

Supplementary Materials

5'- region of *hcp1*

CTCCGCTCCGGCATTATCATTATCATTATCATGGAAAGCCAGAAATTAACCTGGCAAGAAATTGACCTATACCTT
 ATCCGCCACCTGCCCATAGACAAGCGAATAAAAAACGTCAAGTCTAATCTTTTCAGAAAAGTCTACACGTCATGAGA
 ATAAAATGTAGGAAGTACGGCTCTAGATCGCGCCAAAAGACTCCTGTTCTTGCTGCAACCGAAAGCGCAACTTTTG
 GCACACTACCCCTGCCACTAGCCAAGGCAAAAACCTCTTTTACGCCTTGCTAAGCAGCACCATTTCATGCTGCGCAAGA
 AGACGCTGGAGCTGCGCAAAAAATTGCGCATATTTCCGATATATAACGCATACAAAATTATTATTTAACGAGCGCA
 TGCGCACCCCATCAAGGGCCCGCAGACGCGCCTCGCGACCTGGCATGATATTTGTTATAGCGATATCCAGCTTCGC
 ACACATCTCGTTGCGTAGCGCTAATAACCTTCCAGGAGAAACCAATG

5'- region of *hcp2*

TGACTATCTAAGCGAATGAAAAACCTGAATTAACCTATTGAAAAATATAGATTTACCCCTGAAAACCTCAGCA
 GGACGCATACGCTCAAACCGCTCAAAGAAAAAAGATCACGTCTAAGAAAGCGATTAAAATTGATACAA
 ATTCAAGCTTCCATAATGACAAAATCTCTTTACCGACACATCAAAAACGATGTAGGAGATGTCTGAAAT
 TTATCTGGCTGAGTATTTTCGCAGCAATAAGACATGCGTCGGCACAACGCCACCATGCGCAGAAAAGCTGC
 GCACCAAAACTGTTAGTAACCTGTGGATATATCGCCACAGACCTTAGTTAACGAGGGGCACAGCCAAGT
 GCGCAAGAAGTTGCGCAACAGTGCGCAACTTCTTGCGCAAACCAAAAAGCTTTTTTACAACGTAAGGGGA
 TCAAGTCGCCGCGACTGCGGGCGTAGCCAGTTGATTTACATGATTTTTTTGTAACATGGCATGATGATT
 GGAAGATGAGCTGAACCAATGAGCGATTTTCGCCTCCGGGCAACTACATCTATCCAGCAAGGAGCACC
 TCCATG

Supplementary Figure S1. Sequence analysis of the upstream regions of *hcp* genes. In *hcp1* upstream region sequence, tandem repeats and the motif homologous to *hcp2* upstream region are underlined, and putative RhlR motifs are highlighted with turquoise. A potential ribosome binding site is highlighted with grey. In *hcp2* upstream region sequence, the region marked with blue text was not required for promoter activity in reporter gene expression. The long inverted repeat is underlined and highlighted with yellow, and putative RhlR motifs are highlighted with turquoise. The predicted -35 and -10 boxes and a potential ribosome binding site are highlighted with grey. Programs used for sequence analysis: VirtualFootprint, published by Richard Münch (Institute of Microbiology, Technical University of Braunschweig, r.muench(at)tu-bs.de) Link: <http://prodoric.tu-bs.de/vfp/index2.php>; Softberry prediction of bacterial promoters: <http://linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb>; REPFIND, published by JN Betley, MC Frith, JH Graber, S Choo and JO Deshler (2002). A ubiquitous and conserved signal for RNA localization in chordates. Current Biology 12:1756-61. Link: <http://zlab.bu.edu/zlab/gene.shtml>.

Supplementary Table S1. Prediction for protein binding sites by Virtual footprints.**Virtual footprint of *hcp1* 5'-region**

AlgU (-10) <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	146	154	-	5.81	ATTCTCATG
AlgU (-10) <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	134	142	+	5.80	AGTCCTACA
AlgU (-10) <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	116	124	+	5.80	AGTCTAATC
FleQ <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	74	84	-	7.03	TGGCGGATAAG
Fur <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	12	30	+	9.23	CATTATCATTATCATTATC
IHF <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	268	275	-	9.83	CAAGGCGT
LasR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	86	101	-	9.41	CTTGTCTATGGGGCAG
LasR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	86	101	+	9.35	CTGCCCCATAGACAAG
LasR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	115	130	+	8.92	CAGTCTAATCTTTTCAG
NarL <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	93	99	-	5.15	TGTCTAT
NarL <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	439	445	-	5.13	TATCGCT
NarL <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	240	246	+	5.12	TGCCACT
PvdS <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	365	373	-	6.69	GTAAATAA
PvdS <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	262	270	-	5.70	CGTAAAAGA
PvdS <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	45	53	+	5.63	ATTAACCT
RhlR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	390	405	-	10.41	CTGCGGGCCCTTGATG
RhlR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	300	315	-	9.47	CTCCAGCGTCTTCTTG
RhlR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	86	101	+	9.27	CTGCCCCATAGACAAG

Virtual footprint of *hcp2* 5'-region

AlgU (-10) <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	484	492	-	5.84	CTTCCAATC
AlgU (-10) <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	84	92	+	5.82	GCTCAAAC
AlgU (-10) <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	94	102	+	5.81	GCTCAAAGA
Fur <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	130	148	-	8.27	TTGAATTTGTATCAATTTT
LasR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	433	448	-	8.91	CTGGCTACGCCGCAG
NarL <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	334	340	-	5.38	TGCCCT
NarL <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	54	60	+	5.34	TACCCCT
NarL <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	533	539	+	5.25	TACATCT
PvdS <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	49	57	-	6.69	GGTAAATCT
PvdS <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	208	216	-	6.67	GATAAATTT
PvdS <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	450	458	-	6.20	TGTAAATCA
RhlR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	433	448	-	9.50	CTGGCTACGCCGCAG
RhlR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	433	448	+	9.49	CTGCGGGCGTAGCCAG
RhlR <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1)	59	74	-	9.33	CTGCTGAGGTTTTTCAG

Supplementary Table S2. Identification of repeated sequences upstream of the coding regions by Repfind.**REPFIND results for *hcp1* upstream sequence**

Nucleotides 1 to 500
P-value cutoff: 0.0001
Minimum repeat length: 3
Maximum repeat length: infinity
Low complexity filter: on
Statistical background: query sequence
Order of background Markov model: 1

Word: **TATCAT**
Word locations: 15 21 27
Most significant cluster: 15 to 32
P-value: 2.0095e-07

Word: **TTATCA**
Word locations: 14 20 26
Most significant cluster: 14 to 31
P-value: 2.26289e-07

Word: **ATTATC**
Word locations: 13 19 25
Most significant cluster: 13 to 30
P-value: 3.05831e-07

Word: **CATTAT**
Word locations: 12 18 24
Most significant cluster: 12 to 29
P-value: 4.19564e-07

Word: **TGCGCA**
Word locations: 296 317 329 381
Most significant cluster: 296 to 386
P-value: 2.5067e-05

Word: **CATTATCAT**
Word locations: 12 24
Most significant cluster: 12 to 32
P-value: 3.30915e-05

Word: **GCGCA**
Word locations: 217 297 318 330 376 382
Most significant cluster: 297 to 386
P-value: 8.87863e-05

Word: **ACCT**
Word locations: 50 66 72 84 418 483
Most significant cluster: 50 to 87
P-value: 9.87784e-05

REPFIND results for *hcp2* upstream sequence

Nucleotides 1 to 559
P-value cutoff: 0.0001
Minimum repeat length: 3
Maximum repeat length: infinity
Low complexity filter: on
Statistical background: query sequence
Order of background Markov model: 1

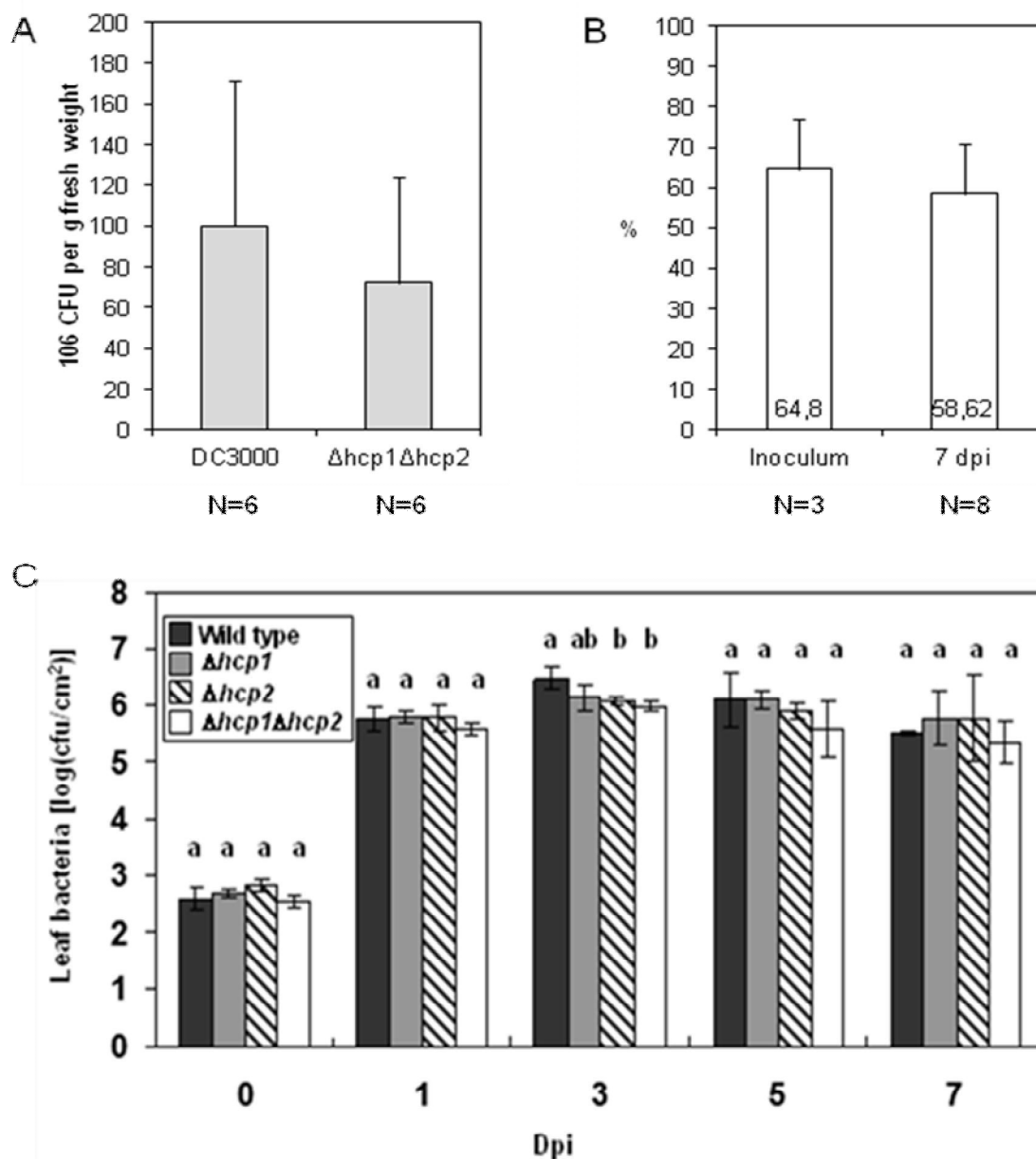
Word: **TGCGCA**
Word locations: 266 278 349 361 371 383
Most significant cluster: 266 to 388
P-value: 3.91261e-09

Word: **TGCGCAA**
Word locations: 349 361 371 383
Most significant cluster: 349 to 389
P-value: 8.07805e-09

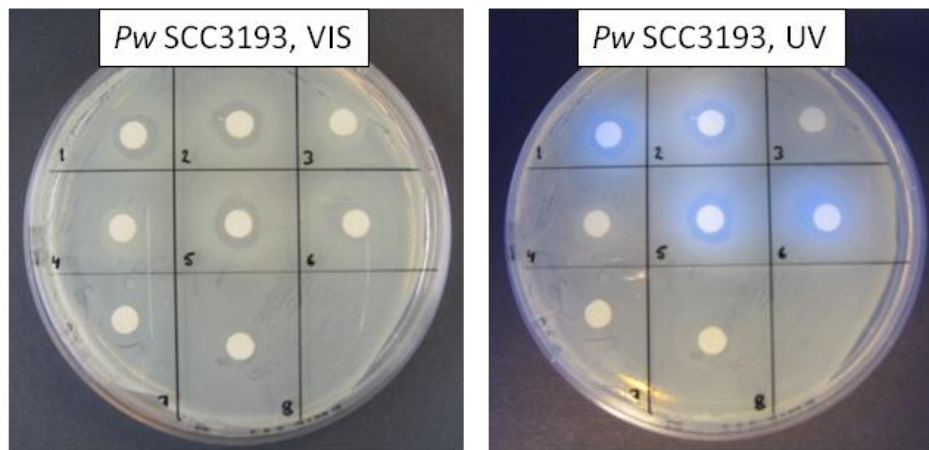
Word: **TGCG**
Word locations: 245 266 278 349 361 371 383 434
Most significant cluster: 245 to 437
P-value: 2.02304e-05

Supplementary Table S3. Promoter predictions by Softberry.

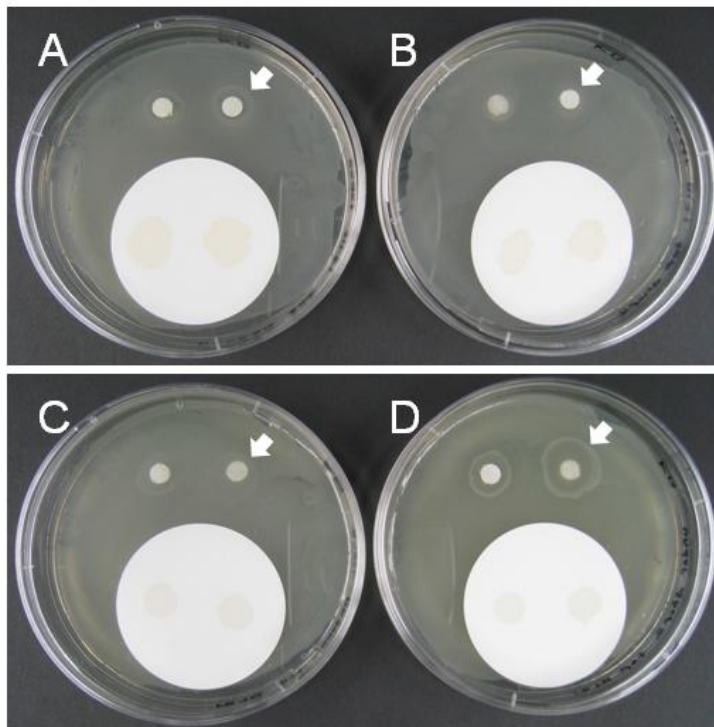
Promoter prediction in <i>hcp1</i> 5'-region	
Promoter Pos: <u>132</u> LDF- 3.89 -10 box at pos. 117 GTCTAATCT Score 47 -35 box at pos. 94 TAGACA Score 29 Promoter Pos: <u>436</u> LDF- 0.98 -10 box at pos. 421 TGGCATGAT Score 52 -35 box at pos. 403 CAGACG Score -12	Oligonucleotides from known TF binding sites: For promoter at 132: arcA: AATAAAAA at position 104 Score - 12 rpoD17: GTCCTACA at position 135 Score - 15 ihf: AATAAAAT at position 152 Score - 10 For promoter at 436: soxS: ATTTGTTA at position 430 Score - 10
Promoter prediction in <i>hcp2</i> 5'-region	
Promoter Pos: <u>482</u> LDF- 4.25 -10 box at pos. 467 TTGTAACAT Score 62 -35 box at pos. 449 TTGATT Score 53	Oligonucleotides from known TF binding sites: Irp: ATTTTTTT at position 461 Score - 11 rpoD15: TTTTGTAA at position 465 Score - 11



Supplementary Figure S2. Deletion of the *hcp1* and *hcp2* genes in *Pst* DC3000 did not affect bacterial growth in the host plants *Arabidopsis* and tomato. A. Separate inoculations of *Pst* DC3000 wild type and $\Delta hcp1\Delta hcp2$ mutant strain in *Arabidopsis thaliana* ecotype Colombia. *Pst* populations in *Arabidopsis* plants were determined 7 days after spray-inoculation. B. Mixed inoculation with wild type and $\Delta hcp1\Delta hcp2$ mutant strain. Proportion of $\Delta hcp1\Delta hcp2$ of the total *Pst* population was determined in the mixed inoculum at time point 0 and in *Arabidopsis* 7 days post inoculation. C. Separate inoculations of *Pst* DC3000 wild type and its mutant derivatives $\Delta hcp1$, $\Delta hcp2$ and $\Delta hcp1\Delta hcp2$ in tomato cultivar Moneymaker. Samples were taken for bacterial counting 0, 1, 3, 5 and 7 days after dip-inoculation of tomato seedlings with 2×10^7 cfu/mL *Pst*. Each value represents mean \pm standard deviation of three replicates. Data analysis was performed using Duncan's multiple range test. Means with the same letter above the bars indicate no difference at a significance level of 5%. This experiment was repeated three times with similar results. Dpi, days post-inoculation.

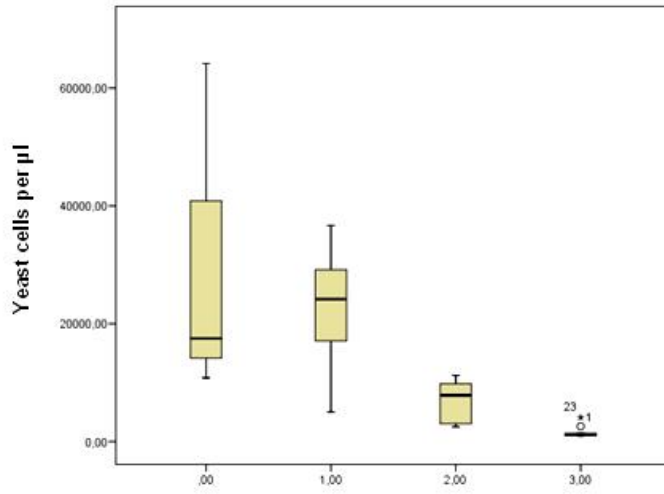


Supplementary Figure S3. The ability of *P. syringae* pv. tomato (*Pst*) DC3000 to survive in mixed culture with *Pectobacterium wasabiae* (*Pw*) SCC3193 is dependent on *hcp2*-encoded function. *Pst* strains applied on top of the *Pc* cell layer: 1 and 2, DC3000 wt; 3 and 4, mutant $\Delta hcp2$; 5 and 6, complemented strain $\Delta hcp2 + hcp2$; 7 and 8, control with no *Pst*. *Pw* cell layer is opaque whereas *Pst* cell layer is translucent, appearing as a clearer zone around the application sites, and under UV illumination *Pst* shows characteristic fluorescence.

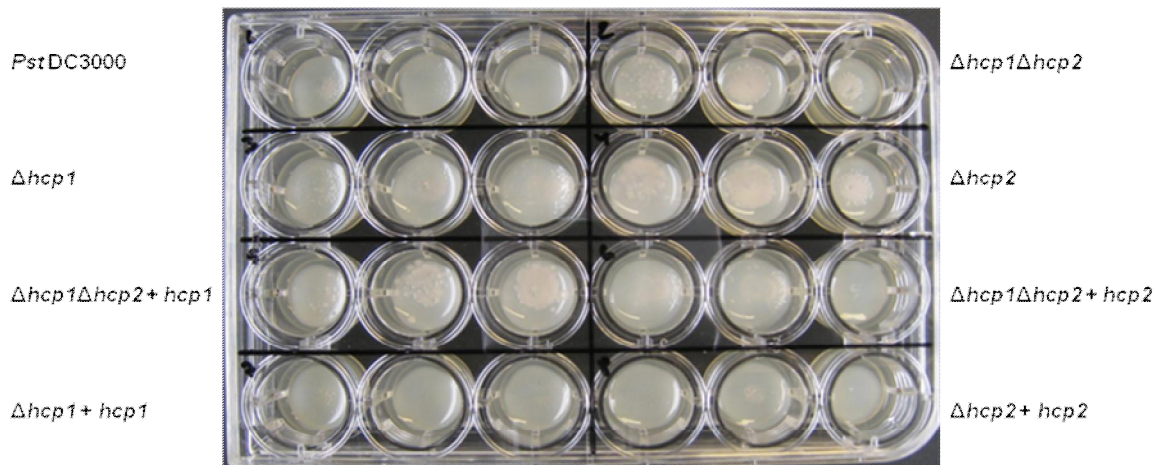


Supplementary Figure S4. *hcp2*-dependent competitive activity of *P. syringae* against enterobacteria requires cell-cell contact. When a cell-impermeable membrane was placed in between the two bacterial cell layers, no difference between the growth of the parent or mutant strain of *Pst* was detected. Without the separating membrane, clear differences were observed (arrows). A. *E. coli* BL21 spread on the plate, *Pst* DC3000 wt applied on the top. B. *E. coli* BL21 spread on the plate, *Pst* $\Delta hcp2$ applied on the top. C. *Pst* DC3000 wt spread on the plate, *E. coli* BL21 applied on the top. D. *Pst* $\Delta hcp2$ spread on the plate, *E. coli* BL21 applied on the top.

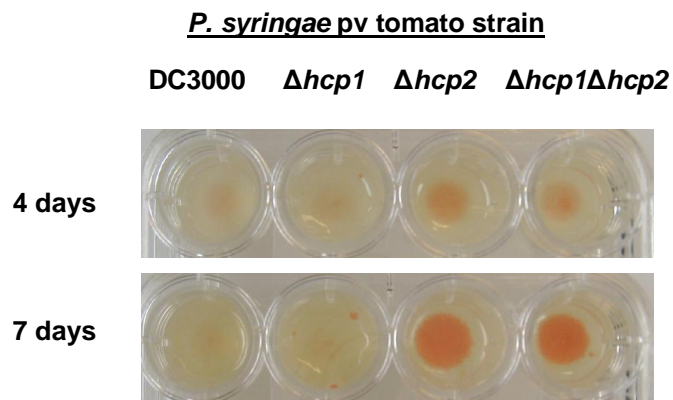
A



B



C



Supplementary Figure S5. Yeast growth suppression by *P. syringae* pv. tomato DC3000 is *hcp*-dependent. **A.** Multiple comparisons of yeast cell number vs. *Pseudomonas hcp* genes in mixed cultures of *P. syringae* pv. tomato (*Pst*) and *Cryptococcus carnescens* reveal strong correlation between the presence of *hcp* genes and suppression of yeast growth. X axis categories: 0=no *hcp* genes, 1=*hcp1* only, 2=*hcp2* only, 3=both *hcp1* and *hcp2*. **B.** Photograph taken of 7-days-old mixed cultures of *Pst* and *Cryptococcus*, which can be seen as pink colonies when growing abundantly. **C.** Suppression of the growth of *Rhodotorula glutinis* by *Pst* in mixed culture is dependent on *hcp2*. *Rhodotorula* growth is bright red in the photographs taken of 4 days and 7 days-old mixed cultures on PYG agar. Each culture was started with 10^5 *Pst* cells and 10^5 *Rhodotorula* cells.