

Supplementary information

A Resident Prophage Metabolically Reprograms Root-Colonizing *Pseudomonas* to Modulate Plant Physiology

Jonelle T.R. Basso^{1,2}, Thai Dao¹, Vlastimil Novak², Maureen Berg¹, Nicolas Grosjean¹, Saray Maeda³, Meghana Faltane¹, Marie Lynde¹, Benjamin P. Bowen², Zhiying Zhao¹, Nathalie H. Elisabeth^{4,5}, Mária Džunková^{1,6}, Katherine Louie^{1,2}, Trent R. Northen^{1,2}, Axel Visel^{1,2}

¹DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA.

²Environmental Genomics and Systems Biology, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA.

³University of California, Merced, California, 95343, USA.

⁴Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA.

⁵Present address: The NSF BioFoundry for Extreme and Exceptional Fungi, Archaea and Bacteria (ExFAB), University of California, Santa Barbara, Santa Barbara, CA, 93106.

⁶Present address: Institute for Integrative Systems Biology (I2SysBio), University of Valencia and Spanish National Research Council (CSIC), Valencia, Spain

Correspondence: Jonelle T.R. Basso (jbasso@lbl.gov)

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Supplementary Figures

Figure S1. Bioinformatic analysis and empirical evidence of resident tailocin, PS417t. **a**, Gene category prediction. **b**, Negative stained TEM micrographs of R-type tailocin-like particles from *P. simiae* WCS417. Headless, rigid rod-shaped particles consistent with tailocins are shown. Particles display a visible tail tube (white arrow) and a thicker sheath (black arrow). No capsids are observed. Scale: 200 nm.

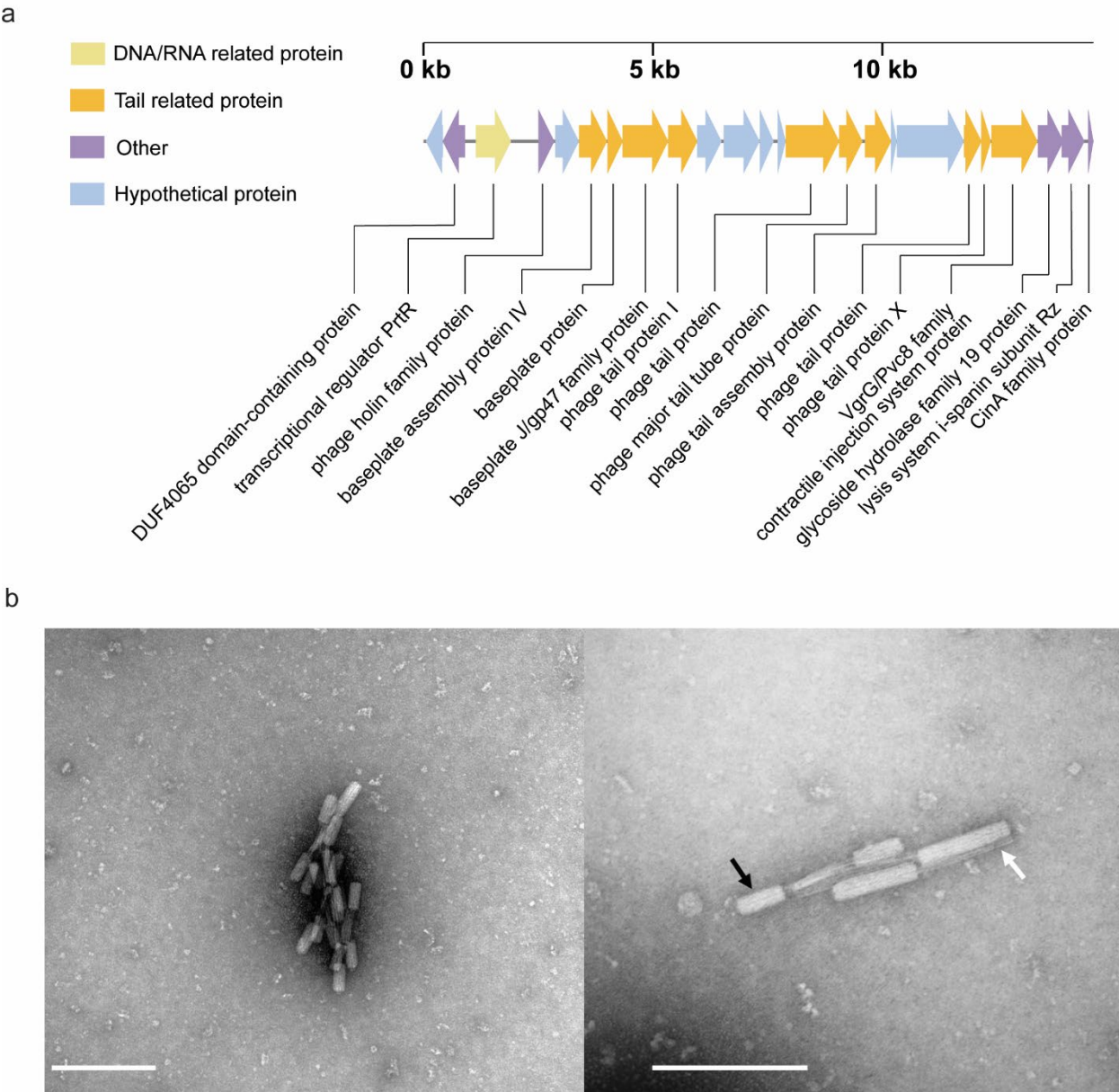


Figure S2. Construction of pTD001 plasmid. Three PCR fragments covering the backbone plasmid pMo130 excluding *KanR* (JB11/17, JB14/15, JB16/18) were combined with the *TetR* CDS amplified from pBR322 (JB12/13) and the 1kb upstream and downstream sequences of *P. simiae* WCS417 locus PS417_10145 (JB19/20, JB21/22) to generate pMo130-145-TcR. A PCR fragment amplified from pMo130-145-TcR excluding the upstream and downstream sequences of PS417_10145 and *XylE* gene (TD001/002) was self-ligated to generate pTD001. pTD001 carries a multiple cloning site (MCS), a *TetR* gene with AmpR promoter encoding tetracyclin resistance, a *sacB* gene providing counter-selection on high-sucrose media, and the ribosomal transcriptional terminator *rrnB* T1. ori and oriT represent a ColE1 origin of replication and an RK2 origin of transfer, respectively.

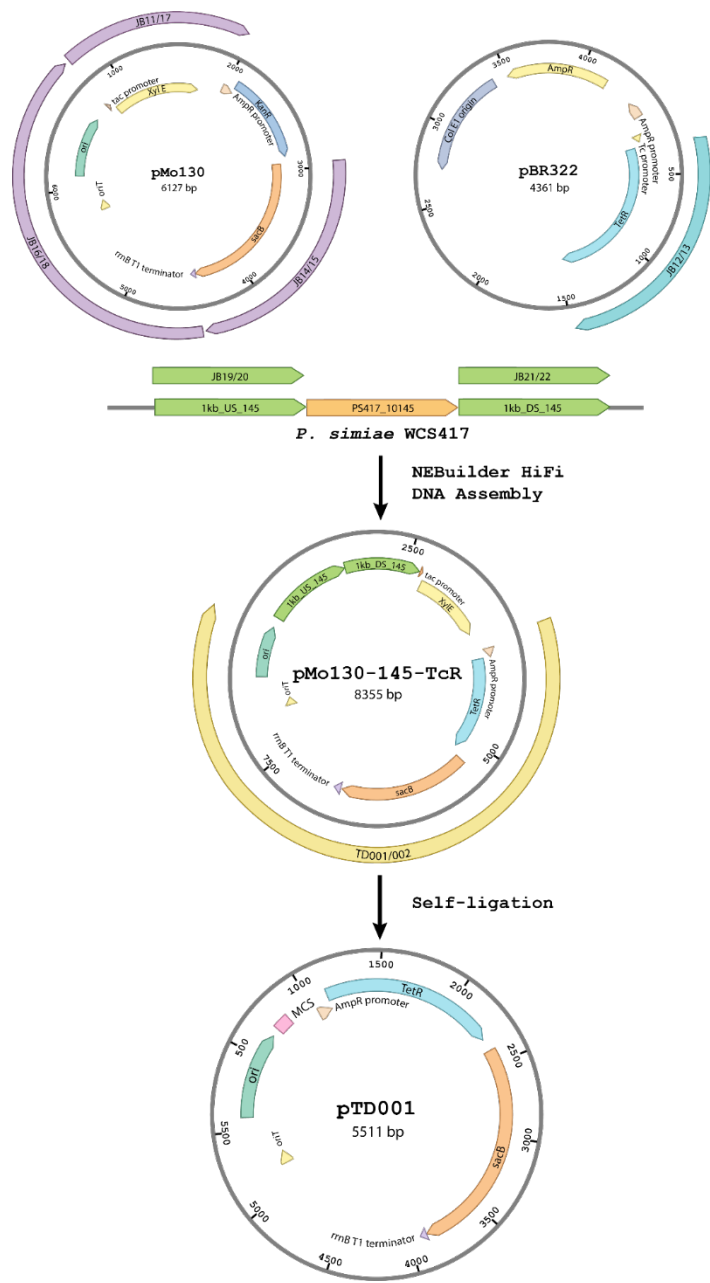


Figure S3. Strategy for prophage deletion by allelic exchange. Allelic exchange to generate phage-deletion mutant in *P. simiae* WCS417. The 1kb upstream and downstream sequences of target phage signature in *P. simiae* were cloned into pTD001 by Twist Bioscience to generate pTD001-phageUS/DS. pTD001-phageUS/DS was introduced into *P. simiae* WCS417 through conjugation and co-integrants were recovered from Kan/Apr/Tet selection. Resolved co-integrants were recovered from high-sucrose/Kan/Apr selection. Colonies were analyzed by PCR to distinguish deletion mutants from wild types. Phage-deletion mutants were confirmed by whole-genome sequencing.

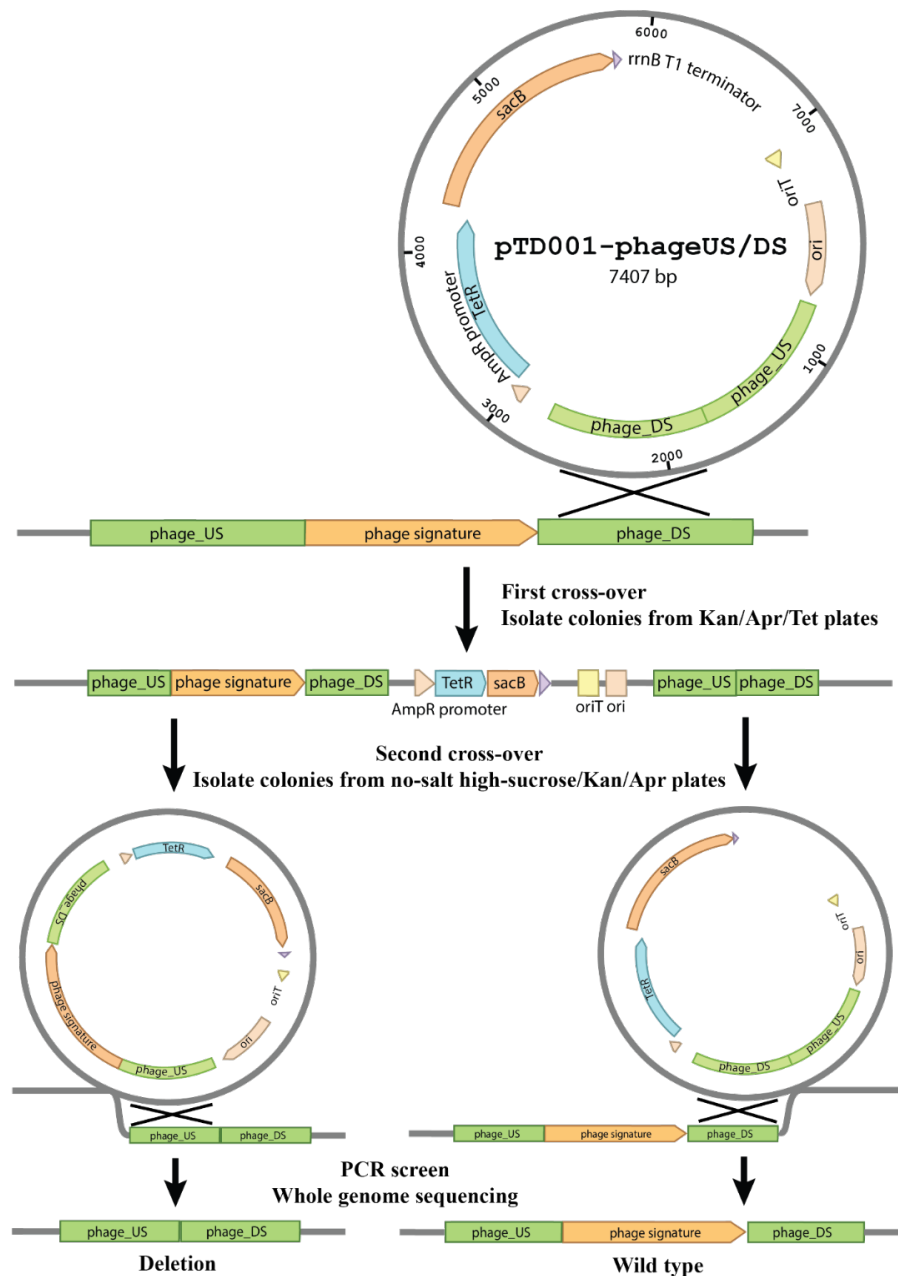


Figure S4. Growth curve comparison on LB growth medium of wild type *P. simiae* WCS417 (teal-colored plots), fluorescently labeled *P. simiae* SB642 (green plots) and fluorescently labeled *P. simiae* Δ phage strain (beige-colored plots). **a**, Media blank control is denoted in red. **b**, Assessment of the comparative growth rate, **c**, lag time in hours and **d**, maximum OD600 was determined.

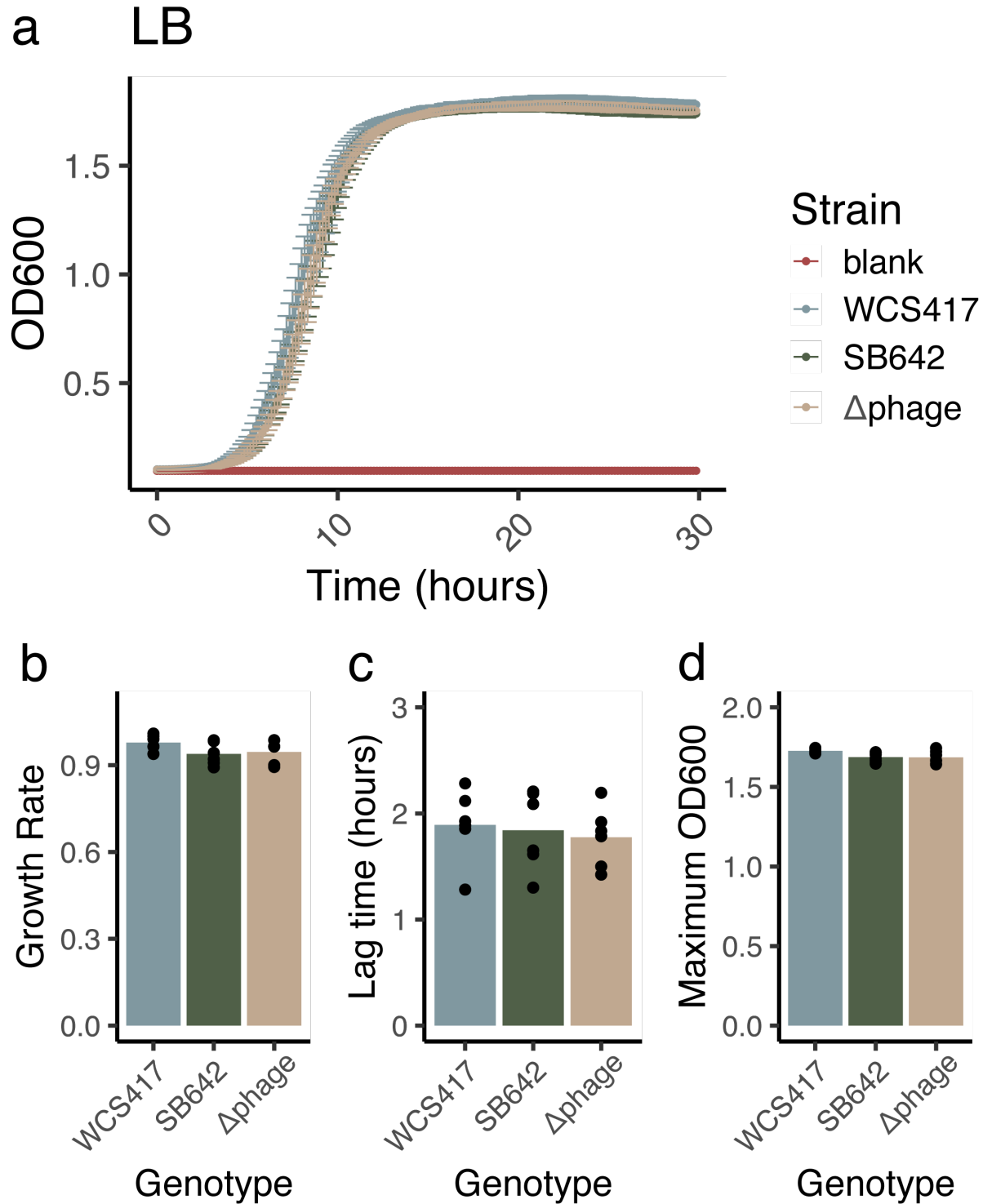


Figure S5. Biolog phenotypic profiling of fluorescently labeled *P. simiae* SB642 and Δ phage strains for 190 substrates, using an automated phenotyping platform, Biolog Odin. Cumulative NADH production was determined for 190 substrates from the carbon utilization microassay plates, **a**, PM01 and **b**, PM2A for both labeled *P. simiae* SB642 and Δ phage strains (NADH production standard measurement wavelength 740 nm). PM2A plates were repeated 2 independent times, each time in biological triplicates (total n=6 for PM2A).

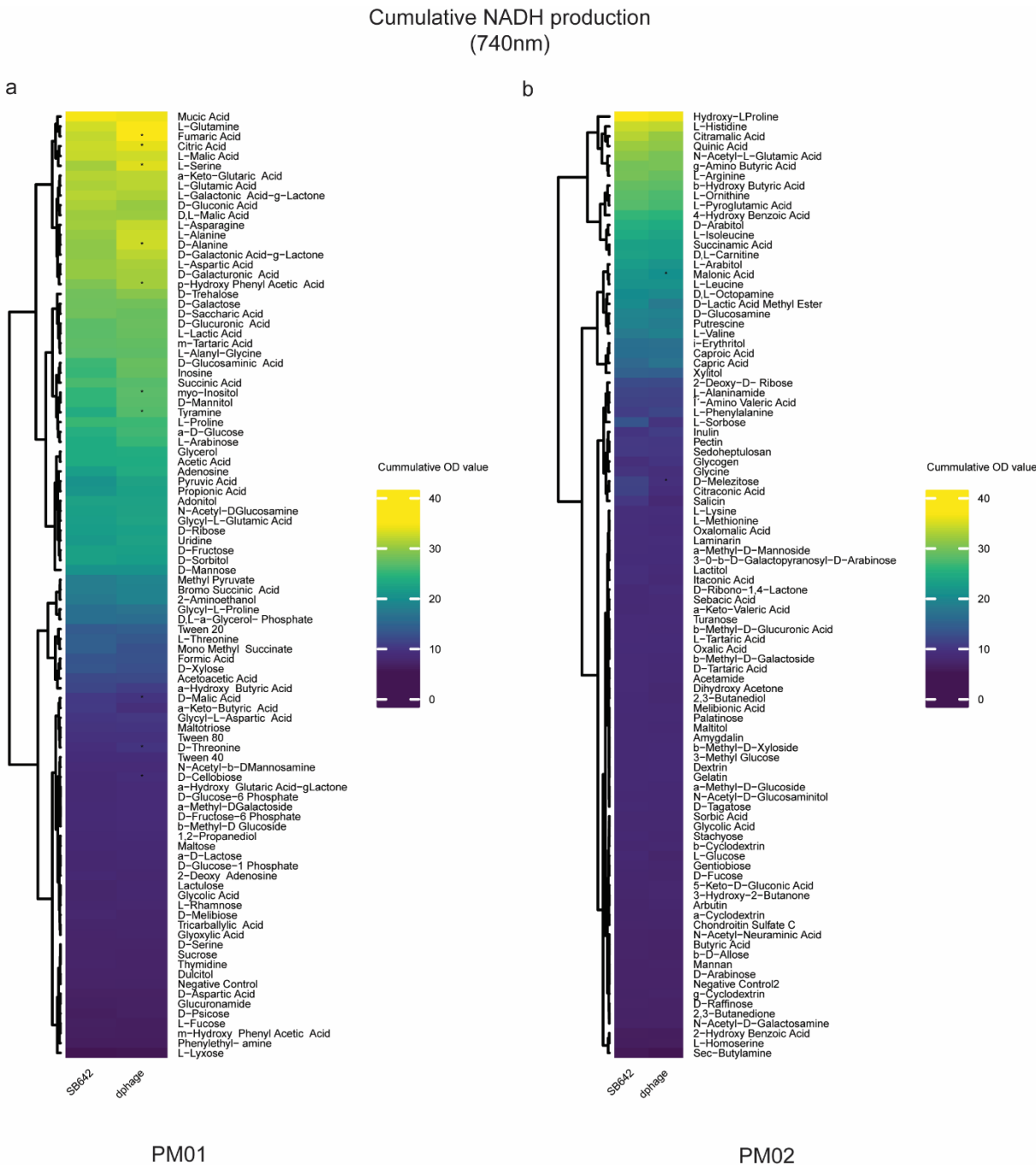
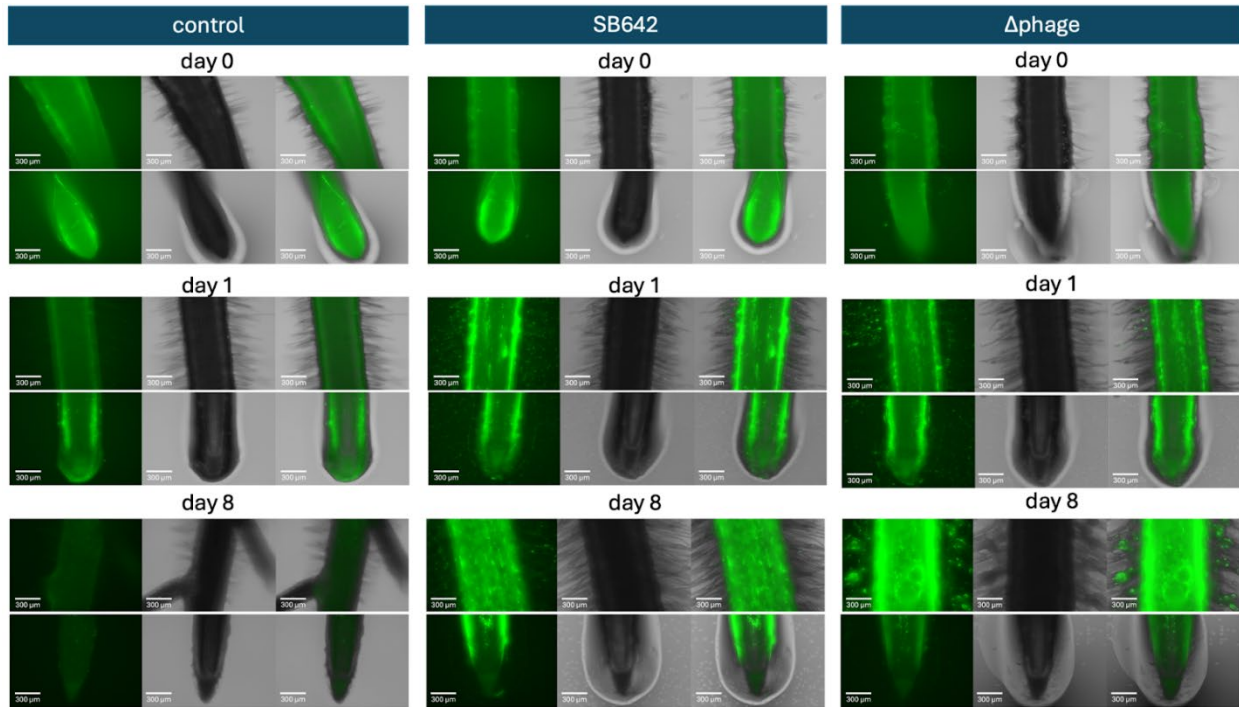


Figure S6. Microscopy validation of bacterial colonization on *B. distachyon* roots. Imaging was conducted on *B. distachyon* roots treated with no bacterial control, *P. simiae* SB642 and Δ phage strains at Day 0, Day 1 and Day 8. Scale bar: 300 μ m.



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Supplementary Tables

156 **Table S1.** List of *P. simiae* WCS417 resident prophage, PS417-V gene annotations. PS417-V genes include
157 63.16% hypothetical proteins, 3.95% peptidases, 1.32% phage capsid proteins, 6.58% phage tail proteins,
158 1.32% integrase proteins (integrase), 2.63% SOS response proteins, 17.11% other phage-like proteins and
159 3.95% regulators.

Gene classification	Gene
Peptidase	Peptidase YpeB-like protein
	YqaJ-like recombinase protein
	HNH endonuclease
	Putative chitinase
	Proteasome lid subunit RPN8/RPN11
	Putative metallopeptidase
Phage capsid protein	SPP1 gp7 family putative phage head morphogenesis protein
Phage tail protein	Tail tube protein
	Lambda family phage tail tape measure protein
	Lambda family phage minor tail protein L
Integrase protein	Integrase
SOS response protein	Putative SOS response-associated YedK
	RecT family protein
	Phage repressor protein C with HTH and peptidase S24 domain
Other phage-like protein	Tail assembly chaperone

Gene classification	Gene
	Uncharacterized protein YjdB
	Signal transduction histidine kinase
	PAPS reductase/FAD synthetase
	NinG protein
	Terminase large submit-like protein
	DnaT-like ssDNA binding protein
Regulator	Phage regulator Rha-like protein
	Regulatory protein CII

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161 *note: PS417-V includes non-annotated hypothetical proteins

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Table S2. List of *P. simiae* WCS417 resident tailocin, PS417t gene annotations. PS417t comprises 52.38% hypothetical proteins, 33.33% phage structural proteins and 14.29% other phage-like proteins.

Gene classification	Gene
Phage structural protein	Baseplate assembly proteins
	phage tail P2 like protein
	bacteriophage tail sheath protein
	bacteriophage tail tube protein
Other phage-like proteins	tail assembly chaperone E/41/14-like protein
	Cro/Ci-type helix-turn-helix DNA binding protein
	holin family Hol protein
	putative chitinase

*note: PS417t includes non-annotated hypothetical proteins

188 **Table S3.** List of all primer sequences used in cloning for strain engineering.

Primer	Note	Sequence
JB11	Pairs with JB17, amplify a 1.36 kb fragment from pMo130	GACGATGAGCGCATTGTTAGATTTCATACTC TTCCTTTTCAATATTATTGAAGC
JB12	Pairs with JB13, amplify the TetR gene from pBR322	TATTGAAAAAGGAAGAGTATGAAATCTAAC AATGCGCTCATCGTCATCCTCGGCACCG
JB13	Pairs with JB12, amplify the TetR gene from pBR322	AACAGCTCACTAGTTTAATTAATCAGGTCG AGGTGGCCCGGCT
JB14	Pairs with JB15, amplify a 1.46 kb fragment from pMo130	CCACCTCGACCTGATTAATTAAGTAGTGAG CTGTTGACAATTAATCATCG
JB15	Pairs with JB14, amplify a 1.46 kb fragment from pMo130	GTCTGCGTAGAATCCTCTGTTTGTCATATAG CTTGTAATCACGAC
JB16	Pairs with JB18, amplify a 2.57 kb fragment from pMo130	CAAGCTATATGACAAACAGAGGATTCTACG CAGACAAACAATCAACGTTTGC
JB17	Pairs with JB11, amplify a 1.36kb fragment from pMo130	AAGATCTGAGCTGTTGACAATTAATCATCG GCTCGTATAATGTGTGGAATATCACAGAA
JB18	Pairs with JB16, amplify a 2.57 kb fragment from pMo130	GGCCTCAGCTGATATCAGCTCACTCAAATA ATACGGTTATCCACAGAATCAATAAAGG
JB19	Pairs with JB20, amplify a 1 kb upstream region of <i>P.simiae</i> PS415_10145	TATTATTGAGTGAGCTGATATCAGCTGAGG CCGTGGCCACTGTGACT
JB20	Pairs with JB19, amplify a 1 kb upstream region of <i>P.simiae</i> PS415_10145	TTGAATAATTGCCTGTGGAGATCTCAACTCA GCAGGTTAGAGTTCCGTCAGTTT
JB21	Pairs with JB22, amplify a 1 kb downstream region of <i>P.simiae</i> PS415_10145	AGTTGAGATCTCCACAGGCAATTATTCAAG GTGAGTCGATGAACATTGCAGAACT

Primer	Note	Sequence
JB22	Pairs with JB21, amplify a 1 kb downstream region of <i>P.simiae</i> PS415_10145	TGATTAATGGTCAACAGCTCAGATCTTAGA ACTAGAATGCCGAACCAGTAGTACCAAT
TD001	Overlaps with TD002, used to generate pTD001 from pMo130-145-TetR	TTGAGTGAGCTGATATCAGGCGGCCGCCCT GCAGCGGATCCCTCTAGATGCATG
TD002	Overlaps with TD001, used to generate pTD001 from pMo130-145-TetR	GCGGCCGCCTGATATCAGCTCACTCAAATA ATACGGTTATCCACAGAATC
TD043	Starts ~ 500 bp upstream of prophage signature, pairs with TD044 to amplify a ~1 kb fragment in $\Delta\phi$ mutant	GAACAAGCTCGCCTCAATG
TD044	Starts ~ 500 bp downstream of prophage signature, pairs with TD043 to amplify a ~1 kb fragment in $\Delta\phi$ mutant	TTGAGCGTGGCGTCTATG
TD054	Pairs with TD043 to amplify a ~1 kb fragment if prophage signature is present	TGCACTCAGGATAACGAGCG
TD055	Pairs with TD044 to amplify a ~1 kb fragment if prophage signature is present	TCCTGTAGAAGTACGGCTCG

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199 **Table S4.** DNA sequences for the upstream and downstream regions of target prophage, PS417-V.

Sequence name: Δφ_US
Note: ~1 kb sequence upstream of prophage signature
Sequence: GTCTTCTTCCAGCTGGCCGAAGTAGTAGAGGTGCTCACCATCCCCACCCATTAGGCTGGGCGGTTGCCAGGA GCGTGCGGCTACGCAGGACGAGCAAGTTTTGAAGGTGGCCATGTCGCCCTCCCAGCATCCGCTGATGAGCTGGT ACTGCTGGCCAGGTTGGATCGGGCCGTAGCATTGCGAGCATTGGTGCTGCTTGCGCGCTACGGGCATGGTTTCCG TTTGAAAGTCGGACATACGAATACCTCGCCCCCACTACCGGCAGGAATCTATGGTGTAATGAGAAGAATTCTTA AAGGAGTGCGAGAAGGATAGTGCCTGGGGGACTGCCTCGTTAACGCGGATGTTTTCGAGCAACCTCAATCGTAA TGCTGGACTCGCGATGTCCCTTTCTTAAGCCTTGATTCTCGGATGGAACGCCAAAGGAAATGCGTTCATGAAGTA CAGCTGGATTGTGATTGCTTTACTGCTTTGTGCCTGCGTTAGCGGCCCAAGCCCTCAACAACCTGCAGAGCAACG TTTATGGGAATATCAGTTGCTTATCCATATCTCTAGGTTCAAGCACTACCCAGAACAAGCTCGCCTCAATGGGCTGG AGGGCACAGTTGCAGTCGAGTTCATCGTCGATGCTCGAGGCAACCTGTCGCACCAGAAAGTCATCAGCTACTCAG GGTCAGAATTTTTTGTCCGGAGGCTAAGGAATTGATTTCGGCTGCATCCCCTGCGCCCGCGCCTCCACCTTCAAT CCTTAAGAATGGTGGTGTTATGTAGTTGCCCCCTTTGTGTATTGCCTCGCACAGGGTATGTGCCAAAAGATCTG GTTACCCATCAATATCGCGGAGAATAGCCCTAAGGCGACTGCGCACCATGTCGACGCTGGCGTCTGGTGACTGCTT CATGACCTCCAAAGCAGTCGCCGAGGTAGATCTGGTACTCACTCATGGCCTTGGCCCCTTGTGGATGAATACGTAG GCGAACCAGAGGGTGCGCA
Sequence name: Δφ_DS
Note: ~1 kb sequence downstream of prophage signature
Sequence: CGGTACGTCCTTACCTGAAAACACATAGAGCTCGGTTATCGGCTGATTTCAACTTTTCTTTATTCAAACGATCAATTT AAACTTGCTTTGTGATGAAGTGCTCGCTCACCACAAAAACGCGTTGTTTATTCCCTGCTTTTGACATGAGCGCCCA CCTGCTGCACAGTGCGCGCCCTCTAATAAGACCTCCTTCACGCCTGCACGCTGGTTGCCACCCGATGGTCGCCTGG GCGCGGGCATGCCTGTTTTTTATGTTTTTCTGCTTGAGGTAACCATGATTAACGCAGTCATTGCCGCGGTGCGCAC CATGCTGGTGCTCAGTTTGTCCCGCGTGCATGTGGTCATCGCCATCATCGTCGGCGCCCTGGTGGGTGGTTTGACC GGTGGCCTGGGCATAGACGCCACGCTCAAAGCCTTCAATGGCGGTTTGGGCGGTGGTGCGACCGTGGCGTTGTC CTACGCCTTGCTCGGCGCTTTCGCCGTGGCAATTGCCAATCCGGCTTGGCCCACGCCCTGGCCGACAAGGCGCT GCTGCTGGTGGACCGCCAGGACGCCAGCGGCGGCGGCCACGTCAAATGGCTGCTGATCGGCCTGCTGTGGGTG GTGGCGATTGCCTCGCAGAACATCCTGCCGATTACATCGCGTTTATTCCGTTGCTGGTGCCGCCGCTGCTCTACG TGCTGACCAAGCTGCAACTGGACCGCCGCTGATCGCCTGCGTGATGACCTTCGGCCTGATCACGCCCTACATGTT TTTGCCGGTGGGCTTCGGCAATATCTTCCTCAACCAGATCCTGCTGGCCAACGTGGCCAAGAGCGGCGTGGATAT CAGCCAGGTCAACGTACCCACGCCATGAGCCTGCCGGCACTCGGCATGGTCGTGGGCTTGTGGTGGCGGTGT TTATCAGTTACCGCAAGAAGCGCGTGTACGACCTGGAGAAAATCGAGCGGGTCGAGCAAGTGCGGTTGCAATAC AACCCGCTGACGCTGCTGGTG

Table S5. List of targeted metabolites detected from *A. thaliana* and *B. distachyon* root colonization assays. These metabolites were categorized by the following classes: i. nucleic acids, nucleotides, nucleosides and their derivatives, ii. organic acids, iii. amino acids and their derivatives, iv. carbohydrates and their derivatives, v. purines and pyrimidines, and vi. assorted metabolites.

Metabolite class	<i>Arabidopsis thaliana</i> detected metabolites	<i>Brachypodium distachyon</i> detected metabolites
i. nucleic acids, nucleotides, nucleosides and their derivatives	uracil	
	cytidine	
	uridine	uridine
	2'-deoxyadenosine	
	5'-deoxyadenosine	
	guanosine	guanosine
	uridine-5-monophosphoric acid	
	thymidine	thymidine
	5-methylcytosine	
	guanosine 3',5'-cyclic monophosphoric acid	
	cytidine 2',3'-cyclic monophosphoric acid	
	deoxycytidine	deoxycytidine
	2',3'-cyclic AMP	
ii. organic acids	4-aminobutanoic acid	

Metabolite class	<i>Arabidopsis thaliana</i> detected metabolites	<i>Brachypodium distachyon</i> detected metabolites
	pipecolic acid	
	malic acid	malic acid
	isonicotinic acid	
	2-hydroxycinnamic acid/ 4-coumaric acid	2-hydroxycinnamic acid
	gluconic acid	gluconic acid
	galacturonic acid/ glucuronic acid	glucuronic acid
	pantothenic acid	
	4-imidazoleacetic acid	4-imidazoleacetic acid
	4-quinolinecarboxylic acid	
	xanthurenic acid	
	4-pyridoxic acid	4-pyridoxic acid
	orotic acid	orotic acid
	6-hydroxynicotinic acid	
	4-hydroxy-2-quinolinecarboxylic acid	4-hydroxy-2-quinolinecarboxylic acid
		4-guanidinobutanoic acid

Metabolite class	<i>Arabidopsis thaliana</i> detected metabolites	<i>Brachypodium distachyon</i> detected metabolites
		abscisic acid
		indole-3-acetic acid
		itaconic acid
		salicylic acid
		syringic acid
		vanillic acid
iii. amino acids and their derivatives	asparagine	
	glutamine	
	citrulline	
	histidine	
	serine	
	lysine	
	glutamic acid	
	isoleucine	
	phenylalanine	
	tyrosine	
	arginine	

Metabolite class	<i>Arabidopsis thaliana</i> detected metabolites	<i>Brachypodium distachyon</i> detected metabolites
	N-trimethyllysine	
	valine	
	N-alpha-acetyl-lysine	
	leucine/ norleucine	
	tryptophan	
	aspartic acid	
	5-oxo-proline	
	proline	
	cysteic acid	
	N-acetyl-glutamic acid	
	N-acetyl-aspartic acid	
	N-acetyl-leucine	
	N-acetyl-phenylalanine	
		3-methoxy-anthranilic acid
		kynurenine
		N-alpha-acetyl-asparagine
	sucrose	

Metabolite class	<i>Arabidopsis thaliana</i> detected metabolites	<i>Brachypodium distachyon</i> detected metabolites
iv. carbohydrates and their derivatives	stachyose	
	raffinose	raffinose
	lactose/ palatinose	
	cellobiose/ lactose/ maltose	
	trehalose	
		galactitol
		mannitol
		N-acetyl-mannosamine
		N-acetyl-mannosamine
v. purines and pyrimidines	guanine	guanine
	adenine	adenine
	3-methyladenine	
	xanthine	xanthine
	thymine	thymine
	cytosine	cytosine
vi. assorted metabolites	agmatine sulfuric acid	
	myo-inositol	

Metabolite class	<i>Arabidopsis thaliana</i> detected metabolites	<i>Brachypodium distachyon</i> detected metabolites
	N-acetylputrescine	
	choline	
	trigonelline	trigonelline
	pyridoxine	pyridoxine
	pterin	
	pyridoxamine	
		4-hydroxy-2-quinolone, histamine
		Indole-3-carboxaldehyde
		myo-inositol
		phenethylamine
		sorbitol

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Table S6. Sterility test plates for treatments used in metabolomics investigations. Five to ten microliters of media were taken from magenta boxes during the 4-week experiment to test for sterility. **a**, Plates were incubated at 28°C for 48-72 hours for media control, no bacteria control, and bacterial treatment with *P. simiae* SB642 and Δ phage strains at day 14 (T1) of the experiment. **b**, Plates were also incubated for media control and no bacteria control treatments at the end (T Final) of the experiment. Sterility test results showed no indication of contamination.

Treatment groups		Sterility results	
Treatment	Replicate	Sterile at T1	Sterile at T Final
Media control	1	Yes	Yes
Media control	2	Yes	Yes
Media control	3	Yes	Yes
Media control	4	Yes	Yes
Media control	5	Yes	Yes
No bacteria	1	Yes	Yes
No bacteria	2	Yes	Yes
No bacteria	3	Yes	Yes
No bacteria	4	Yes	Yes
No bacteria	5	Yes	Yes
SB642	1	Yes	Yes
SB642	2	Yes	Yes
SB642	3	Yes	Yes

Treatment groups		Sterility results	
SB642	4	Yes	Yes
SB642	5	Yes	Yes
Δ phage	1	Yes	Yes
Δ phage	2	Yes	Yes
Δ phage	3	Yes	Yes
Δ phage	4	Yes	Yes
Δ phage	5	Yes	Yes

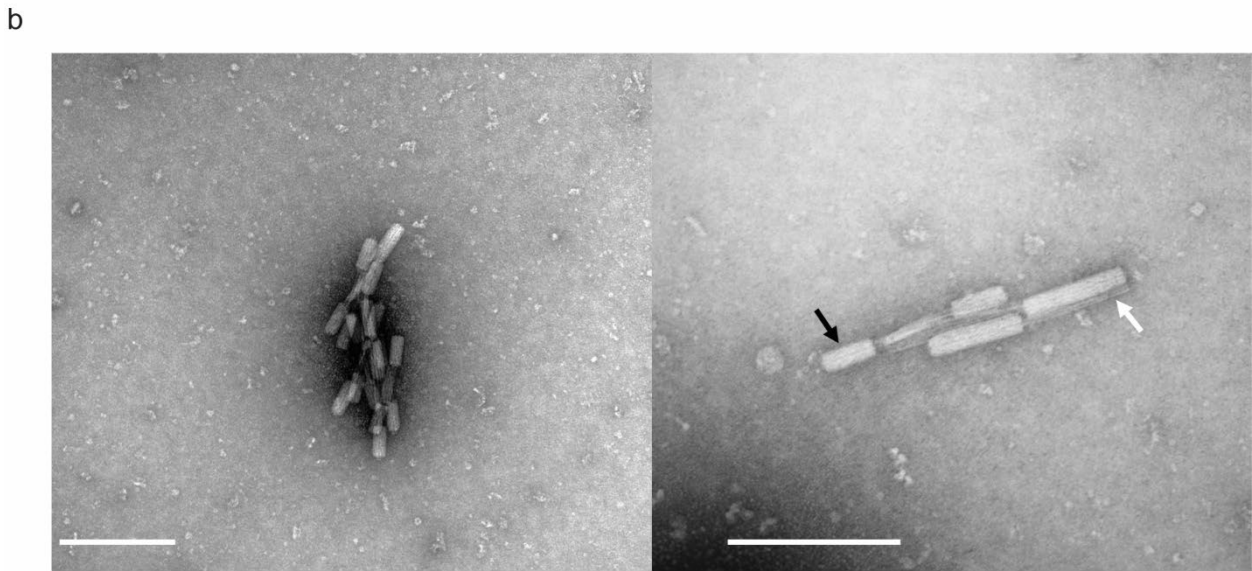
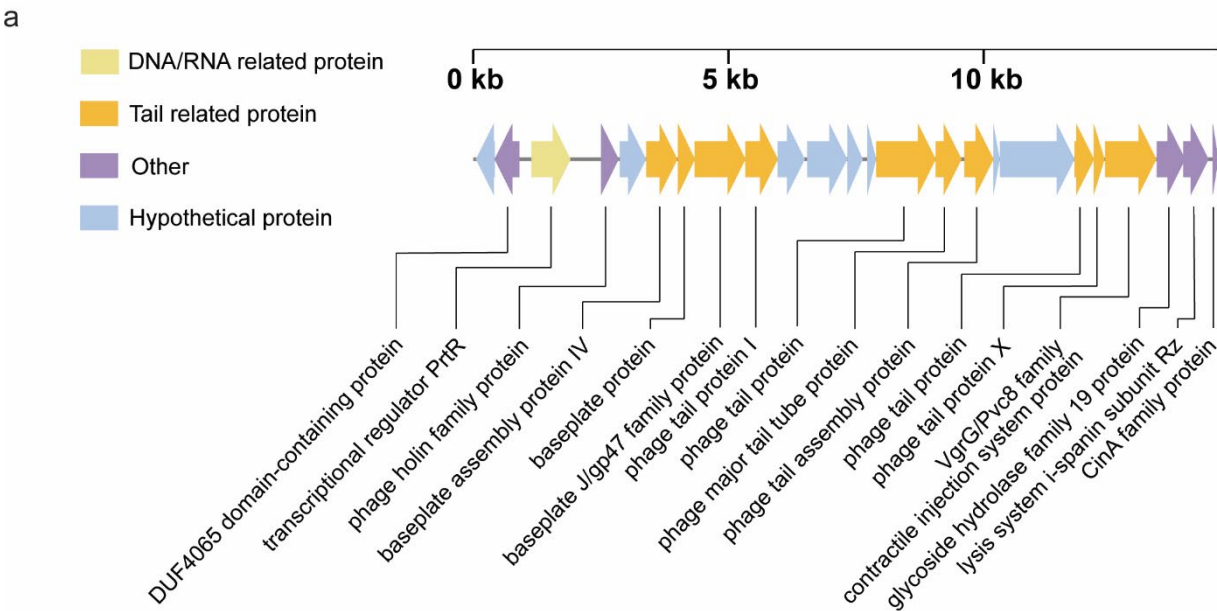
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Supplementary figures 1-7 in higher resolution

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Figure S1



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Figure S2

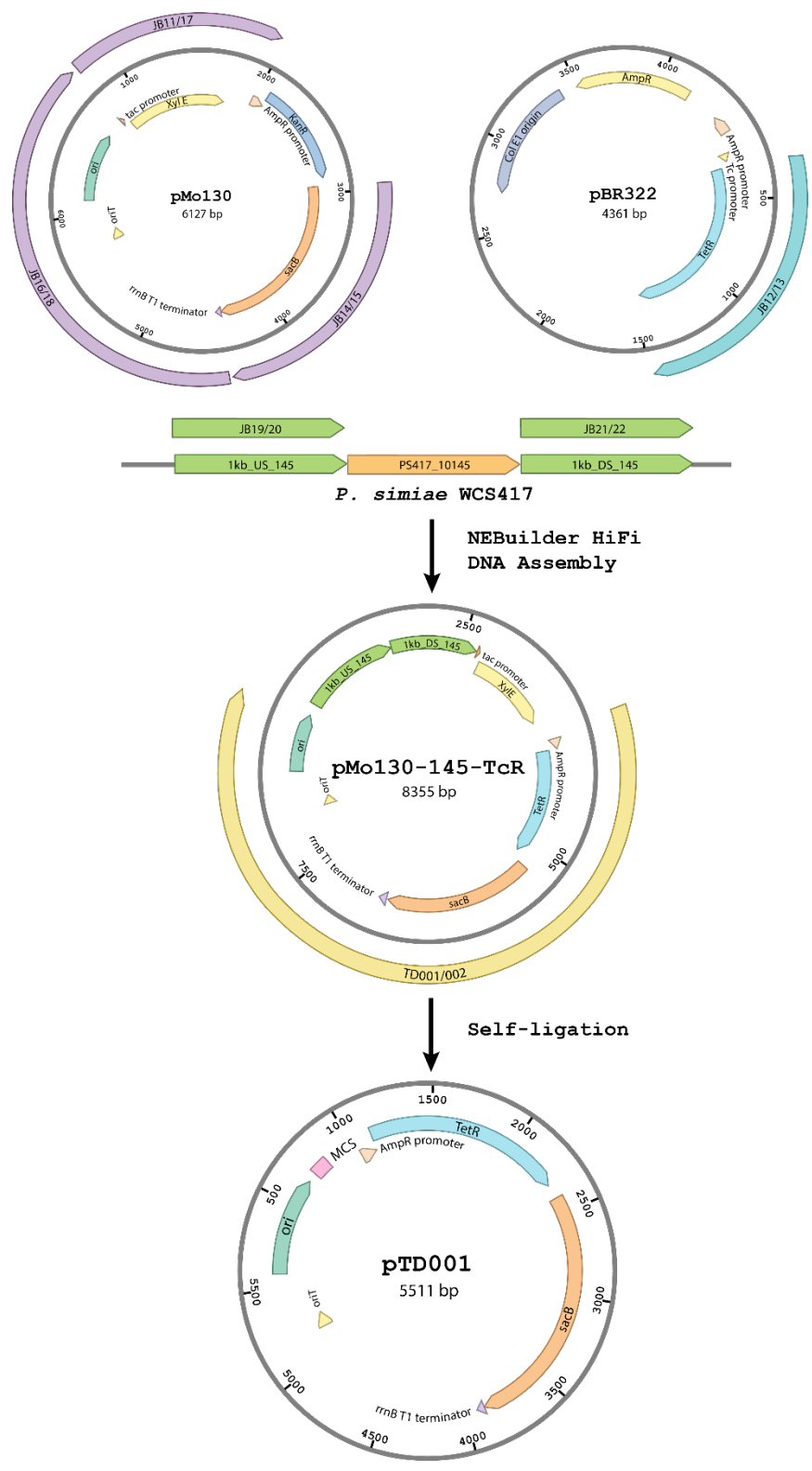
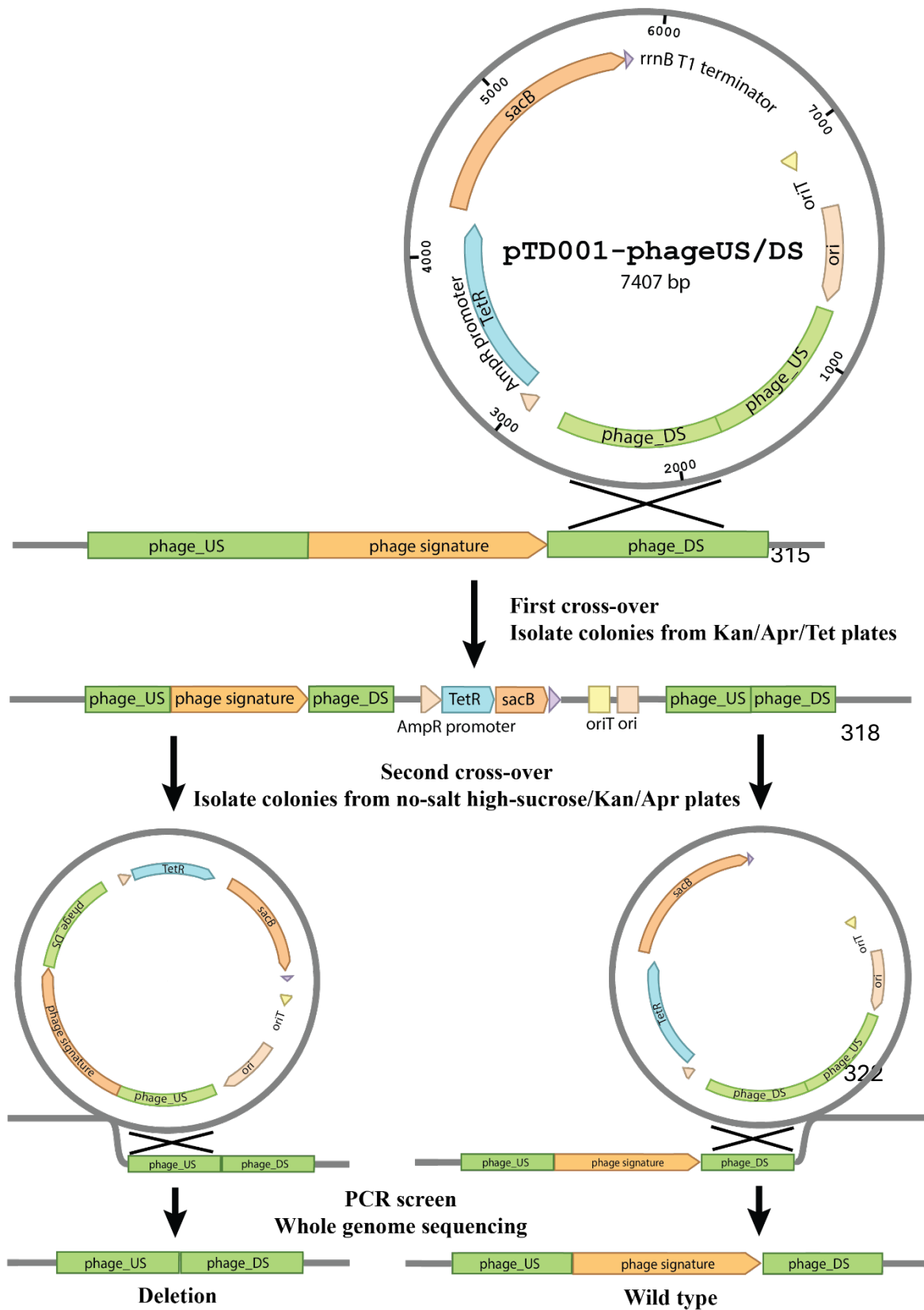


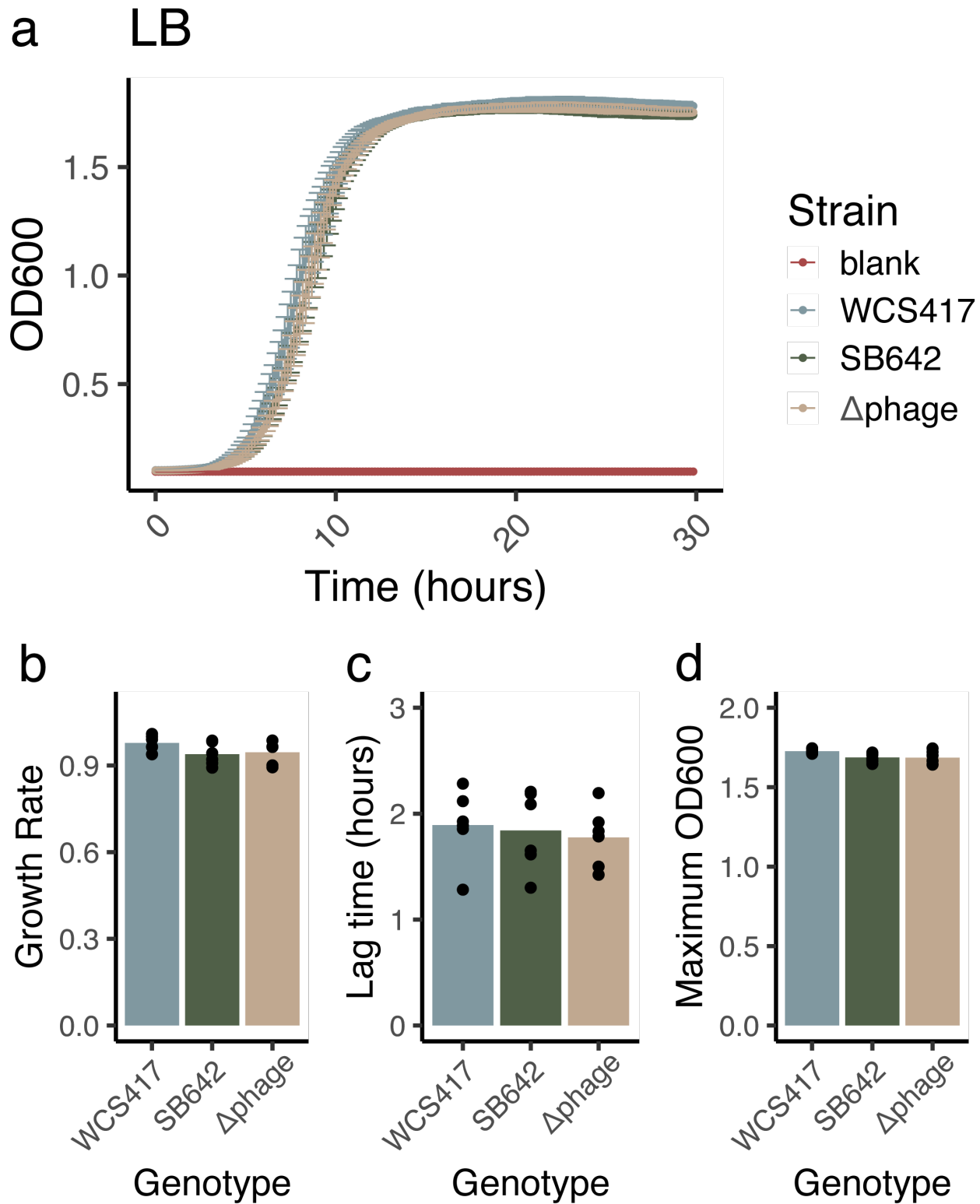
Figure S3



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Figure S4

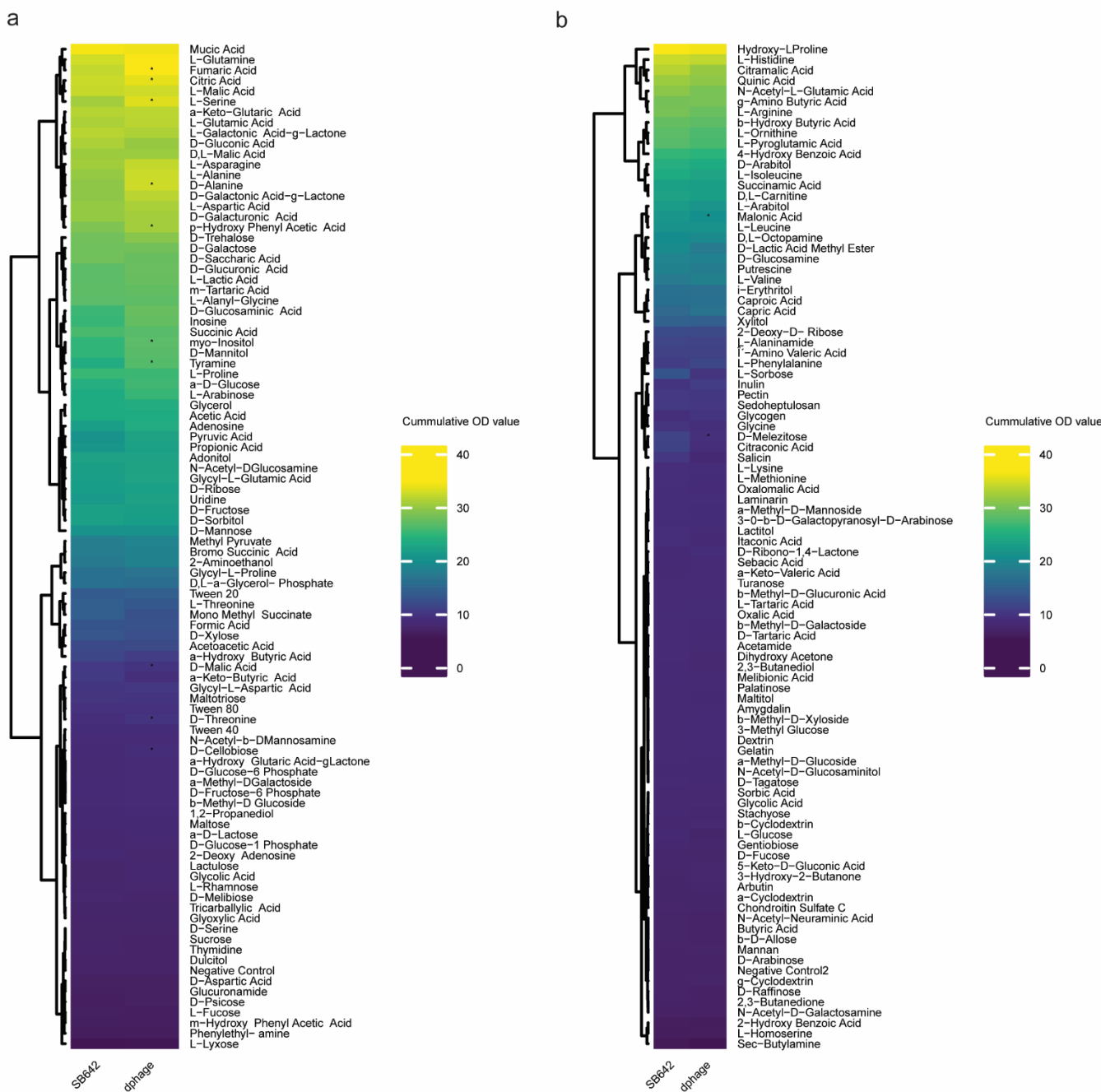
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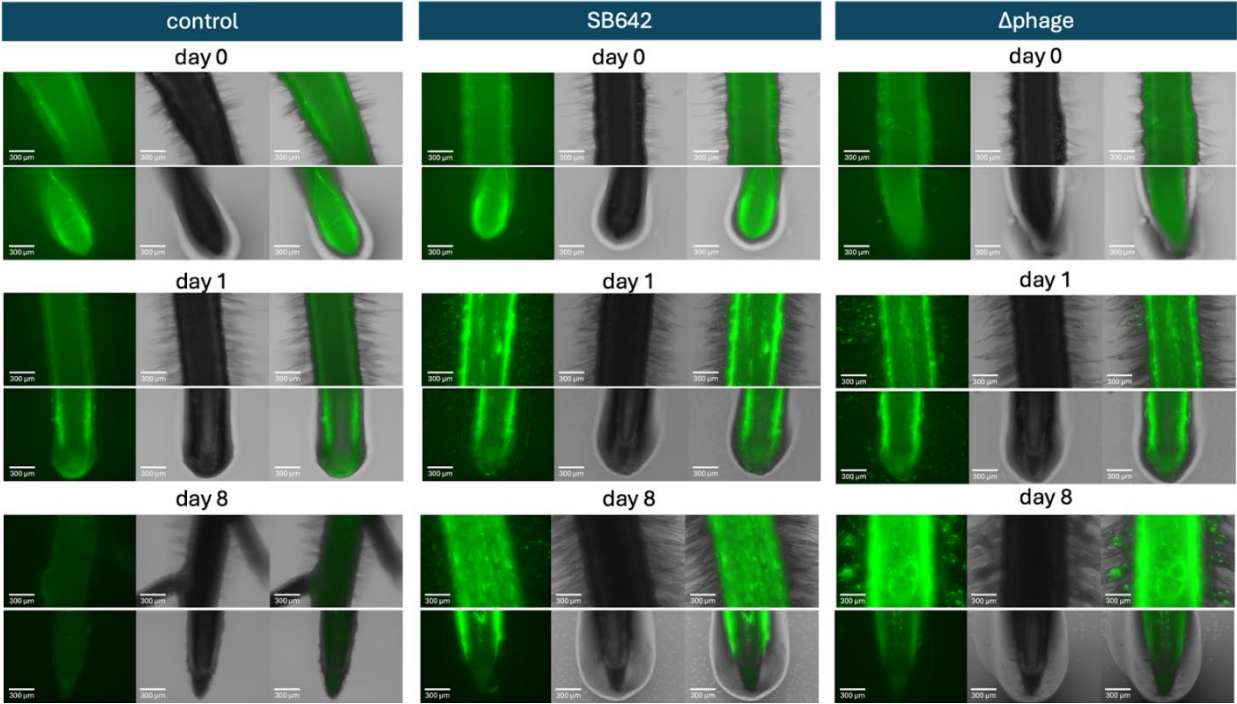
Figure S5

Cumulative NADH production
(740nm)



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Figure S6



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