpC3

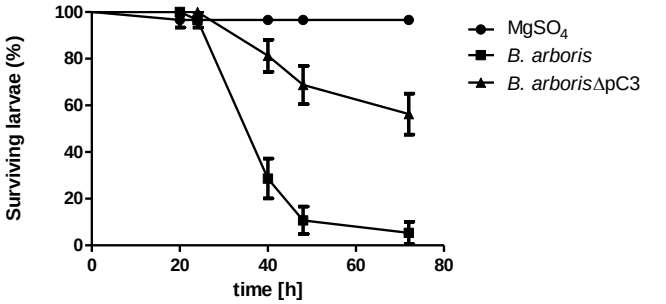

Surviving larvae (%)
time [h]
MgSO4
B. cenocepacia K56-2
B. cenocepacia K56-2ΔpC3


Surviving larvae (%)
time [h]
MgSO4
B. contaminans
B. contaminansΔpC3


Surviving larvae (%)
time [h]
MgSO4
B. metallica
B. metallicaΔpC3


Surviving larvae (%)
time [h]
MgSO4
B. multivorans
B. multivoransΔpC3


Surviving larvae (%)
time [h]
MgSo4
B. stabilis
B. stabilisΔpC3


Surviving larvae (%)
time [h]
MgSO4
B. arboris
B. arborisΔpC3

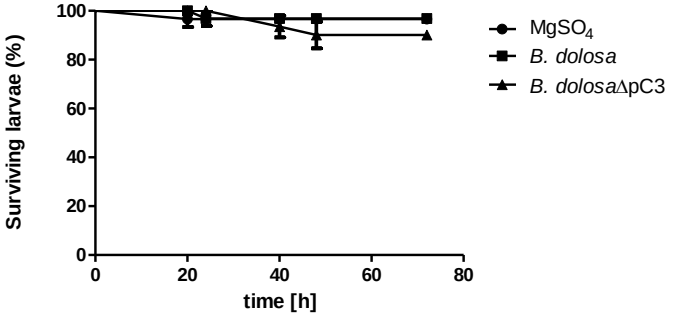

Surviving larvae (%)
time [h]
MgSO4
B. dolosa
B. dolosaΔpC3

FIG S1 WT Bcc strains show higher pathogenicity to *G. mellonella* than their pC3-null derivatives. *G. mellonella* pathogenicity assay with different strains of the Bcc and their ΔpC3 mutants. 10 µl of the particular strains were injected and dead/ live larvae were counted after 20, 24, 40, 48 and 72 hrs post-infection. Each experiment used 10 larvae and was carried out three times at 30°C.

psaB

CTGCAGCGTCGGCGGTGGTCGCCGGTAGCTCGCCCTTGCTTTTTCATATTCCTTACCATAAAGTAAGGAATATTGGAGGTG
CCGCAAATGACAGCAGCTACTATTACTTCAAAGGGTCAGGTGACCATTCCGGTGGATGTACGCAATCAACTAGGCCTGGA
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CGCTCAAGGGCATCGTGAAAAAGCCCGAGAAATCGGTGTCAATCGAGGACATGAATCGGGCCATCGCCGAACAGGGAGC
GTCCGCGCGATGA

psaA

ATGATAGGACTGGATACGAACGTGCTGGTCCGCTATTTCGCTCAAGACGATGCCGTGCAATCAAAGAGGGCGACCGCGCT
GATGGAGTCGTTGTCGGCGGAGCGTCCGGGGTACGTTTCGCAGGTGGCGCTGGTCGAGGTCGTCTGGGTGCTGGGACGT
TGCTACGGCGTAGAGCGCGAACAGATAACAGACATCATCGACTCAATGACCGCTACGAAGGAACTGGCCGTTGAAGGCG
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GCACACGTCGTCGGGTGCGAGTACACAGCAACCTTCGACGTGGCCGCCTCAAAGGTCGCTGGCATGCGGTTGATAAAGTA
A

psbB

ATGTGGTTCTGGCACGCACCAACCTCGGATCGCCAGAACCAATTTGCTGGCCAGTTCACTGGCCAGCGTTTTTTATTCCTCG
AGCCGATCTCGTCGGCTCGTTTGCCGGGCGCTTATCCGGACAAACCGAAAGACCGTTTTGTTCGGGAATTTCGTTCAGAGC
GAGGCGAGCGGTGGCTGGATGACCGCTCGCTTTGCGCGCGATCCTTTATCGGGTAA

psbC

GTGGGAAGTGCCGAATCGCGACGATGTCTTCGCGTTTCCGGGCGCGCGGGCAGACCTGATCGCCTCCGCCAGCCTGGCCT
GAACTGCGATCACAGATTGTTGCGCGGGCGCGGTCCGGGACCGCTCCGATTCTGGGGACGCGGTCGTCTGACGAGCCCG
TCCGTCTGTACGCGCAGGCCGTCTAATGCATGTGGTTCTGGCACGCACCAACCTCGGATCGCCAGAACCAATTTGCTGGCC
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ACCGTTTTGTTCGGGAATTTCGTTCAGAGCGAGGCGAGCGGTGGCTGGATGACCGCTCGCTTTGCGCGCGATCCTTTATCG
GGTAAGGTCAGGCAACGCTGCATCGTCGGTAGCGGCACATCGGACACTGTCCCACGCATCGCGTCAATCGCGTCAGCTCC
ATGCCCCTGA

psbA

CTGCATCGTCGGTAGCGGCACATCGGACACTGTCCCACGCATCGCGTCAATCGCGTCAGCTCCATGCCCCTGATCTTCGTCC
CCGTCACGCACGCCGACGCCGAAGCGCTCGTCGCGATCCGCATCGCCGCGATGCGCGACAGCCTCGCGCGCATCGGCCGT
TTCGATCCGCAGCGTGCGCGCGAGCGGTTTCTCGCGTCGTTCGATCCGGCGCGATGCCGTTTCGTCGAAGTCGACCATTCG
CGTGCGGGTTTCTACACGCTTCGCCCGAGGGCGGATCACTGGTTGCTCGACCATCTGTACATCGTGCCCGCGCATCAGGG
GAAGGGCATCGGCGCCGCCGTGCTGCGCGAGATTTTGGCCGAAGCCGACGAACACCGGATGCCCGTTCACGTCGGCGCG
CTGCGCGGCAGCGACTCGAACCGCTTCTACGAACGGCATGGCTTCGTGCGAACCGGCGAAGCGGAATGGGACATCTACTA
CCGGCGCGAACCGGGCACGGCGACGACGTAG

pscA

ATGCACTAGCGATGCTTTCTTGATCTGGTCCGCGCCGCAACCAGGACAGCATTTGGCCTTTTCTTTTTTGCTCATCTTTTATG
CACCAGAGATCACATGAACGCACATTTCGCCACTTTTAGAGCGCCCCCTATTGGGGCGGCGCTGAATAGTGCGTGGTCGA
GATTATACCCACCCCGCAACATCACGCGGATGAAGACCCACACATCCTTGCGGCAGCGCTTCGGAGCGCGCGTTAGACAG
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CGAAACGGGCAAGGCGAACCCATCACTCGATGCAATTCAAACCATCGCGGAAGGGTTGGGCGTCGAAGTGAGCGCTCTTT
TCGAGGACGACGATTAG

FIG S2 Putative toxin antitoxin gene sequences identified on $pC3_{K56\text{-}2}$ using RASTA Bacteria.

TABLE S4 Phenotypic microarray results showing carbon utilisation phenotypes (Biolog plate PM1) that differ between wt strains and their pC3-null derivatives[12].

Phenotype observed	*B. cenocepacia*				*B. ambifaria*	*B. anthina*	*B. vietnamiensis*	*B. pyrocinia*	*B. lata*	*B. ubonensis*	*B. arboris*	*B. metallica*	*B. seminalis*	*B. contaminans*	*B. diffusa*	*B. stabilis*	*B. multivorans*	*B. latens*	*B. dolosa*
	H111	MCO-3	H12424	K56-2	AMMD	LMG20983	LMG10929	LMG14191	383	LMG20358	LMG24066	LMG24068	LMG24067	LMG23361	LMG24065	LMG7000	LMG18825	LMG24064	LMG18943
PM1																			
D-xylose	+++	+++	+++	+++	+++	+++		+++		+++				+++	+++			+++	
thymidine	+++	+++	+++	+				+	+	+									
tricarballylic acid		+++		+++									+++				+++	+++	
D-aspartic acid					+++			+++			+++				+++				
Succinic acid							++		+										+
D-galacturonic acid							+++						+					+	
p-hydroxyphenylacetic acid									+									+	+
M-tartaric acid									+++							+++			
D-cellobiose	+							++											
m-hydroxyphenylacetic acid						+					-								
2-amino ethanol											-					-			
D-trehalose								+								-			
D-threonine			+																
L-alanyl glycine					-									+					
D-galactonic acid-γ-lactone							+												
D-glucuronic acid							+				-								
L-lactic acid							+												
D-mannitol							+												
D-fructose-6-phosphate							++												
maltose								+											
D-melibiose								++											
α-D-lactose								+											
lactulose								+++											
uridine								+											
α-hydroxy glutaric acid-g-lactone								+											
maltotriose								++											
2-deoxy adenosine								++											
glycolic acid								+											
glyoxylic acid								+											
inosine								+											
N-acetyl-b-D-mannosamine								+											
D-psicose								+											
glucuronamide								+											
Tween 40			+																
D-ribose			+																
tyramine			-																
D-mannose							-												
D-glucose-6-phosphate							+												
dulcitol								+											
D,L-malic acid											-								
L-glutamic acid											-								
L-malic acid											-								
α-keto butyric acid														+					
α-hydroxybutyric acid														+					
formic acid														+					
L-galactonic acid-γ-lactone															+++				
propionic acid															+				
methyl-pyruvate																+			
L-threonine																		+	
pyruvic acid																		+	

[1]Differences in respiration between WT and c3-null derivative have been scored as follows: +++, wt OD_{590} > 4 x c3-null OD_{590}; ++, wt OD_{590} > 3 x c3-null OD_{590}; +, wt OD_{590} > 2 x c3-null OD_{590}; ---, c3-null OD_{590} > 4 x wt OD_{590}; --, c3-null OD_{590} > 3 x wt OD_{590}; -, c3-null OD_{590} > 2 x wt OD_{590}.

[2]Phenotypes which are influenced by c3 in more than two species have been shaded.

TABLE S5 Phenotypic microarray results showing carbon utilisation phenotypes (Biolog plate PM2a) that differ between wt strains and their pC3-null derivatives[1, 2].

Phenotype observed	*B. cenocepacia*				*B. ambifaria*	*B. anthina*	*B. vietnamiensis*	*B. pyrocinia*	*B. lata*	*B. ubonensis*	*B. arboris*	*B. metallica*	*B. seminalis*	*B. contaminans*	*B. diffusa*	*B. stabilis*	*B. multivorans*	*B. latens*	*B. dolosa*
	H111	MCO-3	H12424	K56-2	AMMD	LMG20983	LMG10929	LMG14191	383	LMG20358	LMG24066	LMG24068	LMG24067	LMG23361	LMG24065	LMG7000	LMG18825	LMG24064	LMG18943
PM2a																			
caproic acid	+++	+	++		+++	+++		+	+	+	+++	+		+++	+++	+++	+	+++	
butyric acid	+++				++	+		+	+		+	++	+	++	+	+		+	++
sorbic acid	+				++	+	-			++	+	++		+	+	++	+		
L-isoleucine	+++	+++	+++		++	+++		++		++	+++						+		
L-lysine			+	++		+		+	+		+	+		+					
2-deoxy-D-ribose				++				+	++	+++			+			++			
putrescine	++			++				+	+++							+++			
hydroxy-L-proline					+++	+++									+++		+++	+++	
gelatin	+++	+	+++								++					+			
citraconic acid	+++	+	++											+					
capric acid					++			++	++						+++				
salicin								+	++			+++		+++					
δ-Amino Valeric Acid		+++	+++					+											
pectin	+++		-					++						+					
2,3-butanediol			+					+								+			
L-ornithine	+++		+++								+								
arbutin									+++			+++		+++					
l-arabitol								+					++						
Itaconic acid							-									+++			
succinaminic acid			+					+											
L-methionine			++					++											
2,3-butanone			+					+											
5-keto-D-gluconic acid	+		-																
D-ribono-1,4-lactone		+																	
2-hydroxybenzoic acid									+										
laminarin								+											
l-erythritol								+											
β-methyl-D-xyloside								+											
palatinose								+											
β-methyl-D-galactoside								+											
γ-hydroxy butyric acid								+											
L-leucine								++											
oxalomalic acid								+											
3-hydroxy-2-butanone								++											
lactitol			+																
γ-cyclodextrin								+											
maltitol								+											
L-pyroglutamic acid									+										
Citramalic acid									+										
D-arabitol							-												
L-tartaric acid											-								
quinic acid																		+++	
D-arabinose																			+
arginine				++															
D-lactic acid methyl ester				-															

[1]Differences in respiration between WT and c3-null derivative have been scored as follows: +++, wt OD_{590} > 4 x c3-null OD_{590}; ++, wt OD_{590} > 3 x c3-null OD_{590}; +, wt OD_{590} > 2 x c3-null OD_{590}; ---, c3-null OD_{590} > 4 x wt OD_{590}; --, c3-null OD_{590} > 3 x wt OD_{590}; -, c3-null OD_{590} > 2 x wt OD_{590}.

[2]Phenotypes which are influenced by c3 in more than two species have been shaded.

TABLE S6 Phenotypic microarray results showing nitrogen utilisation phenotypes (Biolog plate PM3b) that differ between wt strains and their pC3-null derivatives[12].

Phenotype observed	*B. cenocepacia* H111	*B. cenocepacia* MCO-3	*B. cenocepacia* H12424	*B. cenocepacia* K56-2	*B. ambifaria* AMMD	*B. anthina* LMG20983	*B. vietnamiensis* LMG10929	*B. pyrocinia* LMG14191	*B. lata* 383	*B. ubonensis* LMG20358	*B. arboris* LMG24066	*B. metallica* LMG24068	*B. seminalis* LMG24067	*B. contaminans* LMG23361	*B. diffusa* LMG24065	*B. stabilis* LMG7000	*B. multivorans* LMG18825	*B. latens* LMG24064	*B. dolosa* LMG18943
PM3b																			
histamine	+++	+++	+++	+++	++			+++	+++		+++	++	+	++		++	+		
uracil	+++	+++	+++			++		++	+	++								+	
cytidine	+		+							++	+			+		+			
thymidine	+	+++	+++					++		++									
thymine	+++	++	+++					++											
L-methionine		-		+	-		-												
ammonia			+++				+			-							++		
alloxan	-	-	+																
cytosine						+				+			++						
xanthine												+	+				-		
D-aspartic acid					++			+++							++				
ε-Amino-N-caproic acid		-			-														
nitrate					-	-													
biuret		- - -					-												
guanosine					-		++												
Ala-Asp							+						+						
putrescine									++									--	
N-acetyl-D-galactosamine	+																		
D-galactosamine	+++																		
xanthosine		-																	
N-acetyl-D-mannosamine		-																	
uridine		+																	
Gly-Glu							+												
Met-Ala							++												
urea									+										
Ala-gly					-														
Ethylenediamine								++											
D-L-α-amino-N-butyric acid								+											
adenine									+										
L-pyroglutamic acid							-												
D-asparagine							-												
agmatine											-							+	
ala-glu													+						
acetamide													+						
inosine														+					
α-amino-N-valeric acid																-			
formamide																	+		
L-isoleucine				+															
L-leucine				+															

[1]Differences in respiration between WT and c3-null derivative have been scored as follows: +++, wt OD_{590} > 4 x c3-null OD_{590}; ++, wt OD_{590} > 3 x c3-null OD_{590}; +, wt OD_{590} > 2 x c3-null OD_{590}; ---, c3-null OD_{590} > 4 x wt OD_{590}; --, c3-null OD_{590} > 3 x wt OD_{590}; -, c3-null OD_{590} > 2 x wt OD_{590}.

[2]Phenotypes which are influenced by c3 in more than two species have been shaded.

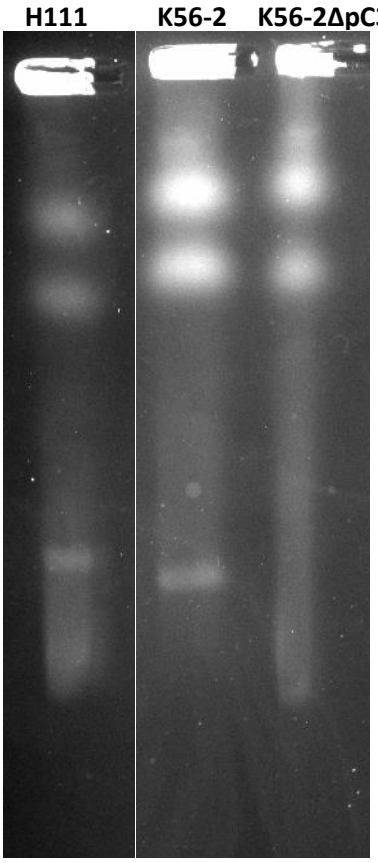

FIG S3 Visualisation of undigested genomic DNA from wild-type *B. cenocepacia* K56-2 and its pC3-null derivative. Genomic DNA was separated by pulsed-field gel electrophoresis. *B. cenocepacia* H111 was included as a standard (left-most lane), since the size of each replicon is known for this strain. Lanes between those showing H111 and K56-2 and it's pC3-null counterpart have been deleted for clarity, but band positions have not been altered.

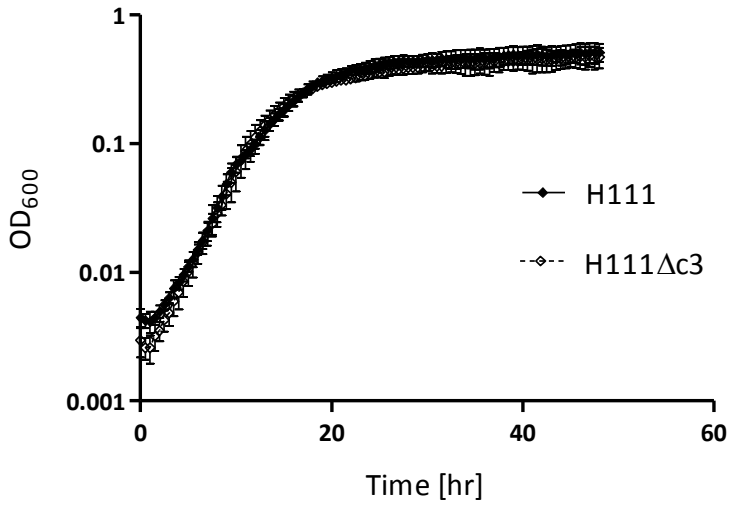

FIG S4 H111 and its pC3-null mutant grow equally well in minimal salts medium. Growth curves were carried out in M9 minimal salts broth at 37 ^{o}C, and OD_{600} was read every 30 mins. Average growth rates were as follows: H111, 0.47 doublings h^{-1}; H111Δc3, 0.51 doublings h^{-1}. Average saturation OD_{600} H111, 0.49; H111Δc3, 0.45.

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