

Supplementary Information

Viunalikeviruses are environmentally common agents of horizontal gene transfer in pathogens and biocontrol bacteria

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Supplementary Table S1: Frequency of transduction of different markers using the *Salmonella* phage Vi1

Donor strain	Relevant marker/plasmid	Recipient strain	m.o.i.	Transduction efficiency ^{a,b,c}	Standard deviation ^b
BDR948	pECA1039-Km3	BDR948	10	$<1.0 \times 10^{-10}$	-
	pECA1039-Km3	BDR948	1	3.7×10^{-7}	7.7×10^{-8}
	pECA1039-Km3	BDR948	0.1	1.3×10^{-5}	1.1×10^{-6}
	pECA1039-Km3	BDR948	0.01	2.3×10^{-5}	2.5×10^{-6}
SW114	<i>STY1890-1891::cat</i>	BDR948	10	$<1.0 \times 10^{-10}$	-
	<i>STY1890-1891::cat</i>	BDR948	1	1.1×10^{-7}	8.8×10^{-9}
	<i>STY1890-1891::cat</i>	BDR948	0.1	5.6×10^{-6}	7.1×10^{-7}
	<i>STY1890-1891::cat</i>	BDR948	0.01	1.2×10^{-5}	3.5×10^{-6}
BA231	<i>STY3632::cat</i>	BDR948	10	$<1.0 \times 10^{-10}$	-
	<i>STY3632::cat</i>	BDR948	1	4.5×10^{-8}	5.7×10^{-8}
	<i>STY3632::cat</i>	BDR948	0.1	5.2×10^{-6}	2.8×10^{-7}
	<i>STY3632::cat</i>	BDR948	0.01	4.6×10^{-5}	8.1×10^{-6}

^aTransduction efficiency is expressed as the number of transductants per plaque forming unit (p.f.u.)

^bMean and standard deviation of three independent experiments are shown.

^cTransduction experiments were performed using 10^9 bacterial cells.

Supplementary Table S2. Phages, bacteria, oomycete, and plasmids used in this study

Phage/Bacteria/oomycete/plasmids	Genotype or relevant characteristic ^a	Reference or source
Phages		
ViI	<i>Salmonella</i> Typhi infecting phage	Pickard <i>et al.</i> , (2010)
LIMEstone1	<i>Dickeya solani</i> infecting phage	Adriaenssens <i>et al.</i> , (2012)
LIMEstone2	<i>Dickeya solani</i> infecting phage	Adriaenssens <i>et al.</i> , (2012)
CBA120	<i>Escherichia coli</i> O157:H7 infecting phage	Kutter <i>et al.</i> , (2011)
φXF1	<i>Dickeya solani</i> infecting phage	This study
φXF3	<i>Dickeya solani</i> infecting phage	This study
φXF4	<i>Dickeya solani</i> infecting phage	This study
φXF28	<i>Dickeya solani</i> infecting phage	This study
Bacteria strains		
<i>Salmonella</i> Typhi BRD948	ViI host strain; Ty2 Δ aroC aroD htrA; Wild type “vaccine strain”	Bishop <i>et al.</i> , (2008)
<i>Salmonella</i> Typhi SW114	BDR948 mutant <i>STY1890-1891::cat</i> ; Cm ^r	D. Pickard
<i>Salmonella</i> Typhi SW231	BDR948 mutant <i>STY3632::cat</i> ; Cm ^r	D. Pickard
<i>Escherichia coli</i> β2163	F ⁻ RP4-2-Tc::Mu Δ dapA::(<i>erm-pir</i>), Km ^r Em ^r	Demarre <i>et al.</i> , (2005)
<i>E. coli</i> O157:H7 NCTC 12900	CBA120 host strain; The strain lacks the shiga-toxin (Stx) genes; Km ^r	ATCC 700728
<i>Dickeya solani</i> MK10	Wild type strain isolated from potato in Israel; LIMEstone phage host strain	Pritchard <i>et al.</i> , (2013)
MK10P1	MK10 transposon mutant defective in secreted protease C precursor <i>spp::Tn-KRCPN1</i> ; Protease-negative, Km ^r	This study
MK10P7	MK10 transposon mutant defective in alkaline protease inhibitor precursor <i>api::Tn-KRCPN1</i> ; Protease-negative, Km ^r	This study
MK1610P1	MK10 transposon mutant defective in secreted protease C precursor <i>spp::mini-Tn5Sm/Sp</i> ; Transduced from MK16P1 using LIMEstone phages; Protease-negative, Sm ^r	This study
MK10MM1	MK10 transposon mutant defective in 3-isopropylmalate dehydratase (large subunit) <i>ipd::Tn-KRCPN1</i> ; auxotrophic, Km ^r	This study
MK10MM2	MK10 transposon mutant defective in glutamate synthase (large subunit) <i>gts::mini-Tn5Sm/Sp</i> ; auxotrophic, Sm ^r	This study
MK10oocF	MK10 transposon mutant <i>oocF::Tn-KRCPN1</i> ; oocydin A negative, Km ^r	This study
MK10oocN	MK10 transposon mutant <i>oocN::Tn-KRCPN1</i> ; oocydin A negative, Km ^r	This study
<i>Dickeya solani</i> MK16	Wild type strain isolated from river water in the United Kingdom; LIMEstone phage host strain	Pritchard <i>et al.</i> , (2013)
MK16P1	MK16 transposon mutant defective in secreted protease C precursor <i>spp::mini-Tn5Sm/Sp</i> ; Protease-negative, Sm ^r	This study
MK16P2	MK16 transposon insertion in promoter region of gene for secreted protease C precursor <i>spp::mini-Tn5Sm/Sp</i> ; Protease-negative, Sm ^r	This study
MK16MM1	MK16 transposon mutant defective in dihydroorotate dehydrogenase <i>dhd::mini-Tn5Sm/Sp</i> ; auxotrophic, Sm ^r	This study
MK16MM3	MK16 transposon mutant defective in anthranilate phosphoribosyltransferase <i>apr::mini-Tn5Sm/Sp</i> ; auxotrophic, Sm ^r	This study
MK16MM4	MK16 transposon mutant defective in nitrite/sulfite reductase (beta-component) <i>nsr::mini-Tn5Sm/Sp</i> ; auxotrophic, Sm ^r	This study
MK16MM5	MK16 transposon mutant defective in phosphoribosylaminoimidazole carboxylase <i>ppc::mini-Tn5Sm/Sp</i> ; auxotrophic, Sm ^r	This study
MK1016P1	MK16 transposon mutant defective in secreted protease C precursor <i>spp::Tn-KRCPN1</i> ; Transduced from MK10P1 using LIMEstone phages; Protease-negative, Km ^r	This study
1016MM2	MK16 transposon mutant defective in glutamate synthase (large subunit) <i>gts::mini-Tn5Sm/Sp</i> ; Transduced from MK10MM2 using LIMEstone phages; auxotrophic, Sm ^r	This study
MK16oocF	MK16 transposon mutant <i>oocF::Tn-KRCPN1</i> ; Transduced from MK10oocF using LIMEstone phages; oocydin A negative, Km ^r	This study
MK16oocN	MK16 transposon mutant <i>oocN::Tn-KRCPN1</i> ; Transduced from MK10oocN using LIMEstone phages; oocydin A negative, Km ^r	This study
<i>Dickeya solani</i> IPO 2222	Wild type strain isolated from potato in the Netherlands; LIMEstone host strain	Pritchard <i>et al.</i> , (2013)
10IPOP1	IPO 2222 transposon mutant defective in secreted protease C precursor <i>spp::Tn-KRCPN1</i> ; Transduced from MK10P1 using LIMEstone phages; Protease-negative, Km ^r	This study

IPOoocF	IPO 2222 transposon mutant <i>oocF::Tn-KRCPN1</i> ; Transduced from MK10oocF using LIMEstone phages; oocydin A negative, Km ^r	This study
IPOoocN	IPO 2222 transposon mutant <i>oocN::Tn-KRCPN1</i> ; Transduced from MK10oocN using LIMEstone phages; oocydin A negative, Km ^r	This study
<u>Oomycete strains</u>		
<i>Pythium ultimum</i>	Wild type, plant pathogen	R. Cooper
<u>Plasmids</u>		
pKRCPN1	Km ^r , Tc ^r ; Derivative of pDS1028 <i>uidA</i> with the <i>uidA</i> and <i>cat</i> genes replaced with <i>lacZ</i> and <i>km</i> genes. Plasmid used in transposon mutagenesis	K.J. Roberts (2010)
pUTmini-Tn5Sm/Sp	Ap ^r , Sm ^r , Sp ^r ; Delivery plasmid for mini-Tn5Sm/Sp; Plasmid used in transposon mutagenesis	De Lorenzo <i>et al.</i> , (1990)
pACYC184	Cm ^r , Tc ^r ; Plasmid cloning vector	Chang and Cohen (1978)
pECA1039-Km3	Km ^r ; Transposon-marked derivative of the original ToxIN plasmid, pECA1039, from <i>Pectobacterium atrosepticum</i>	Fineran <i>et al.</i> , (2009)
pFR2	Ap ^r ; pBR322 containing the full <i>tenpin</i> locus from <i>Photorhabdus luminescens</i> TT01	Blower <i>et al.</i> , (2012)
p34S-Km3	Km ^r , Ap ^r <i>km3</i> antibiotic resistance cassette	Dennis and Zylstra (1998)
pMAMV116	Km ^r ; 0.99-kb PstI fragment containing <i>km3</i> cassette of p34S-Km3 was inserted into PstI site of <i>amp</i> resistance gene in pFR2	Matilla and Salmond (submitted for publication)
pTA46	Ap ^r ; pBR322 containing the full toxin locus from <i>Pectobacterium atrosepticum</i>	Fineran <i>et al.</i> , (2009)
pMAMV114	Km ^r ; 0.99-kb PstI fragment containing <i>km3</i> antibiotic resistance cassette of p34S-Km3 inserted into PstI site of <i>amp</i> resistance gene in pTA46	Matilla and Salmond (submitted for publication)

^aAp, ampicillin; Km, kanamycin; Cm, chloramphenicol; Sm, streptomycin; Sp, spectinomycin; Tc, tetracycline; Em, erythromycin; ATCC, American Type Culture Collection.

Supplementary Table S3: Oligonucleotide PCR primers used in this study

Name	Sequence (5'-3')	Note
PF106	GACCACACGTCGACTAGTGCNNNNNNNNNNAGAG	Random primed PCR primer 1
PF107	GACCACACGTCGACTAGTGCNNNNNNNNNNACGCC	Random primed PCR primer 2
PF108	GACCACACGTCGACTAGTGCNNNNNNNNNNGATAC	Random primed PCR primer 3
PF109	GACCACACGTCGACTAGTGC	Random primed PCR adapter primer
MAMV1-KRCPN1	GGAATTGATCCGGTGGATG	TnKRCPN1 specific primer
MAMV2-KRCPN1	GCATAAAGCTTGCTCAATCAATCAC	TnKRCPN1 specific primer
MAMV3-Sm/Sp	CTAAGCTGATCCGGTGGATG	mini-Tn5Sm/Sp specific primer
MAMV4-Sm/Sp	AACGGTTTACAAGCATAAAGC	mini-Tn5Sm/Sp specific primer
MJ7	TTTTGAATTCGTTTTATCGACATTGTGAACC	pECA1039-Km3 specific primer
KD02	TTTTAAGCTTTTACGATCTTCAGTATGGG	pECA1039-Km3 specific primer
pTRB58	TGATGTCGGCGATATAGG	pACYC184 specific primer
pTRB59	AGAGATTACGCGCAGACC	pACYC184 specific primer

Supplementary Table S4: Transduction efficiencies of LIMEstone phages within and between *Dickeya solani* strains.

Donor strain	Relevant marker/plasmid	Recipient strain	Phenotype	Transduction efficiency LS1 ^{a,b,c}	Standard deviation ^b	Transduction efficiency LS2 ^{a,b,c}	Standard deviation ^b
Transduction within <i>Dickeya solani</i> MK10							
MK10P1	<i>spp::Km</i>	MK10	Protease ⁻	5.1 x 10 ⁻⁵	1.4 x 10 ⁻⁷	5.5 x 10 ⁻⁵	4.2 x 10 ⁻⁶
MK10P7	<i>api::Km</i>	MK10	Protease ⁻	6.1 x 10 ⁻⁵	9.2 x 10 ⁻⁶	7.6 x 10 ⁻⁵	1.8 x 10 ⁻⁵
MK10MM1	<i>ipd::Km</i>	MK10	Auxotrophy	6.3 x 10 ⁻⁵	3.4 x 10 ⁻⁶	6.2 x 10 ⁻⁵	9.9 x 10 ⁻⁷
MK10MM2	<i>gts::Sm</i>	MK10	Auxotrophy	1.2 x 10 ⁻⁵	1.0 x 10 ⁻⁶	1.7 x 10 ⁻⁵	3.2 x 10 ⁻⁶
MK10oocF	<i>oocF::Km</i>	MK10	Oocydin A ⁻	2.5 x 10 ⁻⁵	2.5 x 10 ⁻⁶	3.5 x 10 ⁻⁵	1.7 x 10 ⁻⁶
MK10oocN	<i>oocN::Km</i>	MK10	Oocydin A ⁻	3.5 x 10 ⁻⁵	7.5 x 10 ⁻⁷	3.3 x 10 ⁻⁵	1.3 x 10 ⁻⁶
MK10	pECA1039-km3	MK10	ToxIN ⁺	5.1 x 10 ⁻⁵	9.8 x 10 ⁻⁶	4.7 x 10 ⁻⁵	4.0 x 10 ⁻⁶
Transduction within <i>Dickeya solani</i> MK16							
MK16P1	<i>spp::Sm</i>	MK16	Protease ⁻	2.2 x 10 ⁻⁵	1.2 x 10 ⁻⁶	2.8 x 10 ⁻⁵	3.5 x 10 ⁻⁷
MK16P2	P _{<i>spp</i>} ::Sm	MK16	Protease ⁻	2.5 x 10 ⁻⁵	2.1 x 10 ⁻⁶	3.2 x 10 ⁻⁵	4.2 x 10 ⁻⁶
MK16MM1	<i>dhd::Sm</i>	MK16	Auxotrophy	1.9 x 10 ⁻⁵	3.1 x 10 ⁻⁶	2.9 x 10 ⁻⁵	3.3 x 10 ⁻⁶
MK16MM3	<i>apr::Sm</i>	MK16	Auxotrophy	1.0 x 10 ⁻⁵	6.6 x 10 ⁻⁷	9.1 x 10 ⁻⁶	1.3 x 10 ⁻⁶
MK16MM4	<i>nsr::Sm</i>	MK16	Auxotrophy	1.7 x 10 ⁻⁵	7.1 x 10 ⁻⁷	1.7 x 10 ⁻⁵	1.8 x 10 ⁻⁶
MK16MM5	<i>ppc::Sm</i>	MK16	Auxotrophy	1.8 x 10 ⁻⁵	2.4 x 10 ⁻⁶	2.3 x 10 ⁻⁵	1.9 x 10 ⁻⁶
MK16	pECA1039-km3	MK16	ToxIN ⁺	4.1 x 10 ⁻⁵	3.5 x 10 ⁻⁷	4.4 x 10 ⁻⁵	3.4 x 10 ⁻⁶
Interstrain transduction							
MK10P1	<i>spp::Km</i>	MK16	Protease ⁻	1.7 x 10 ⁻⁵	4.2 x 10 ⁻⁷	3.3 x 10 ⁻⁵	4.3 x 10 ⁻⁶
MK10P1	<i>spp::Km</i>	IPO 2222	Protease ⁻	1.5 x 10 ⁻⁵	5.7 x 10 ⁻⁷	1.2 x 10 ⁻⁵	6.4 x 10 ⁻⁶
MK10MM2	<i>gts::Sm</i>	MK16	Auxotrophy	9.6 x 10 ⁻⁶	1.1 x 10 ⁻⁶	1.2 x 10 ⁻⁵	1.3 x 10 ⁻⁶
MK10oocF	<i>oocF::Km</i>	MK16	Oocydin A ⁻	9.3 x 10 ⁻⁶	1.8 x 10 ⁻⁶	1.1 x 10 ⁻⁵	1.4 x 10 ⁻⁶
MK10oocF	<i>oocF::Km</i>	IPO 2222	Oocydin A ⁻	1.1 x 10 ⁻⁵	1.6 x 10 ⁻⁶	1.4 x 10 ⁻⁵	2.3 x 10 ⁻⁶
MK10oocN	<i>oocN::Km</i>	MK16	Oocydin A ⁻	1.5 x 10 ⁻⁵	1.9 x 10 ⁻⁷	1.2 x 10 ⁻⁵	1.5 x 10 ⁻⁶
MK10oocN	<i>oocN::Km</i>	IPO 2222	Oocydin A ⁻	1.6 x 10 ⁻⁵	4.7 x 10 ⁻⁶	1.4 x 10 ⁻⁵	8.5 x 10 ⁻⁷
MK10	pECA1039-km3	MK16	ToxIN ⁺	2.4 x 10 ⁻⁵	1.1 x 10 ⁻⁶	2.3 x 10 ⁻⁵	2.8 x 10 ⁻⁶
MK10	pECA1039-km3	IPO 2222	ToxIN ⁺	1.5 x 10 ⁻⁵	8.5 x 10 ⁻⁷	1.3 x 10 ⁻⁵	1.3 x 10 ⁻⁶
MK16P1	<i>spp::Sm</i>	MK10	Protease ⁻	3.9 x 10 ⁻⁵	9.9 x 10 ⁻⁷	3.7 x 10 ⁻⁵	1.3 x 10 ⁻⁶
MK16	pECA1039-km3	MK10	ToxIN ⁺	7.0 x 10 ⁻⁵	9.5 x 10 ⁻⁶	6.6 x 10 ⁻⁵	3.0 x 10 ⁻⁶
MK16	pECA1039-km3	IPO 2222	ToxIN ⁺	4.3 x 10 ⁻⁵	2.2 x 10 ⁻⁶	4.1 x 10 ⁻⁵	4.5 x 10 ⁻⁶

^aTransduction efficiency is expressed as the number of transductants per plaque forming unit (p.f.u.)

^bMean and standard deviation of three independent experiments are shown.

^cTransduction experiments were performed using 10⁹ cells with LIMEstone1 (LS1) and LIMEstone2 (LS2) at an m.o.i. of 0.1.

Supplementary Table S5: Transduction efficiency of LIMEstone phages

Number of cells	m.o.i.	Transduction efficiency LS1 ^{a,b}	Standard deviation LS1 ^b	Transduction efficiency LS2 ^{a,b}	Standard deviation LS2 ^b
1 x 10 ¹⁰	10	< 1.0 x 10 ⁻¹¹	-	< 1.0 x 10 ⁻¹¹	-
	1	9.9 x 10 ⁻⁶	2.3 x 10 ⁻⁶	9.3 x 10 ⁻⁶	1.1 x 10 ⁻⁶
	0.1	4.1 x 10 ⁻⁵	1.1 x 10 ⁻⁶	7.6 x 10 ⁻⁵	4.5 x 10 ⁻⁶
	0.01	7.4 x 10 ⁻⁵	9.9 x 10 ⁻⁶	7.5 x 10 ⁻⁵	5.7 x 10 ⁻⁶
1 x 10 ⁹	10	< 1.0 x 10 ⁻¹⁰	-	< 1.0 x 10 ⁻¹⁰	-
	1	1.1 x 10 ⁻⁶	3.0 x 10 ⁻⁷	1.2 x 10 ⁻⁶	2.1 x 10 ⁻⁷
	0.1	4.0 x 10 ⁻⁵	2.1 x 10 ⁻⁶	6.5 x 10 ⁻⁵	1.4 x 10 ⁻⁶
	0.01	7.5 x 10 ⁻⁵	5.3 x 10 ⁻⁶	1.3 x 10 ⁻⁴	3.5 x 10 ⁻⁶
1 x 10 ⁸	10	< 1.0 x 10 ⁻⁹	-	< 1.0 x 10 ⁻⁹	-
	1	< 1.0 x 10 ⁻⁸	-	< 1.0 x 10 ⁻⁸	-
	0.1	1.1 x 10 ⁻⁶	3.5 x 10 ⁻⁶	1.0 x 10 ⁻⁶	3.2 x 10 ⁻⁷
	0.01	9.0 x 10 ⁻⁵	7.1 x 10 ⁻⁶	1.4 x 10 ⁻⁴	1.1 x 10 ⁻⁵

^aTransduction efficiency of LIMEstone1 (LS1) and LIMEstone2 (LS2) phages, defined as the number of transductants obtained per p.f.u., of *api*::Tn-KRCPN1 into MK10.

^bMean and standard deviation of three independent experiments are shown.

Supplementary Table S6: Frequency of transduction of different markers using the *Escherichia coli* phage CBA120

Donor strain ^a	Relevant marker/plasmid	Recipient strain ^a	m.o.i.	Transduction efficiency ^{b,c,d}	Standard deviation ^c
O157:H7	pACYC184	O157:H7	10	<1.0 x 10 ⁻¹⁰	-
	pACYC184	O157:H7	1	5.8 x 10 ⁻⁵	9.3 x 10 ⁻⁷
	pACYC184	O157:H7	0.1	4.5 x 10 ⁻⁵	1.2 x 10 ⁻⁵
	pACYC184	O157:H7	0.01	1.4 x 10 ⁻⁴	1.6 x 10 ⁻⁶

^a *Escherichia coli* O157:H7 NCTC 12900.

^bTransduction efficiency is expressed as the number of transductants per plaque forming unit (p.f.u.)

^cMean and standard deviation of three independent experiments are shown.

^dTransduction experiments were performed using 10⁹ bacterial cells.

Supplementary Table S7: Frequency of transduction of different markers using the *Dickeya* phages ϕ XF1, ϕ XF3 and ϕ XF4.

Donor strain	Relevant marker/plasmid	Recipient strain	m.o.i.	ϕ XF1 Transduction efficiency ^{a,b,c}	ϕ XF1 standard deviation ^c	ϕ XF3 Transduction efficiency ^{a,b,c}	ϕ XF3 standard deviation ^c	ϕ XF4 Transduction efficiency ^{a,b,c}	ϕ XF4 standard deviation ^c
MK10P1	<i>spp::Km</i>	MK10	10	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-
	<i>spp::Km</i>	MK10	1	2.6×10^{-8}	5.5×10^{-9}	1.3×10^{-8}	4.0×10^{-9}	1.8×10^{-8}	6.0×10^{-9}
	<i>spp::Km</i>	MK10	0.1	1.1×10^{-6}	7.0×10^{-8}	8.5×10^{-7}	1.3×10^{-8}	8.3×10^{-7}	7.3×10^{-8}
	<i>spp::Km</i>	MK10	0.01	3.5×10^{-6}	5.0×10^{-8}	2.4×10^{-6}	1.4×10^{-7}	2.1×10^{-6}	3.2×10^{-7}
MK10P1	<i>spp::Km</i>	MK16	10	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-
	<i>spp::Km</i>	MK16	1	6.6×10^{-8}	4.0×10^{-9}	2.6×10^{-8}	5.5×10^{-9}	2.9×10^{-8}	9.1×10^{-9}
	<i>spp::Km</i>	MK16	0.1	9.6×10^{-7}	1.0×10^{-8}	1.0×10^{-6}	5.0×10^{-8}	1.1×10^{-6}	9.1×10^{-8}
	<i>spp::Km</i>	MK16	0.01	2.6×10^{-6}	4.5×10^{-7}	2.0×10^{-6}	3.2×10^{-7}	2.0×10^{-6}	4.1×10^{-7}
MK10P1	<i>spp::Km</i>	IPO 2222	10	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-
	<i>spp::Km</i>	IPO 2222	1	2.6×10^{-8}	5.5×10^{-9}	8.2×10^{-9}	1.8×10^{-9}	4.6×10^{-9}	2.7×10^{-9}
	<i>spp::Km</i>	IPO 2222	0.1	1.3×10^{-6}	3.0×10^{-8}	1.1×10^{-6}	7.7×10^{-8}	7.4×10^{-7}	5.9×10^{-8}
	<i>spp::Km</i>	IPO 2222	0.01	2.4×10^{-6}	2.5×10^{-7}	2.1×10^{-6}	5.9×10^{-7}	1.4×10^{-6}	1.4×10^{-7}
MK10	pECA1039-km3	MK10	10	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-	$<1.0 \times 10^{-10}$	-
	pECA1039-km3	MK10	1	2.0×10^{-8}	1.3×10^{-9}	1.4×10^{-8}	2.3×10^{-9}	4.1×10^{-9}	4.6×10^{-10}
	pECA1039-km3	MK10	0.1	9.1×10^{-7}	4.2×10^{-9}	9.2×10^{-7}	6.4×10^{-8}	1.4×10^{-6}	9.6×10^{-8}
	pECA1039-km3	MK10	0.01	2.6×10^{-6}	4.2×10^{-8}	2.2×10^{-6}	2.3×10^{-7}	1.4×10^{-6}	2.3×10^{-7}

^aTransduction experiments were performed using 10^9 bacterial cells.

^bTransduction efficiency defined as the number of transductants obtained per p.f.u.

^cMean and standard deviation of three independent experiments are shown.

Supplementary Figure S1: Nucleotide sequence alignments of structural and non-structural genes from LIMEstone 1, ϕ XF1, ϕ XF3, and ϕ XF4. Alignments of DNA polymerase (non-structural) (A) and tail-spike protein (structural) (B) encoding genes are shown. Sequences of the genes encoding the tail-spike protein Orf158 (genome coordinates 119416-120966) and DNA polymerase Orf180 (genome coordinates 141743-142810) of LIMEstone1 (LS1) phage were used for the alignments. Multiple sequence alignments were carried out with ClustalW2 (European Bioinformatics Institute).

XF1 CAATGGATCAAAGACAACACTACCAGCCCGAGACCAATGAGTGGGCGCAGCTTCTGTGCAAC 420
 XF3 CAATGGATCAAAGACAACACTACCAGCCCGAGACCAATGAGTGGGCGCAGCTTCTGTGCAAC 420
 XF4 CAATGGATCAAAGACAACACTACCAGCCCGAGACCAATGAGTGGGCGCAGCTTCTGTGCAAC 420
 LS1 CAATGGATTAAGGACAACACTATCAGCCTAAGACCAATGAGTGGGCACAGCTTCTGTGCAAC 420
 ***** ** ***** ***** ***** ***** ***** *****

XF1 ACCATGAACGGGTTTCGAGCAGCGCATGGTGTGGGAGCGTGAAGTAATTGCATCAGCCGCC 480
 XF3 ACCATGAACGGGTTTCGAGCAGCGCATGGTGTGGGAGCGTGAAGTAATTGCATCAGCCGCC 480
 XF4 ACCATGAACGGGTTTCGAGCAGCGCATGGTGTGGGAGCGTGAAGTAATTGCATCAGCCGCC 480
 LS1 ACCATGAATGGGTTTCGAGCAGCGCATGGTGTGGGAGCGTGAAGTAATTGCATCATCCGCC 480
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 LS1 GTCTGGCGAGCCAAGAAGATGTATGCGATGGCCGTGTATGACAGCGAGGGCATCAAGTAT 540
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XF1 GAGAAGTCGAAGATCAAGTTCAAAGGTCTTGAAGCCCGTAAGTCCACCACGCCCGAATGG 600
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 LS1 GAGAAGCCGAAGATTAATTCAAAGGTCTGGAAGCCCGTAAGTCCACCACACTGAATGG 600
 ***** ***** ** ***** ***** ***** ***** ***** *****

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 ***** ***** ***** ***** ***** ** ***** *****

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B

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XF3 GCGTGGTTATTAAGTTTGTGGTGCTTATTTAAGAGTAGATAAATCATTTTGCACGTT 1080

XF4 GCGTGGTTATTAAGTTTGTGGTGCTTATTTAAGAGTAGATAAATCATTTTGCACGTT 1080

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 XF4 AGGATCATAAATTTTTATCCAAGTGGTAGGAGAAATACCACCACTTGCCTCTGGTGAAGA 1200
 LS1 AGGATCATAAATTTTTATCCAAGTGGTAGGAGAAATACCACCACTTGCCTCTGGTGAAGA 1200

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 LS1 ACCAAGAGGTACAATTTTAGGGAATGTGCCACCCATCGATATTTACCATCATCGTGGGT 1260

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 XF4 AATGGCATTGTGTTAAGGAGATGAAGACGGAGTCGGAAGGTAAGTCGGAAGGTAAAGCATA 1440
 LS1 AATGGCATTGTGTTAAGGAGATGAAGACGGAGTCGGAAGGTAAGTCGGAAGGTAAAGCATA 1440

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XF1          AGAACGCTCTGTGTTCTTATCGTAGATTACTTTATACCCAGATAAAGATACGCCCGCTTT 1500
XF3          AGAACGCTCTGTGTTCTTATCGTAGATTACTTTATACCCAGATAAAGATACGCCCGCTTT 1500
XF4          AGAACGCTCTGTGTTCTTATCGTAGATTACTTTATACCCAGATAAAGATACGCCCGCTTT 1500
LS1          AGAACGCTCTGTGTTCTTATCGTAGATTACTTTATACCCAGATAAAGATACGCCCGCTTT 1500
*****

XF1          GGCATACAATACCTCAGACTTCTTACACCCGAATTTGCGGGCGATGGAATC 1551
XF3          GGCATACAATACCTCAGACTTCTTACACCCGAATTTGCGGGCGATGGAATC 1551
XF4          GGCATACAATACCTCAGACTTCTTACACCCGAATTTGCGGGCGATGGAATC 1551
LS1          GGCATACAATACCTCAGACTTCTTACACCCGAATTTGCGGGCGATGGAATC 1551
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Materials and methods

Bacterial strains, plasmids, phages, culture media and growth conditions.

Bacterial strains and plasmids used in this study are listed in Supplementary Tables 2.

Salmonella, *Dickeya* and their derivative strains were routinely grown at 30 °C in Luria Broth (LB; 5 g yeast extract l⁻¹, 10 g Bacto tryptone l⁻¹ and 5 g NaCl l⁻¹), Potato Dextrose (24 g potato dextrose broth l⁻¹) or minimal medium (0.1%, w/v, (NH₄)₂SO₄, 0.41 mM MgSO₄, 0.2% (w/v) glucose, 40 mM K₂HPO₄, 14.7 mM KH₂PO₄, pH 6.9–7.1). *S. Typhi* BRD948 LB broth was supplemented with a mixture of aromatic amino acids (0.01 g para-aminobenzoic acid l⁻¹, 0.04 g tryptophan l⁻¹, 0.04 g phenylalanine l⁻¹ and 0.01 g dihydrobenzoic acid l⁻¹) and 0.04 g tyrosine l⁻¹, as described previously (Bishop *et al.*, 2008). *Escherichia coli* strains were grown at 37 °C in LB. Media for propagation of *E. coli* β2163 were supplemented with 300 μM 2,6-diaminopimelic acid (DAPA). When appropriate, antibiotics were used at the following final concentrations (in μg ml⁻¹): kanamycin, 25 (*E. coli* strains) and 50 (*Dickeya* strains); streptomycin, 50; chloramphenicol, 25; tetracycline, 10. Phages were stored at 4 °C in phage buffer (10 mM Tris-HCl pH 7.4, 10 mM MgSO₄, 0.01% w/v gelatine) over a few drops of NaHCO₃-saturated chloroform.

Isolation of novel bacteriophages and phage lysate preparation. Treated sewage effluent was collected from Milton (Cambridge, United Kingdom). To prepare phage enrichment, a 10 ml sample of the effluent was filter sterilized and added to 10 ml 2X LB broth. Then, 500 μ L of an overnight culture of *Dickeya solani* MK10 was added to 250 ml flasks and incubated at 30 °C with shaking at 225 rpm. After overnight incubation, 1 ml of the sample enrichment was mixed with 100 μ L of chloroform and vortexed vigorously for 1 min to kill bacteria. The sample was centrifuge at 16,000 x g for 1 min and 100 μ L dilution series of the sterilized supernatant was mixed with 200 μ L of a *D. solani* MK10 overnight culture and 4 mL of top LB-agar (LBA, 0.35% w/v agar) and poured as an overlay onto LBA plates (1.5% w/v agar). Plates were incubated overnight at 30 °C and single phage plaques were picked with a sterile toothpick into 0.2 ml phage buffer and shaken with 20 μ L of chloroform to kill any bacteria. Phages obtained were plaque-purified three times. High-titre phage lysates were then obtained as described Petty *et al.* (2006). Phages were titrated by serial dilutions in phage buffer and the phage titre determined in plaque-forming units (p.f.u.) per millilitre. Phages ϕ XF1, ϕ XF3, ϕ XF4 and ϕ XF28 were isolated from four independent sewage effluent samples from Milton (Cambridge, UK).

Electron microscopy

High-titre lysates for transmission electron microscopy were obtained as described by Petty *et al.* (2006) using 0.35% (w/v) LB agarose instead of 0.35% (w/v) LB agar overlays. Phage samples were negatively stained by placing the grids in 25 μ l drops of 2% phosphotungstic acid for 1 min. Phages were examined by transmission electron microscopy, in the Multi-Imaging Centre (Department of Physiology, Development and Neuroscience, University of Cambridge) using a FEI Tecnai G2 transmission electron microscope (FEI, Oregon, USA). The accelerating voltage was 120.0 kV and images were captured with an AMT XR60B digital camera running Deben software.

Transposon mutagenesis and isolation of auxotrophic, oocydin A negative and protease negative mutants.

Random transposon mutagenesis of *Dickeya solani* MK10 and MK16 using Tn-KRCPN1 and mini-Tn5Sm/Sp were performed as follows. In a biparental conjugal mating, 500 µl of overnight cultures of *E. coli* β2163 and *D. solani* were mixed, collected by centrifugation, resuspended in 30 µl of fresh LB, and spotted on an LB agar plate supplemented with 300 µM DAPA. After overnight incubation at 30 °C, cells were scraped off the plate and resuspended in 1 ml of LB. Serial dilutions were plated on LB agar medium containing the appropriate antibiotic, kanamycin and streptomycin for Tn-KRCPN1 and mini-Tn5Sm/Sp, respectively. 2,6-Diaminopimelic acid was not added to the LB agar medium, to allow counterselection of the *E. coli* donor. Auxotrophic, oocydin A negative and protease negative mutants were identified for their inability to grow on M9 glucose minimal medium agar, inhibit *Pythium ultimum* growth in potato dextrose agar and produce haloes when plated onto skimmed milk agar (nutrient broth agar, 10 g of Marvel skimmed milk powder per liter), respectively. Identification of the transposon insertion sites in mutants was determined using random primed PCR following the method described previously (Fineran *et al.*, 2005) and using primers described in supplemental Table S3.

Transduction assays

Phage lysates were prepared on bacterial strains carrying the desired mutation or plasmid. For the transduction assays, an appropriate volume of a high-titre lysate was added to an overnight culture of the recipient strain to give the desired m.o.i. The mixture was incubated at 30 °C (*Dickeya* and *Salmonella*) or 37 °C (*E. coli*) for 1 h, pelleted by centrifugation (4.000 g for 10 min at 4 °C), washed twice with LB to remove remaining phage and 100 µL aliquots of the phage-host mixture were spread on LBA plates containing the appropriate antibiotic. The drug-resistant transductants were restreaked three times to reduce any bacteriophage carry-over. The number of

transductants was determined by co-inheritance of the antibiotic resistance and a secondary phenotype of either anti-*Pythium* activities, protease production or auxotrophy. The transposon insertion sites in the final transductants were confirmed by random primed PCR and sequencing, as described above. In the case of transduction of plasmids, the presence of the plasmids was confirmed by colony PCR in 10 of the final transductants per experiment, using primers listed in supplementary table S3. For the transduction assays, the protocol was optimized for incubation time, temperature, multiplicity of infection (m.o.i.) and number of bacterial cells. During the transduction assays, routine controls for spontaneous resistance to antibiotics were determined by spreading 100 µl of the overnight culture onto LBA plates containing the relevant antibiotic. A 100 µl volume of the high-titre lysate was also spread on non-LBA plates to confirm lysate sterility. Transduction efficiency was defined as the number of transductants obtained per p.f.u.

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