Supplemental materials

Lysobacter enzymogenes predates soilborne bacteria using the type IV secretion system

Xi Shen[a], Bingxin Wang[a], Nianda Yang[a], Lulu Zhang[a], Danyu Shen[a], Huijun Wu[a], Ying Dong [b], Ben Niu[b], Shan-Ho Chou[c], Gerardo Puopolo[d,e], Jiaqin Fan[a], Guoliang Qian[a]*

Containing 4 supplemental tables and 11 supplemental figures.

Table S1 GenBank accession numbers of T4SS components and effectors

Gene ID of *L. enzymogenes* OH11	Gene	Gene ID of *X. citri* 306	Similarity %	Identity %	GenBank accession number of *L. enzymogenes* OH11 genes
Le4215	VirB7	XAC2622	48.2	32.4	MW052476
Le4216	VirB8	XAC2621	60.3	45.3	MW052477
Le4217	VirB9	XAC2620	80.8	65.2	MW052478
Le4218	VirB10	XAC2619	66.0	48.2	MW052479
Le4219	VirB11	XAC2618	91.9	83.8	MW052480
Le4220	VirB1	XAC2617	59.6	48.1	MW052481
Le4221	VirB2	XAC2616	52.3	34.1	MW052482
Le4222	VirB3	XAC2615	68.0	55.3	MW052483
Le4223	VirB4	XAC2614	91.1	80.0	MW052484
Le4224	VirB5a	XAC2613	38.5	22.4	MW052485
Le4225	VirB5b	XAC2613	43.6	23.3	MW052486
Le4226	VirB6	XAC2612	57.7	39.1	MW052487
Le4238	VirD4	XAC2623	86.9	75.8	MW052492
Le0908	-				MW052467
Le0989	-				MW052468
Le1288	-				MW052469
Le1408	-				MW052470
Le1519	-				MW052471
Le1841	-				MW052472
Le3180	-				MW052473
Le3316	-				MW052474
Le3432	-				MW052475
Le4230	-				MW052488
Le4232	-				MW052489
Le4235	-				MW052490
Le4236	-				MW052491
Le4253	-				MW052493
Le4949	-				MW052495
Le4579	-				MW052494

Table S2 Contact-dependent killing activity of *L. enzymogenes* strains against the

*** indicates *E. coli* was LacZ labelled.**

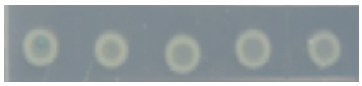
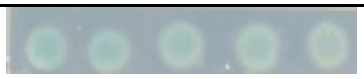
Gene	Mutant	Protein Function	*E. coli*: mutant* 15:1 10:1 7:1 5:1 3:1
tolC	Δ*tolC*	an outer membrane protein of type I secretion system	
xpsD	Δ*xpsD*	an outer membrane pathway protein of type II secretion system	
xpsF	Δ*xpsF*	an inner membrane protein of type II secretion system	
virD4	Δ*virD4*	a T4SS-specific ATPase	
hcp	Δ*hcp*	an inner tube protein of T6SS	
clpV	Δ*clpV*	a T6SS-specific ATPase	
waps1	Δ*wapS1*	a nonribosomal peptide synthetase responsible for antibacterial WAP-8294A2 biosynthesis	
lafB	Δ*lafB*	encoding a hybrid PKS/NRPS (polyketide synthase/nonribosomal peptide synthetase) responsible for HSAF biosynthesis	

Table S3. Strains and plasmids used in this study

Strains and plasmids	Characteristics[a]	Source
Lysobacter enzymogenes		
OH11	Wild type, Km^R	Qian *et al*., 2009
Δ*virD4*	In-frame deletion of *virD4*, Km^R	This study
Δ*tolC*	In-frame deletion of *tolC*, Km^R	This study
Δ*xpsD*	In-frame deletion of *xpsD*, Km^R	This study
Δ*xpsF*	In-frame deletion of *xpsF*, Km^R	This study
Δ*hcp*	In-frame deletion of *hcp*, Km^R	Yang *et al*., 2020
Δ*clpV*	In-frame deletion of *clpV*, Km^R	Yang *et al*., 2020
Δ*pks*	Antifungal antibiotic HSAF-deficient mutant, Km^R	Lou *et al*., 2011
Δ*wapS1*	Antibacterial antibiotic WAP8294A2 deficient mutant, Km^R	Zhang *et al*., 2011
Δ*lafB*	In-frame deletion of *lafB*, Km^R	Wang *et al*., 2017
OH11(mCherry)	OH11 harboring plasmid pYC12-mCherry, Gm^R, Km^R	This study
Δ*virD4*(mCherry)	Δ*virD4* harboring plasmid pYC12-mCherry, Gm^R, Km^R	This study
Δ*virD4(virD4)*	Δ*virD4* harboring plasmid pEX18GM-chiA-*virD4*, Gm^R, Km^R	This study
Escherichia coli		
DH5α	Host strain for molecular cloning	Qian *et al*., 2013
BL21(DE3)	Host strain for molecular cloning	Su *et al*., 2017
E. coli-LacZ	LacZ-labelled *E. coli* BL21	Wang *et al*., 2020
E. coli (GFP)	*E. coli* harboring pSMC21-GFP vector, Km^R	This study
Fungus		
Valsa pyri SXYL134	A fungal pathogen causing pear Valsa canker	Yin *et al*., 2018
Pectobacterium carotovorum		
PccS1	Wild type, Rif^R	Wang *et al*., 2018
PccS1 (GFP)	PccS1 harboring pBBR1-MCS5-GFP vector, Rif^R , Gm^R	This study
Pseudomonas fluorescence		
2P24	Wild type, Km^R	Wang *et al*., 2020

2P24-LacZ	LacZ-labelled strain, Km^R	Wang *et al*., 2020
2P24 (GFP)	2P24 harboring pBBR1-MCS5-GFP vector, Km^R, Gm^R	This study
Pseudomonas protegens		
Pf-5	Wild type, Amp^R	A gift strain from Prof. Huijun Wu
Pf-5 (GFP)	Pf-5 harboring pBBR1-MCS5-GFP vector, Amp^R, Gm^R	This study
Pf-5Δ*tssA*	In-frame deletion of *tssA*, Amp^R	A gift strain from Prof. Huijun Wu
Bacillus subtilis		
NCD-2	Wild type	Guo *et al.*, 2019
NCD-2 (GFP)	NCD-2 harboring pC-1-GFP vector, Chl^R	Dong *et al.*, 2020
Plasmids		
pEX18GM	Suicide vector with a *sacB* gene, Gm^R	Kovach *et al*., 1995
pEX18GM-*virD4*	pEX18GM with two flanking fragments of *virD4*, Gm^R	This study
pEX18GM-*tolC*	pEX18GM with two flanking fragments of *tolC*, Gm^R	This study
pEX18GM-*xpsD*	pEX18GM with two flanking fragments of *xpsD*, Gm^R	This study
pEX18GM-*xpsF*	pEX18GM with two flanking fragments of *xpsF*, Gm^R	This study
pBBR1-MCS5	Broad-host-range vector with a P_{lac} promoter, Gm^R	Xu *et al*., 2018
pBBR1-GFP	pBBR1-MCS5 containing the coding region of GFP, Gm^R	This study
pEX18GM-ChiA	chromosomally-integrated suicide vector, Gm^R	Xu *et al*., 2015
pEX18GM-ChiA-*virD4*	pEX18GM-ChiA containing the coding region of *virD4* and its native promoter, Gm^R	This study
pET22 b (+)	Vector for IPTG-inducible gene expression, Amp^R	Jiang *et al*., 2014
pET22 b (+)-PldB	pET22 b (+)containing the coding region of PldB, Amp^R	Jiang *et al*., 2014
pET22 b (+)-Le4232	pET22 b (+)containing the coding region of Le4232, Amp^R	This study
pET22 b (+)-Le0989	pET22 b (+)containing the coding region of Le0989, Amp^R	This study
pET22 b (+)-Le0908	pET22 b (+)containing the coding region of Le0908, Amp^R	This study
pET22 b (+)-Le4230	pET22 b (+)containing the coding region of Le4230, Amp^R	This study
pET22 b (+)-Le1288	pET22 b (+)containing the coding region of Le1288, Amp^R	This study
pET22 b (+)-Le3316	pET22 b (+)containing the coding region of PldB, Amp^R	This study
pBAD/Myc-His A	Vector for arabinose-inducible gene expression, Amp^R	Xu *et al*., 2018
pBAD-AvrRXO1	pBAD containing the coding region of AvrRXO1, Amp^R	Wang *et al*., 2020
pBAD-Le4232	pBAD containing the coding region of Le4232, Amp^R	This study
pBAD-Le0989	pBAD containing the coding region of Le0989, Amp^R	This study
pBAD-Le0908	pBAD containing the coding region of Le0908, Amp^R	This study

pBAD-Le4230	pBAD containing the coding region of Le4230, Amp^R	This study
pBAD-Le1288	pBAD containing the coding region of Le1288, Amp^R	This study
pBAD-Le3316	pBAD containing the coding region of Le3316, Amp^R	This study
pBAD-PelB	pBAD containing the coding region of signal peptide PelB, Amp^R	This study
pBAD-PelB- Le0908	pBAD-PelB containing the coding region of Le0908, Amp^R	This study
pBADGM	Vector for arabinose-inducible gene expression, Gm^R	This study
pBADGM- Le0907	pBADGM containing the coding region of Le0907, Gm^R	This study
pBADGM- Le0909	pBADGM containing the coding region of Le0909, Gm^R	This study
pUT18C	The plasmid used for protein expression in bacterial two-hybridization assay, Amp^R	Xia *et al*., 2018
pUT18C-Le0909-FLAG	pUT18C containing the coding region of Le0909 with C-terminal FLAG tag and its native promoter, Amp^R	This study
pUT18C-VirD4-FLAG	pUT18C containing the coding region of VirD4 with C-terminal FLAG tag and its native promoter, Amp^R	This study
pUT18C-GFP-FLAG	pUT18C containing the coding region of GFP with C-terminal FLAG tag and its native promoter, Amp^R	This study
pKT25	The plasmid used for protein expression in bacterial two-hybridization assay, Km^R	Xia *et al*., 2018
pKT25-Le0908-His	pKT25 containing the coding region of Le0908 with C-terminal FLAG tag and its native promoter, Km^R	This study
pKT25-mCherry-His	pKT25 containing the coding region of mCherry with C-terminal FLAG tag and its native promoter, Km^R	This study
pYC12-mCherry	pYC12 containing the coding region of mCherry, Gm^R	Ling *et al*., 2016

Table S4. Primers used in this study

Primer	Sequence (5'-3')[a]	Purpose

In-frame deletion		
virD4-F1	GGAATTCCAGGAGGACCGATGAGCAAC (*EcoR*I)	To amplify a 418-bp fragment upstream of *virD4*
virD4-R1	CCCAAGCTTTCAGATAGCCCGACAGGTAC (*Hind*III)	
virD4-F2	CCCAAGCTTCTGCTGCCGAAGGTCAACGT (*Hind*III)	To amplify a 441-bp fragment upstream of *virD4*
virD4-R2	GCTCTAGATGGAACGGATGCTCTGGGTC (*Xba*I)	
tolC-F1	GGAATTCATGGTGTGCTCCTGATTGGG (*EcoR*I)	To amplify a 566-bp fragment upstream of *tolC*
tolC -R1	CCCAAGCTTCAACTTCCTGCTCAACCGCC (*Hind*III)	
tolC -F2	CCCAAGCTTCGCTGGCTTCGCTGTCGTTG (*Hind*III)	To amplify a 582-bp fragment upstream of *tolC*
tolC -R2	CGAGCTCGAACTACAGCACCGACCGCC (*Sac*I)	
xpsD-F1	GGAATTCATCGGCAGTTCGCTCATCGC (*EcoR*I)	To amplify a 424-bp fragment upstream of *xpsD*
xpsD-R1	CCCAAGCTTTGAACCCGAAGCCGAAGAAG (*Hind*III)	
xpsD-F2	CCCAAGCTTGCGACCGAGTTGTTGATGAC (*Hind*III)	To amplify a 457-bp fragment upstream of *xpsD*
xpsD-R2	CGAGCTCCAACAACCGCAACCCCAGCC (*Sac*I)	
xpsF-F1	GGAATTCTCGTCGTCATCCGTTGTTGG (*EcoR*I)	To amplify a 392-bp fragment upstream of *xpsF*
xpsF-R1	CCCAAGCTTGTGGTGATGGCGGTGCTGTT (*Hind*III)	
xpsF-F2	CCCAAGCTTCGCTTGGTAACGGTAGAGGG (*Hind*III)	To amplify a 450-bp fragment upstream of *xpsF*
xpsF-R2	CGAGCTCGAAAAGGACAACCAAGAGGC (*Sac*I)	
Complementation		
cp-VirD4-F	GGGGTACCTTGTCCAACGGCAGCCTTTC (*Kpn*I)	To amplify a 1804-bp fragment containing coding region of VirD4 and its promoter region
cp-VirD4-R	GCTCTAGATCAGCCGACCTTGATGTCCA (*Xba*I)	
Pull-down assays		
Le0908-His-F	GAAACAGCTATGACCCGCATGGATGGAAGAACCAC	To amplify a 1440-bp fragment containing coding region of Le0908
Le0908-His-R	TGGTGGTGCTCGAGTGCGGCCGCAACCCGCATGATCGAAGCCT	
Le0908T-His-F	GAAACAGCTATGACCCGCATGGATGGAAGAACCAC	To amplify a 1099-bp fragment containing coding region of Le0908
Le0908T-His-R	TGGTGGTGCTCGAGTGCGGCCGCACTGGCAAACGCCTGCGGCA	
Le0909-FLAG-F	GAAACAGCTATGACCAATGAGGCGACACCGCCCGC	To amplify a 575-bp fragment containing coding region of Le0909
Le0909-FLAG-R	CTTGTAATCACTAGTGGATCCCTCTTGCCACCGCTTGCACT	
VirD4-His-F	GAAACAGCTATGACCGTGGCGCTGCTGGCGACCGC	To amplify a 1650-bp fragment containing coding region of VirD4
VirD4-His-R	CTTGTAATCACTAGTGGATCCGCCGACCTTGATGTCCAGCA	
VirD4T-His-F	GAAACAGCTATGACCATGCCCAAGAAGCAGTCCAT	To amplify a 1425-bp fragment containing coding region of VirD4
VirD4T-His-R	CTTGTAATCACTAGTGGATCCGCCGACCTTGATGTCCAGCA	
mCherry-His-F	GAAACAGCTATGACCATGGTGAGCAAGGGCGAGGA	

mCherry-His-R	TGGTGGTGCTCGAGTGCGGCCGCCTTGTACAGCTCGTCCATGC	To amplify a 711-bp fragment containing coding region of mCherry
GFP-FLAG-F	GAAACAGCTATGACCAGTAAAGGTGAAGAACTGTT	To amplify a 717-bp fragment containing coding region of GFP
GFP-FLAG-R	CTTGTAATCACTAGTGGATCCTTTGTAGAGTTCATCCATGC	
Periplasmic expression in *Escherichia coli*		
pET22b-PldB- F	CCAGCCGGCGATGGCCATGGATATGAGCGACCTGTACAAACC	To amplify a 2238-bp fragment containing coding region of PldB
pET22b-PldB- R	GTTTTTGTTCGGGCCCAAGCTTGTCCACCGTGAGACCGGGGC	
pET22b-Le4232-F	CATGCCATGGATATGGGCAATAAGGAAGAATT (*Nco*I)	To amplify a 1188-bp fragment containing coding region of Le4232
pET22b-Le4232-R	CCCAAGCTTTCAGGCGGTCATCGTCTTCG (*Hind*III)	
pET22b-Le0989-F	CCGGAATTCGATGAGCGGAAGGTACCGCAT (*EcoR*I)	To amplify a 2289-bp fragment containing coding region of Le0989
pET22b-Le0989-R	CCCAAGCTTTTACTGCGCCATGGCCTTGG (*Hind*III)	
pET22b-Le0908-F	CATGCCATGGATGTGCGCATGGATGGAAGAAC (*Nco*I)	To amplify a 1440-bp fragment containing coding region of Le0908
pET22b-Le0908-R	CGCGGATCCTCAAACCCGCATGATCGAAG (*BamH*I)	
pET22b-Le4230-F	CATGCCATGGATTTGGACGACGCGATGACCCG (*Nco*I)	To amplify a 1245-bp fragment containing coding region of Le4230
pET22b-Le4230-R	CGCGGATCCTCAGAGGCCCTGGCTTCGGT (*BamH*I)	
pET22b-Le1288-F	CATGCCATGGATATGACGCTGCCCAGCCGCGC (*Nco*I)	To amplify a 1623-bp fragment containing coding region of Le1288
pET22b-Le1288-R	CGCGGATCCTCAGCCCATCGAGCGCGAGC (*BamH*I)	
pET22b-Le3316-F	CATGCCATGGATGTGCAGTACCGCGCCGGTCT (*Nco*I)	To amplify a 780-bp fragment containing coding region of Le3316
pET22b-Le3316-R	CGCGGATCCTCAGGCCGGCAGCTTCGGGG (*BamH*I)	
pBAD-pel-Le0908-F	CCCAGCCGGCGATGGCCATGGATCGCATGGATGGAAGAACCAC	To amplify a 1440-bp fragment containing coding region of Le0908
pBAD-pel-Le0908-R	TTTTTGTTCGGGCCCAAGCTTAACCCGCATGATCGAAGCCT	
pBADGM-Le0907-F	AACAGGAGGAATTAACCATGGATGTGAGCCCATCCGATCGGCC	To amplify a 300-bp fragment containing coding region of Le0907
pBADGM-Le0907-F	TTTTTGTTCGGGCCCAAGCTTCCGCACCGCAATCGCATCCT	

pBADGM-Le0907-F	AACAGGAGGAATTAACCATGGATATGCTGGTGACCAGCGCGCT	To amplify a 618-bp fragment containing coding region of Le0909
pBADGM-Le0907-F	TTTTTGTTCGGGCCCAAGCTTCTCTTGCCACCGCTTGCACT	
Cytoplasmic expression in *Escherichia coli*		
pBAD-Le4232-F	GGGGTACCATGGGCAATAAGGAAGAATT (*Kpn*I)	To amplify a 1188-bp fragment containing coding region of Le4232
pBAD-Le4232-R	CCCAAGCTTTCGGCGGTCATCGTCTTCGGAG (*Hind*III)	
pBAD-Le0989-F	CGAGCTCGATGAGCGGAAGGTACCGCATC (SacI)	To amplify a 2289-bp fragment containing coding region of Le0989
pBAD-Le0989-R	CCAAGCTTGGCTGCGCCATGGCCTTGGCCTG (*Hind*III)	
pBAD-Le0908-F	GGGGTACCGTGCGCATGGATGGAAGAAC (*Kpn*I)	To amplify a 1440-bp fragment containing coding region of Le0908
pBAD-Le0908-R	CCAAGCTTGGAACCCGCATGATCGAAGCCT (*Hind*III)	
pBAD-Le4230-F	GGGGTACCTTGGACGACGCGATGACCCG (*Kpn*I)	To amplify a 1245-bp fragment containing coding region of Le4230
pBAD-Le4230-R	CCCAAGCTTAAGAGGCCCTGGCTTCGGTTTC (*Hind*III)	
pBAD-Le1288-F	GGGGTACCATGACGCTGCCCAGCCGCGC (*Kpn*I)	To amplify a 1623-bp fragment containing coding region of Le1288
pBAD-Le1288-R	CCCAAGCTTAAGCCCATCGAGCGCGAGCGGC (*Hind*III)	
pBAD-Le3316-F	GGGGTACCGTGCAGTACCGCGCCGGTCTG (*Kpn*I)	To amplify a 780-bp fragment containing coding region of Le3316
pBAD-Le3316-R	GCTCTAGAAAGGCCGGCAGCTTCGGGGCGC (*Xba*I)	

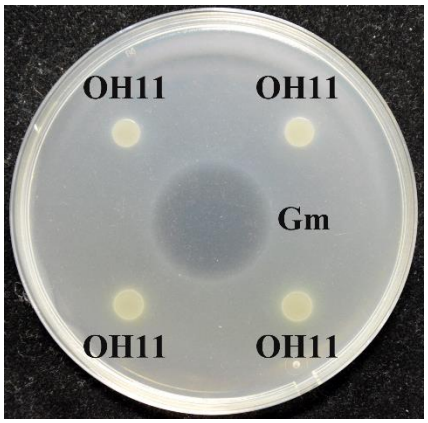


Figure S1. *L. enzymogenes* OH11 failed to kill *E. coli* through secreting diffusible factors. OH11 was inoculated on the surface of LB agar plates containing *E. coli*. The results were observed after 3-day incubation. The antibiotic gentamycin (Gm) was used a positive control.

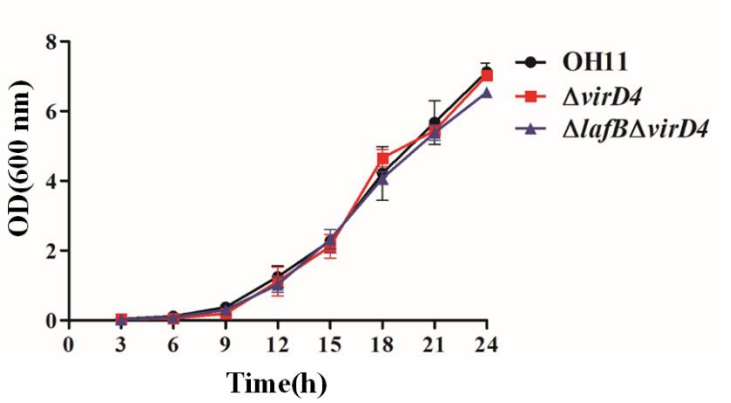


Figure S2. The Δ*virD4* and Δ*lafBvirD4* strains exhibited a growth curve similar to the wild-type OH11 of *L. enzymogenes* in liquid LB broth. Δ*virD4*, the OH11 T4SS-defective mutant with in-frame deletion of *virD4*, a T4SS specific ATPase**;** Δ*lafB*Δ*virD4* – an OH11 derivative with double mutations in *lafB* and *virD4*.

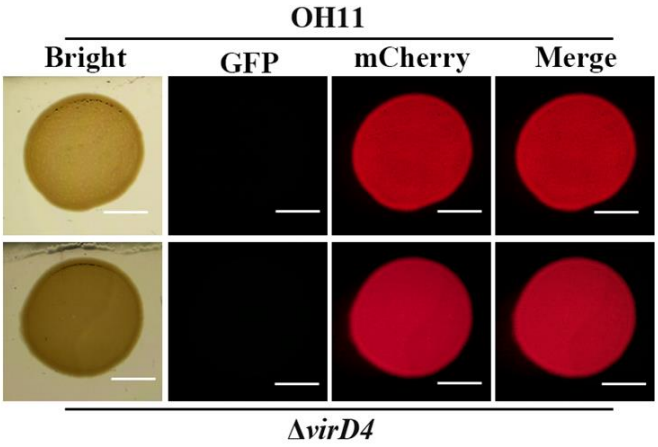


Figure S3. The Δ*virD4* strain displayed a fluorescent signal intensity similar to the wild-type OH11. Both OH11 and Δ*virD4* were labelled by mCherry. The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 mm.

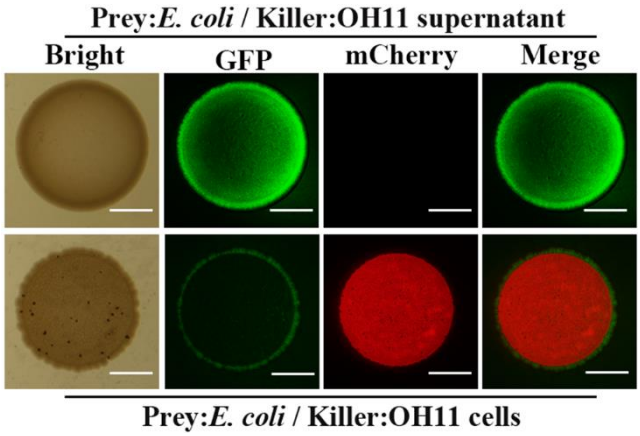


Figure S4. The cell-free supernatant of *L. enzymogenes* OH11 failed to kill *E. coli* when co-culture on LB agar plates in a 1:1 ratio. OH11 and *E. coli* were labelled with mCherry and GFP, respectively. OH11 cells were used here as a positive control. The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 mm.

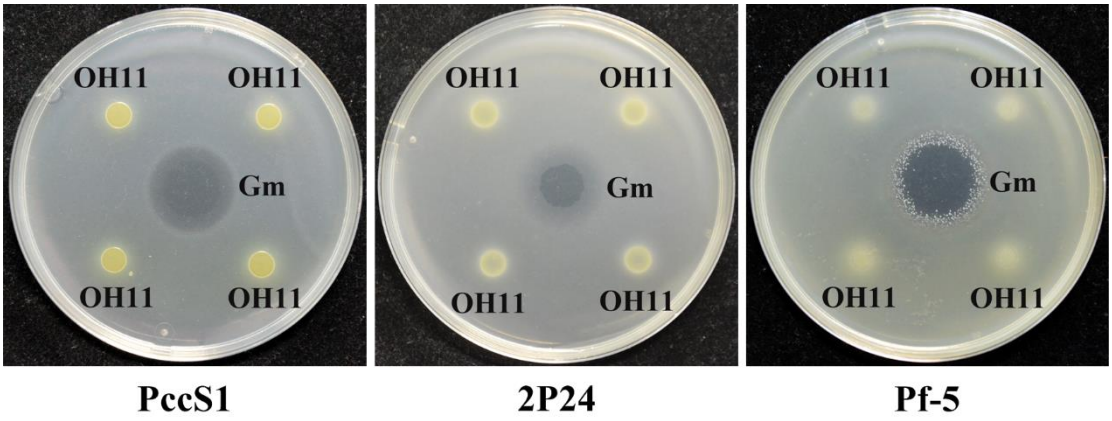


Figure S5. *L. enzymogenes* OH11 failed to kill three soilborne bacteria through secreting diffusible factors. OH11 was inoculated on the surface of LB agar plates containing the soil pathogenic bacterium, *Pectobacterium carotovorum* PccS1 (left), the soil biocontrol bacteria - *Pseudomonas* (*Ps.*) *fluorescens* 2P24 (middle) and *Ps. protegens* Pf-5 (right). The results were observed after 3-day incubation. The antibiotic gentamycin (Gm) was used a positive control.

.

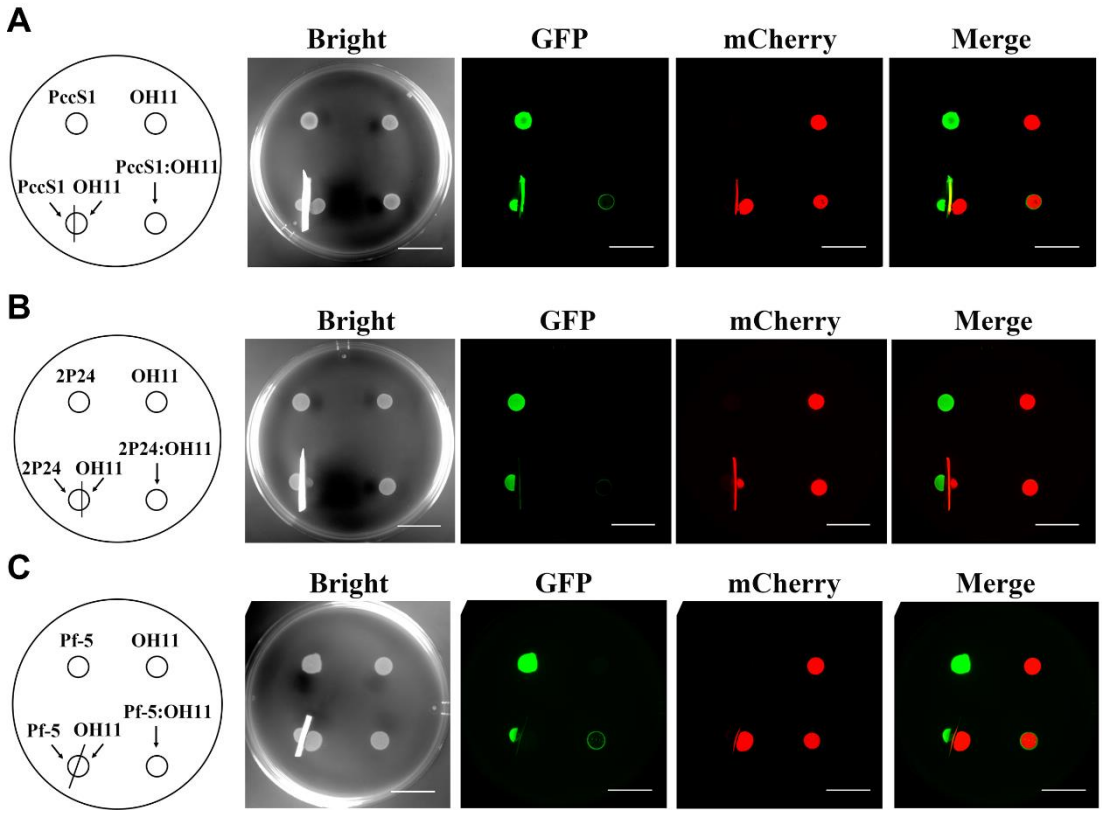


Figure S6. Membrane separation blocked the cell-cell contact-dependent killing of three soilborne bacteria by *L. enzymogenes* OH11. The mCherry-labelled OH11 was co-cultured with each of the GFP-labelled *Pectobacterium carotovorum* PccS1 (**A**), *Pseudomonas* (*Ps.*) *fluorescens* 2P24 (**B**) and *Ps. protegens* Pf-5 (**C**) in a 1:1 ratio. A 0.22-μM filter membrane was used to separate the growth of the mCherry-labelled killer (OH11) and the GFP-labelled preys (PccS1, 2P24 and Pf-5). The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 cm.

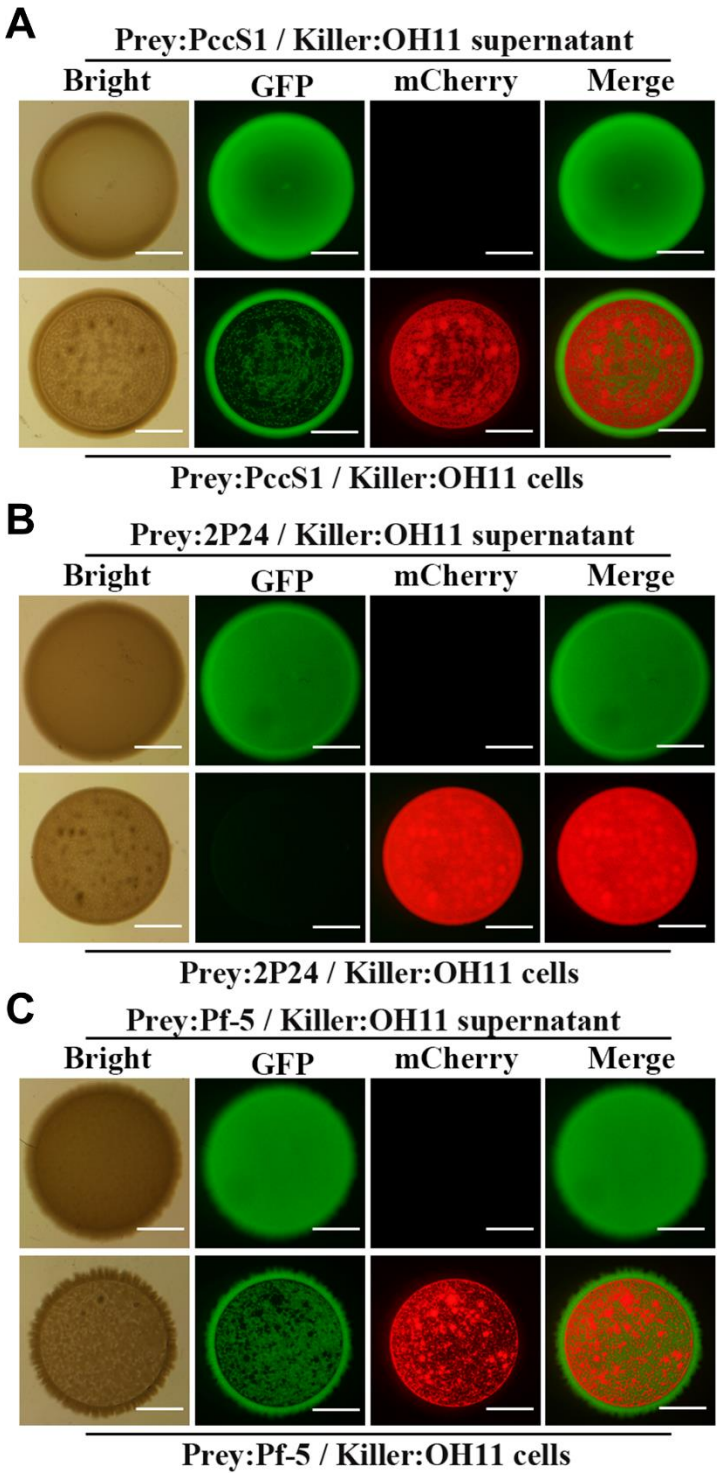


Figure S7. The cell-free supernatant of *L. enzymogenes* OH11 failed to kill three soilborne bacteria when co-culture on LB agar plates in a 1:1 ratio. OH11 was mCheery labelled, while *Pectobacterium carotovorum* PccS1 (**A**), *Pseudomonas* (*Ps.*) *fluorescens* 2P24 (**B**) and *Ps. protegens* Pf-5 (**C**) were GFP labelled, respectively. OH11 cells were used here as a positive control. The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 mm.

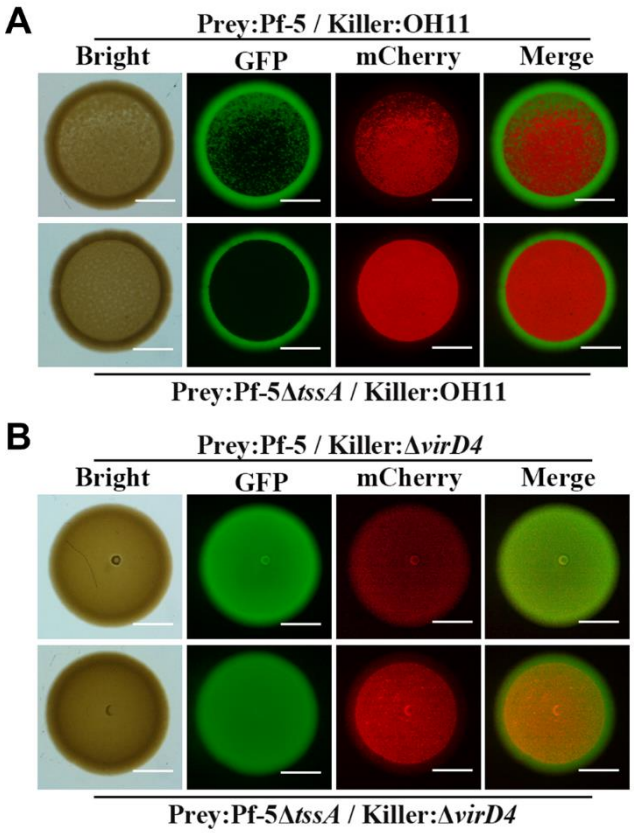


Figure S8. **Evidence of the counterattack of *Pseudomonas protegens* Pf-5 against *L. enzymogenes* OH11 via type VI secretion system (T6SS)**. Compared to the co-culture with the GFP-labelled wild-type Pf-5, the mCherry fluorescence in the *L. enzymogenes* wild-type OH11 (**A**) or the T4SS-defective strain, Δ*virD4* (**B**) was visibly increased when they were co-cultured with the GFP-labelled Δ*tssA* that is a T6SS-inactive mutant of *Ps. protegens* Pf-5, respectively. The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 mm.

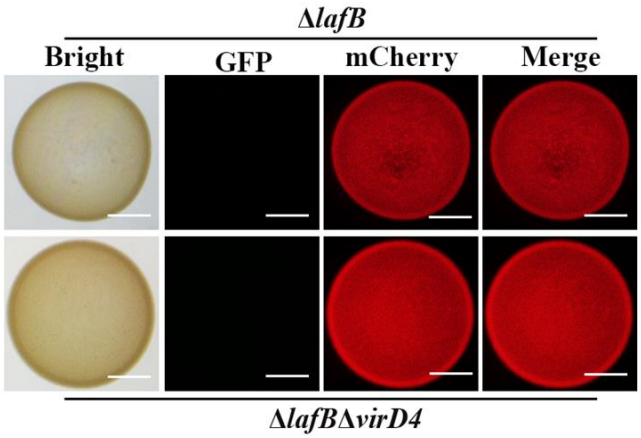


Figure S9. The Δ*lafB*Δ*virD4* strain displayed a fluorescent signal intensity similar to Δ*virD4*. Both Δ*lafB*Δ*virD4* and Δ*virD4* were labelled by mCherry. Δ*virD4*, the OH11 T4SS-defective mutant with in-frame deletion of *virD4*; Δ*lafB*Δ*virD4* – an OH11 derivative with double mutations in *lafB* and *virD4*. The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 mm.

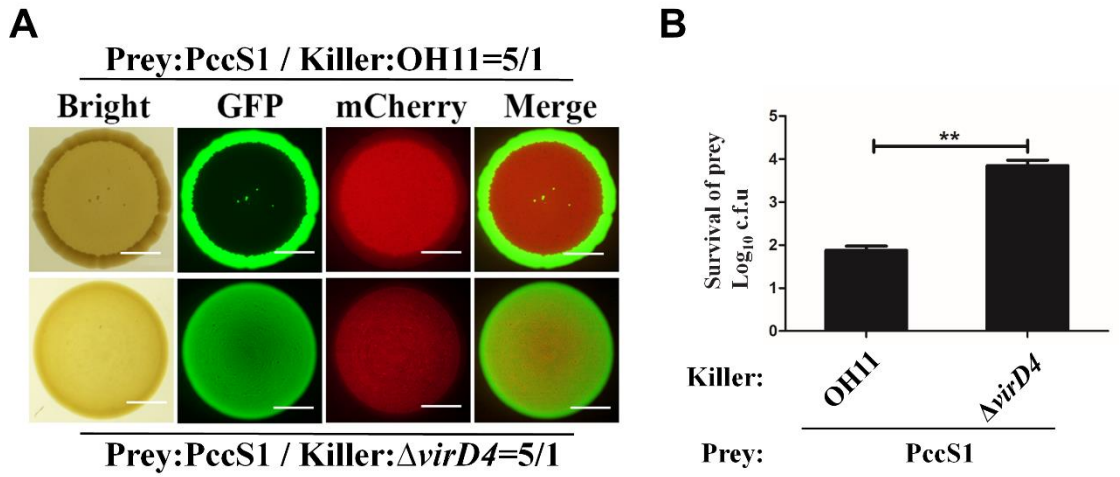


Figure S10. Fluorescent observation of the contact-dependent killing of the mCherry-labelled *L. enzymogenes* OH11 or Δ*virD4* against the GFP-labelled PccS1 that was co-inoculated on agar plates in a 1:5 ratio. The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 mm.

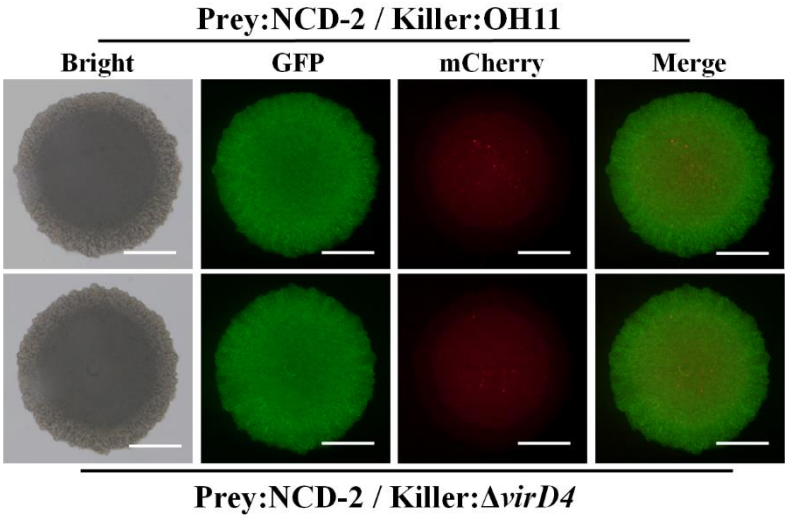


Figure S11. The mCherry-labelled *L. enzymogenes* OH11 was unable to kill the GFP-labelled *Bacillus subtilis* NCD-2 when both strains were co-cultured on agar plates in a 1:1 ratio. NCD-2, a soilboren, gram-positive, biocontrol agent (Guo *et al*., 2019). The fluorescence signals were observed after 24-hour incubation. Bars indicate 2 mm.

References:

Dong, L., Guo, Q., Wang, P., Zhang, X., Su, Z., and Zhao, W. *et al*. (2020) Qualitative and quantitative analyses of the colonization characteristics of *Bacillus subtilis* Strain NCD-2 on Cotton Root. *Curr Microbiol* **77**: 1600-1609.

Guo, S., Zhang, J., Dong, L., Li, X., Asif, M., and Guo, Q. *et al*. (2019) Fengycin produced by *Bacillus subtilis* NCD-2 is involved in suppression of clubroot on Chinese cabbage. *Biol Control* **136**: 104001.

Jiang, F., Waterfield, N.R., Yang, J., Yang, G., and Jin, Q. (2014) A *Pseudomonas aeruginosa* type VI secretion phospholipase D effector targets both prokaryotic and eukaryotic cells. *Cell Host Microbe* **15**: 600-610.

Kovach, M.E., Elzer, P.H., Steven, H.D., Robertson, G.T., Farris, M.A., and Roop, R, M. *et al* (1995) Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**: 175-176.

Ling, J., Wang, H., Wu, P., Li, T., Tang, Y., and Naseer, N. *et al*. (2016) Plant nodulation inducers enhance horizontal gene transfer of *Azorhizobium caulinodans* symbiosis island. *Proc Natl Acad Sci U S A* **113**: 13875-13880.

Lou, L., Qian, G., Xie, Y., Hang, J., Chen, H., and Zaleta-Rivera, K. *et al* (2011). Biosynthesis of HSAF, a tetramic acid-containing macrolactam from *Lysobacter enzymogenes*. *J Am Chem Soc* **133**: 643-645.

Qian, G., Hu, B., Jiang, Y., and Liu, F. (2009) Identification and characterization of *Lysobacter enzymogenes* as a biological control agent against some fungal pathogens. *Agr Sci China* **8**: 68-75.

Qian, G., Wang, Y., Liu, Y., Xu, F., He, Y., and Du, L. *et al*. (2013) *Lysobacter enzymogenes* uses two distinct cell-cell signaling systems for differential regulation of secondary-metabolite biosynthesis and colony morphology. *Appl Environ Microbiol* **79**: 6604-6616.

Su, Z., Chen, H., Wang, P., Tombosa, S., Du, L., and Han, Y. *et al* (2017) 4-Hydroxybenzoic acid is a diffusible factor that connects metabolic shikimate pathway to the biosynthesis of a unique antifungal metabolite in *Lysobacter enzymogenes*. *Mol Microbiol* **104**: 163-178.

Wang, C., Pu, T., Lou, W., Wang, Y., Gao, Z., and Hu, B. *et al*. (2018) Hfq, a RNA chaperone, contributes to virulence by regulating plant cell wall-degrading enzyme production, type VI secretion system expression, bacterial competition, and suppressing host defense response in *Pectobacterium carotovorum*. *Mol Plant Microbe Interact* **31**:1166-1178.

Wang, J., Shen, D., Ge, C., Du, Y., Lin, L., and Liu, J. *et al* (2020) Filamentous *Phytophthora* pathogens deploy effectors to interfere with bacterial growth and motility. *Front Microbiol* **11**: 581511

Wang, P., Chen, H., Qian, G., and Liu, F. (2017) LetR is a TetR family transcription factor from *Lysobacter* controlling antifungal antibiotic biosynthesis. *Appl Microbiol Biotechnol* **101**: 3273-3282.

Xia, J., Chen, J., Chen, Y., Qian, G., and Liu. F. (2018) Type IV pilus biogenesis genes and their roles in biofilm formation in the biological control agent *Lysobacter enzymogenes* OH11. *Appl Microbiol Biotechnol* **102**: 833-846.

Xu, G., Han, S., Huo, C., Chin, K., Chou, S., and Gomelsky, M. *et al.* (2018) Signaling specificity in the c-di-GMP-dependent network regulating antibiotic synthesis in *Lysobacter*. *Nucleic Acids Res* **46**: 9276-9288.
Xu, G., Zhao, Y., Du, L., Qian, G., and Liu, F. (2015) Hfq regulates antibacterial antibiotic biosynthesis and extracellular lytic-enzyme production in *Lysobacter enzymogenes* OH11. *Microb Biotechnol* **8**: 499-509.
Yang, M., Ren, S., Shen, D., Yang, N., Wang, B., and Han, S. *et al*. (2020) An intrinsic mechanism for coordinated production of the contact-dependent and contact-independent weapon systems in a soil bacterium. *PLoS Pathog* **16**: e1008967.
Yin, Z., Liu, H., Li, Z., Ke, X., Dou, D., and Gao, X. *et al* (2018) Genome sequence of *Valsa* canker pathogens uncovers a potential adaptation of colonization of woody bark. *New Phytol* **208**:1202-1216.
Zhang, W., Li, Y., Qian, G., Wang, Y., Chen, H., and Li, Y. *et al*. (2011) Identification and characterization of the anti-methicillin-resistant *Staphylococcus aureus* WAP-8294A2 biosynthetic gene cluster from *Lysobacter enzymogenes* OH11. *Antimicrob Agents Chemother* **55**: 5581-5589.