Supplementary Information for

Genome-wide identification of *Pseudomonas syringae* genes required for fitness during colonization of the leaf surface and apoplast

Tyler C. Helmann, Adam M. Deutschbauer, and Steven E. Lindow

Steven E. Lindow
Email: icelab@berkeley.edu

This PDF file includes:

Other supplementary materials for this manuscript include the following:

Supplementary data and figures

Fig. 3 Expanded.

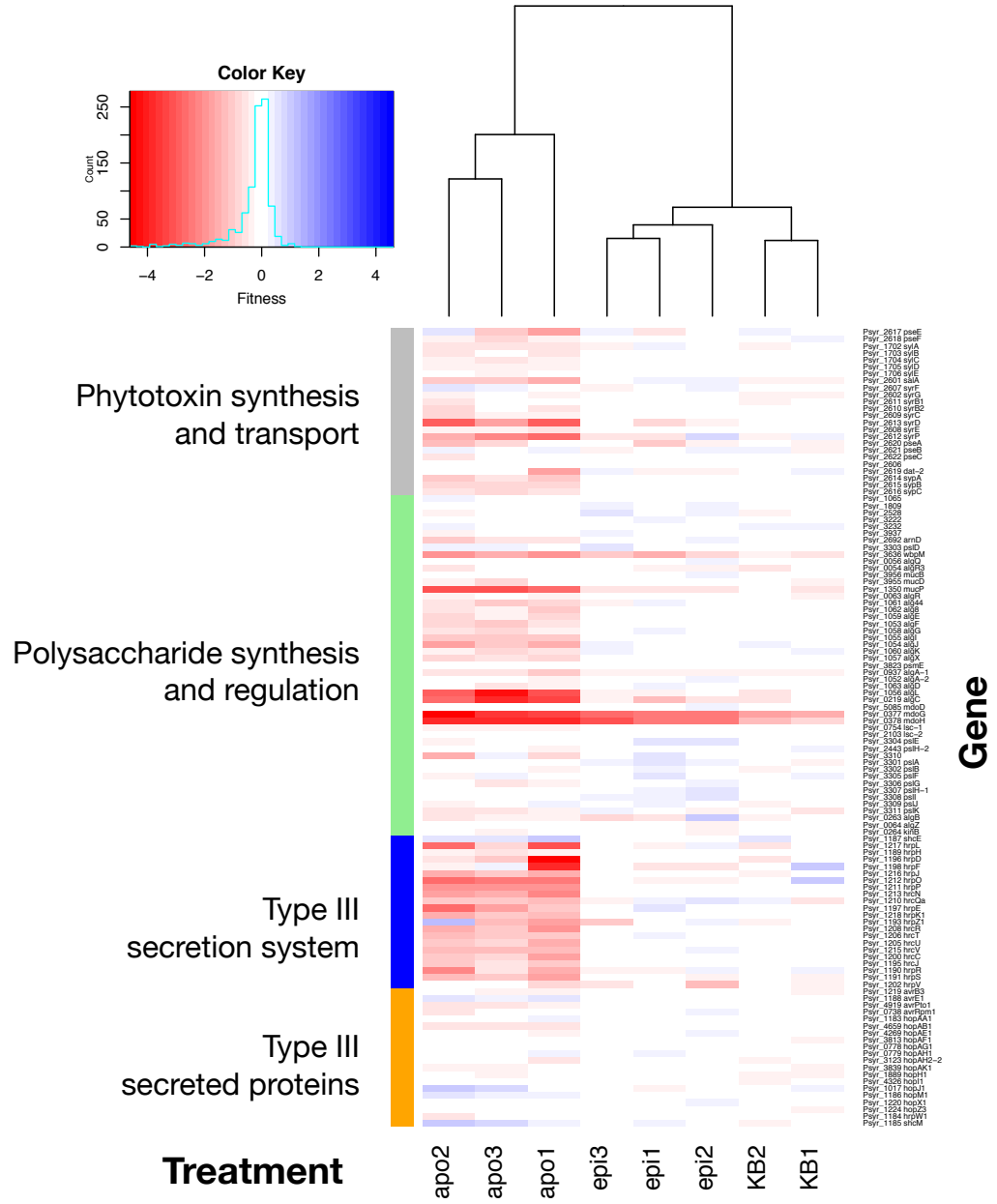
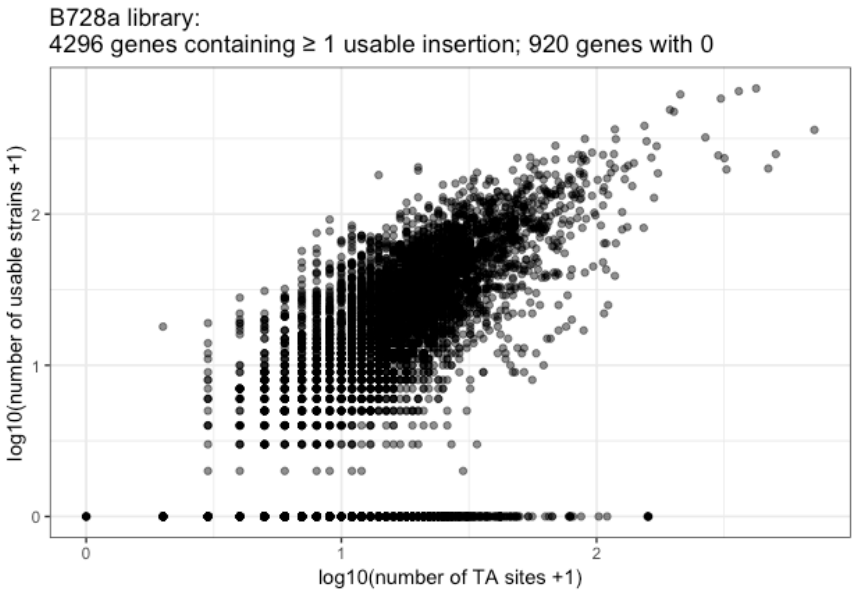


Fig. 3 Expanded. Fitness contributions of genes involved in phytotoxin synthesis and transport, the type III secretion system, and polysaccharide synthesis and regulation are required for apoplastic colonization. Gene fitness values are shown from 3 replicate epiphytic experiments (epi), 3 replicate apoplastic experiments (apo), and 2 replicate experiments in King's B medium (KB). The treatment ordering and corresponding dendrogram are calculated based on these genes only.

Fig. S1.

A.



B.

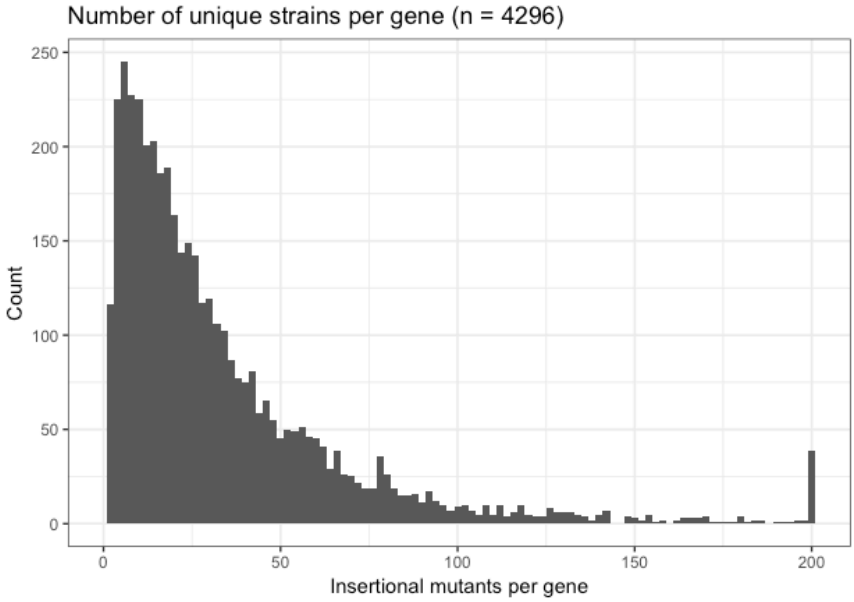


Fig. S1. The B728a *mariner* transposon library contains 281,417 mapped insertions, 169,826 of which lie within the central 10 – 90 % of a gene. Only these central insertions are used to calculate fitness. (A) The number of usable insertions for each gene is correlated with the number of TA dinucleotide sites within each coding region. The Pearson correlation coefficient calculated for all genes having at least 1 usable insertion strain: r = 0.71. (B) 4,296 genes contained at least one usable insertion. Genes for which their contribution to fitness could be calculated are represented by a median of 24 insertion strains per gene. 38 genes with > 200 insertions each (range = 203 to 676) are plotted at 200 for clarity.

Fig. S2.

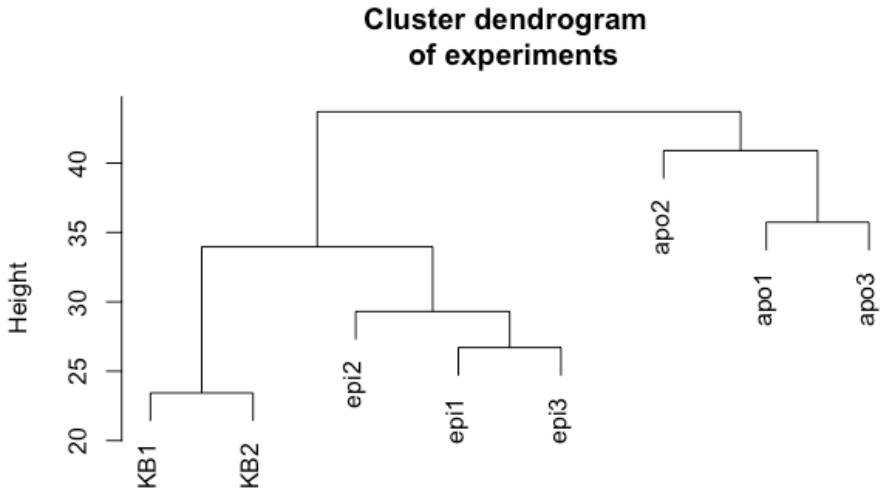


Fig. S2. Dendrogram of experiments, generated using *P. syringae* gene fitness values determined in experimental replicates of the three conditions tested. Rich medium King's B (KB) experiments cluster more closely with the epiphytic experiments (epi) than the apoplastic experiments (apo).

Fig. S3.

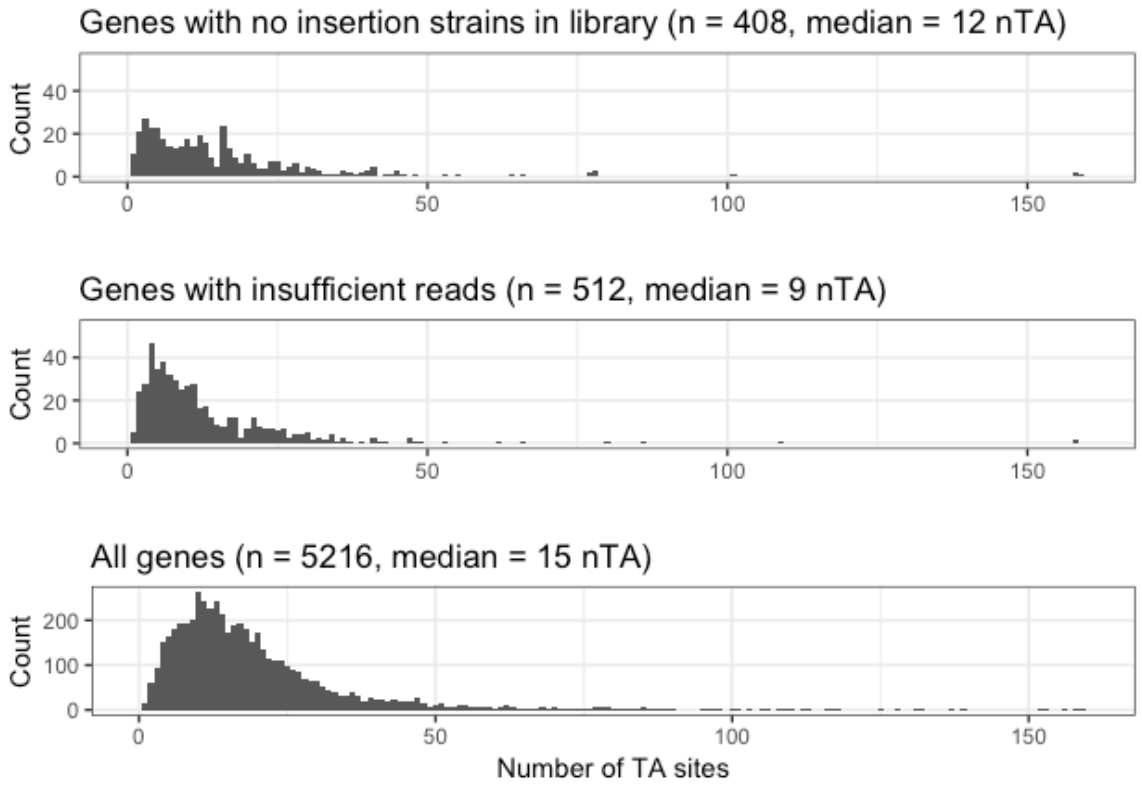


Fig. S3. Of the 920 genes for which their fitness contribution could not be calculated, 408 have no insertion strains in the library, while 512 have at least one insertion but an insufficient number of sequencing reads in time0 samples to calculate fitness. These 920 genes range in size from 73 bp to 4199 bp, with a median size of 575 bp. 5 genes have no TA sites. The histogram of all genes excludes 17 genes containing 163 to 713 TA sites.

Fig. S4.

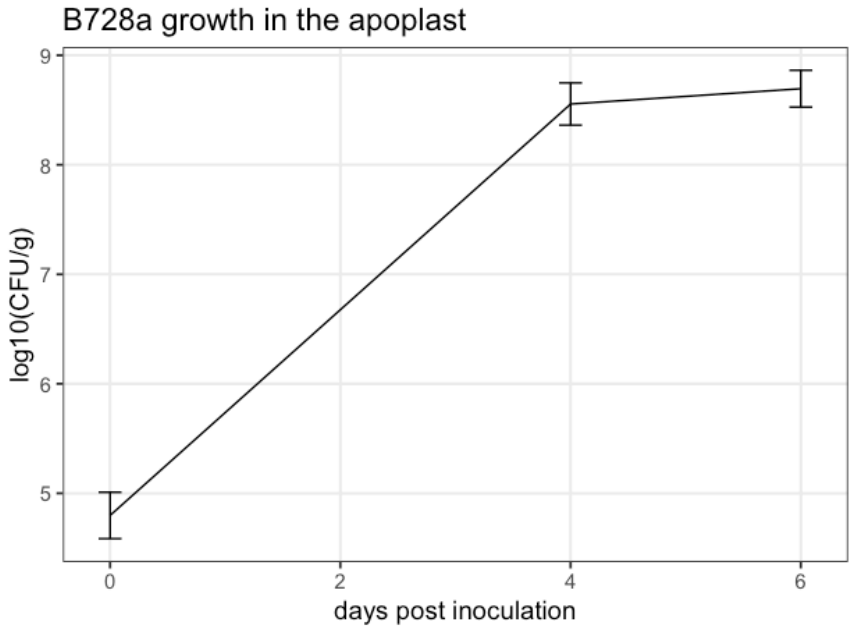
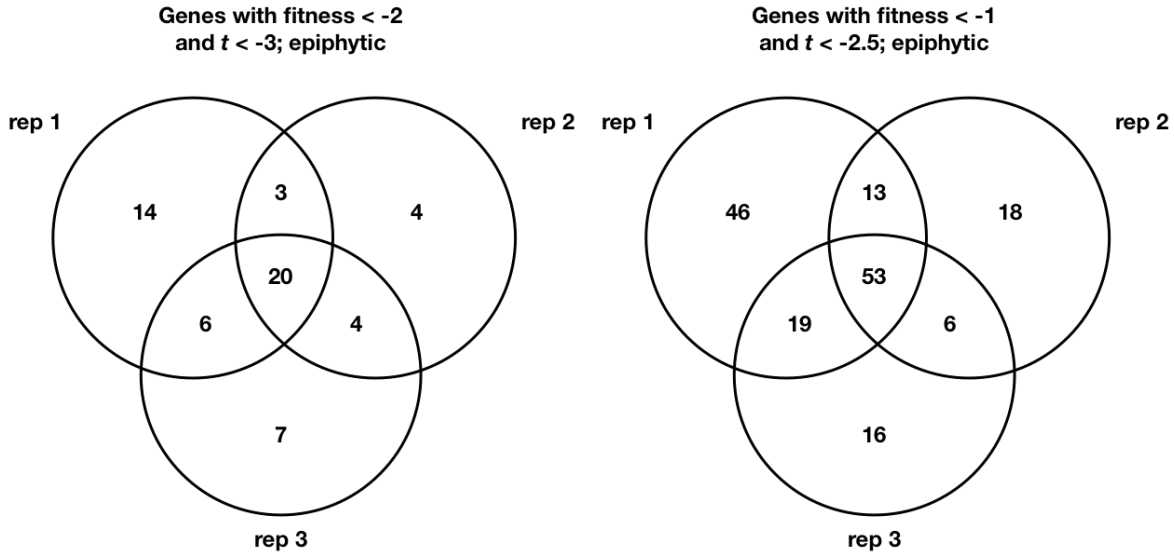


Fig. S4. Representative growth of B728a in the apoplast. B728a WT cells were inoculated into the apoplast by submerging the plants in 1.5 L of inoculum containing 10^5 CFU/ml in 1 mM KPO_4 buffer and subjecting the plants to a vacuum for 1.25 minutes. Rapidly restoring atmospheric pressure forced the bacterial suspension into the intercellular spaces of the leaves. The plants were allowed to dry on a laboratory bench for at least five hours, and then moved to the greenhouse. Cells were recovered from 8 to 12 replicate leaves at each sampling time by homogenization of the leaves, dilution plating of the homogenate on selective medium, followed by enumeration of colonies. Error bars represent the standard error of the mean of log-transformed population size.

Fig. S5.

A.



B.

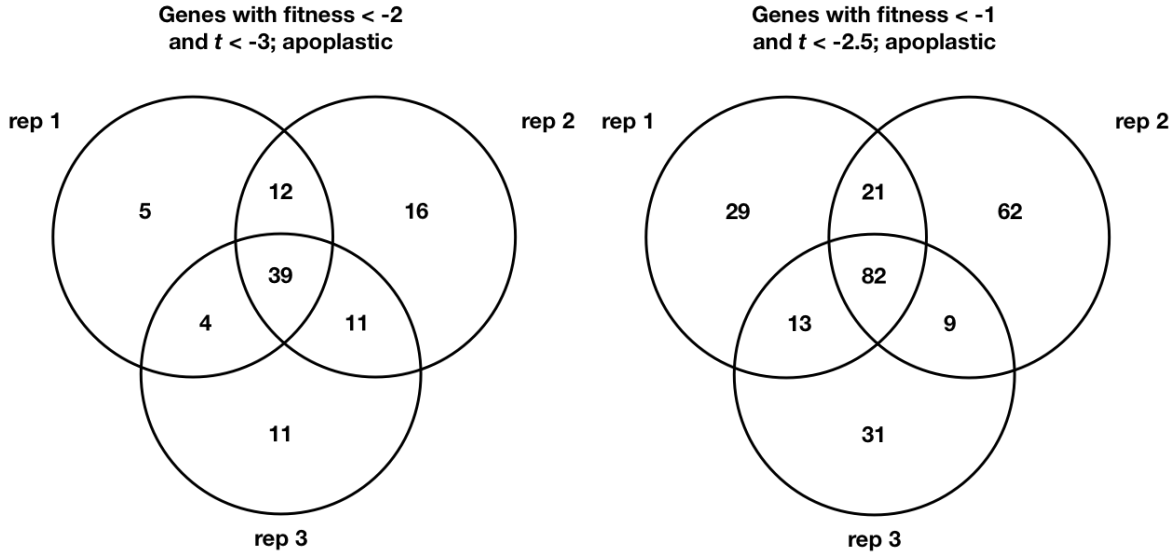


Fig. S5. Venn diagrams showing consistency in identifying genes contributing to epiphytic (A) and apoplastic growth (B) in different replicate experiments. Fitness contribution thresholds are fitness < -2, *t* < -3 (left) and fitness < -1, *t* < -2.5 (right).

Fig. S6.

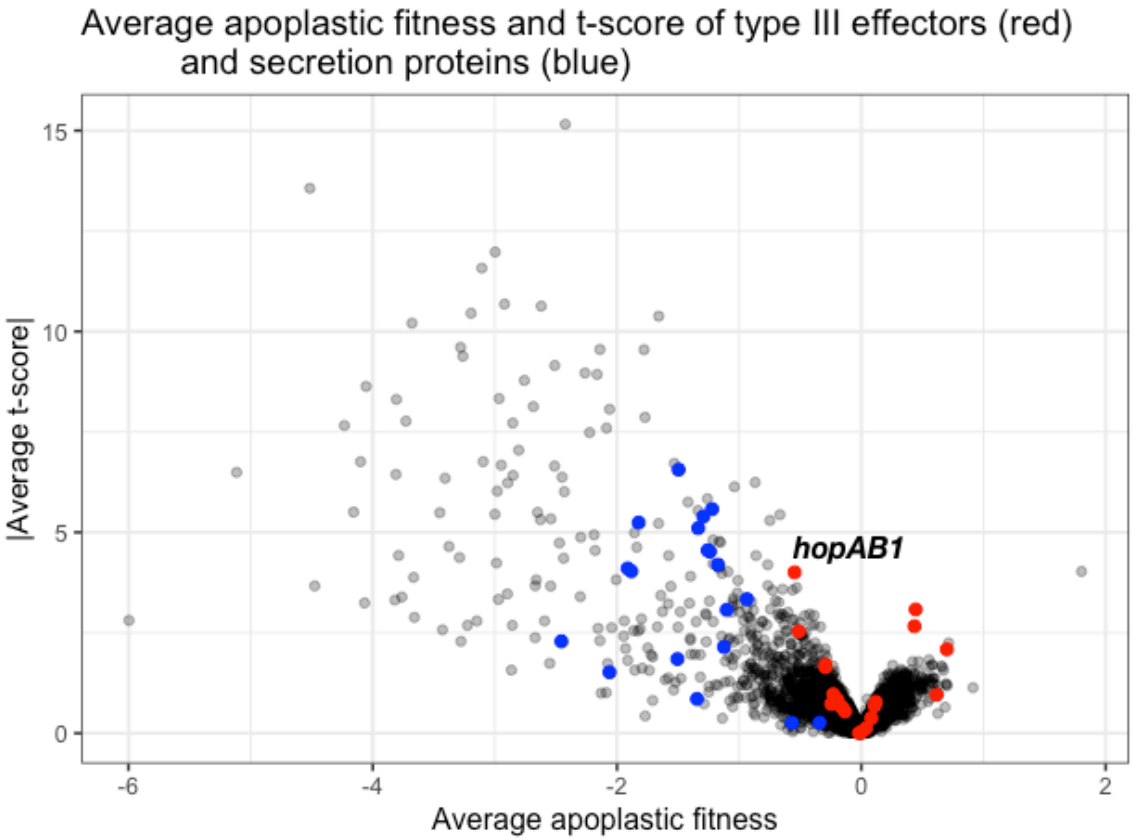
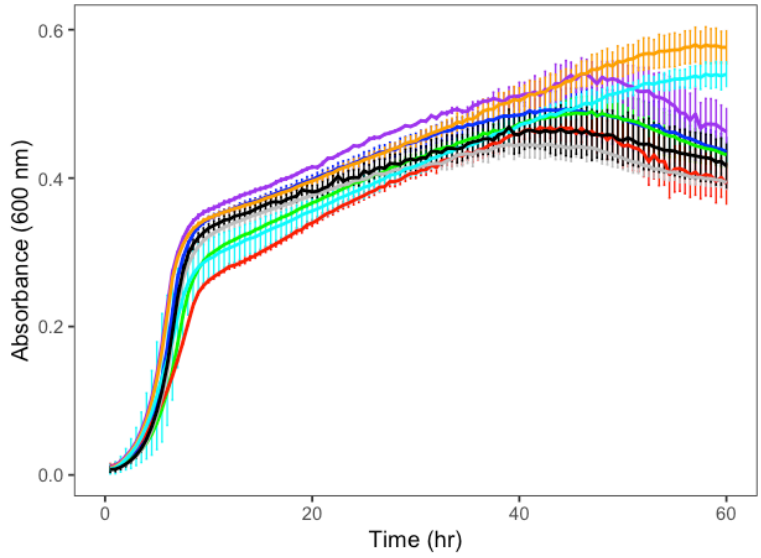


Fig. S6. Scatterplot of average apoplastic fitness and absolute average *t*-score for all genes, averaging values from three replicate experiments. Genes encoding type III secreted proteins are shown in red, with those encoding type III secretion system components and regulatory elements shown in blue. Of the secreted effector proteins, HopAB1 has a consistent but relatively small contribution to apoplastic fitness. Disruptions in individual type III secretion system pilus components have large effects. All other genes are shown in grey.

Fig. S7.

A.



B.

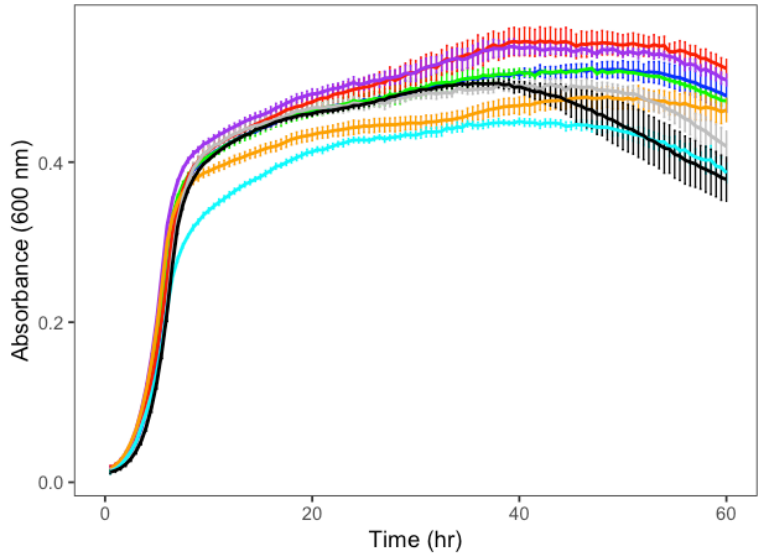
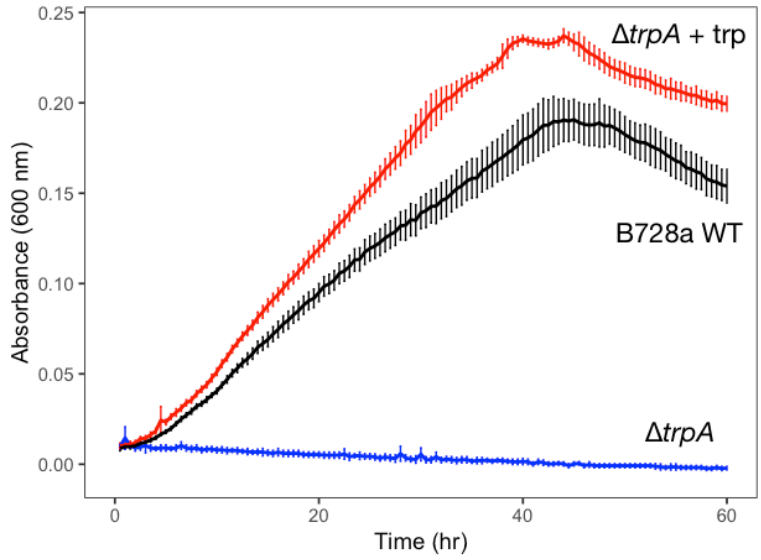


Fig. S7. Growth of deletion mutants in KB (A) and LB (B). Absorbance at 600 nm was measured in 4 to 5 replicate wells per strain. Strains shown are B728a WT (black), Δ*syrP* (blue), Δ*hisD* (red), Δ*trpA* (green), Δ*hrpL* (purple), Δ*eftA* (grey), Δ*Psyr_0532* (orange), and Δ*Psyr_0920* (cyan). Mean absorbance of 4 replicate wells per strain is shown. Vertical lines indicate the standard deviation.

Fig. S8.

A.



B.

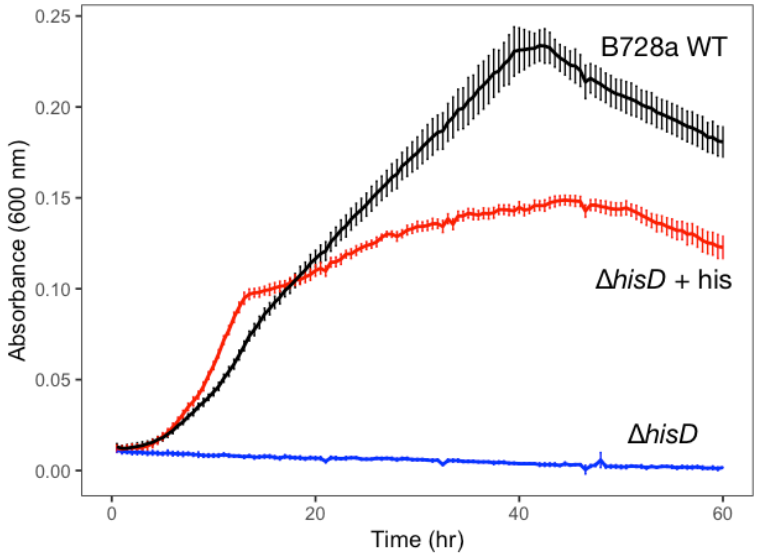


Fig. S8. Growth of B728a auxotrophic mutant strains Δ*trpA* (A) and Δ*hisD* (B) in M9 minimal medium supplemented by 0.2% glycerol as a carbon source. Auxotrophic deletion strains were complemented by the addition of 20.8 µg/ml tryptophan or 62 µg/ml histidine. Mean absorbance of 3 replicate wells per strain is shown. Vertical lines indicate the standard deviation.

Fig. S9.

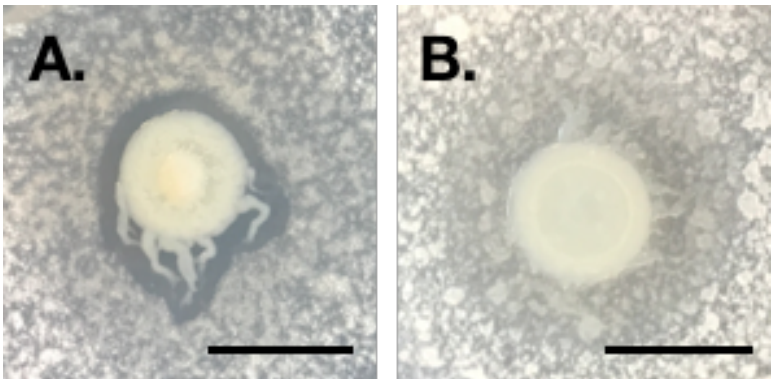


Fig. S9. Inhibition of *Geotrichum candidum* Fr260 growth by B728a WT (A) and Δ*syrP* (B). Scale bars are 10 mm in length.

Table S1. Number of genes within each functional category predicted to be essential or nearly essential in LB (N = 392), based on the TnSeq data. Protein coding genes were considered essential or nearly essential for growth in LB if normalized read density (reads/nucleotides across the entire gene) and normalized insertion density (sites/nucleotides in the central 10 – 90% of the gene) were below 0.2 (1). Genes shorter than 325 bp were excluded from this analysis. Functional category annotations for B728a genes are primarily based on COG (2) and KEGG (3) annotations, with manual additions and corrections originally published by Yu *et al.* (4).

Category	Predicted essential genes
Translation	66
None	38
Cofactor metabolism	36
Energy generation	30
Amino acid metabolism and transport	22
Nucleotide metabolism and transport	22
Replication and DNA repair	20
LPS synthesis and transport	19
Hypothetical	15
Secretion/Efflux/Export	15
Peptidoglycan/cell wall polymers	12
Fatty acid metabolism	10
Sulfur metabolism and transport	8
Terpenoid backbone synthesis	8
Carbohydrate metabolism and transport	7
Cell division	7
Siderophore synthesis and transport	7
Transcription	5
Chaperones/Heat shock proteins	4
Nitrogen metabolism	4
Phospholipid metabolism	4
Transcription - Sigma factor	4
Transport (organic compounds)	3
Iron metabolism and transport	2
Iron-sulfur proteins	2
Organic acid metabolism and transport	2
Outer membrane proteins	2
Oxidative stress tolerance	2
Post-translational modification	2
Stress resistance	2
Transcriptional regulation	2
Compatible solute synthesis	1
Osmosensing & regulation	1
Oxidative stress tolerance (Antioxidant enzyme)	1
Phage & IS elements	1
Phosphate metabolism and transport	1
Polysaccharide synthesis and regulation	1
Proteases	1

Signal transduction mechanisms	1
Transport	1
Transport (inorganic ions)	1

Table S2. B728a genes predicted to be essential compared to their predicted homologs in *P. aeruginosa* PAO1 (A), *P. simiae* WCS417 (B), and *P. stutzeri* RCH2 (C). Homologs to B728a genes were identified using the IMG database genome-gene best homologs function, at 70% identity (2). Essential vs. nonessential characterization of PAO1 genes was predicted in (5), and predicted for *P. simiae* and *P. stutzeri* in (1). For these studies, all transposon libraries of all strains (including B728a) were generated on LB (1, 6). These genes are listed in Table S3.

A.

	B728a essential	B728a nonessential
P. aeruginosa essential	259	48
P. aeruginosa nonessential	104	1938
No homolog in *P. aeruginosa*	29	2610
Total	392	4596

B.

	B728a essential	B728a nonessential
P. simiae essential	282	133
P. simiae nonessential	75	2070
No homolog in *P. simiae*	35	2393
Total	392	4596

C.

	B728a essential	B728a nonessential
P. stutzeri essential	254	76
P. stutzeri nonessential	63	1057
No homolog in *P. stutzeri*	75	3463
Total	392	4596

Table S3. Genes that when disrupted confer individually large decreases in fitness (average fitness < -2 from two replicate experiments) in King's B medium. All genes represent those in which *t* < -3 in both replicate experiments.

Locus	Name	Description	Fitness in King's B			Classification
			1	2	Average	
Psyr_4581	trpG	anthranilate synthase, component II	-2.47	-1.71	-2.09	Amino acid metabolism and transport
Psyr_0826	pgi	glucose-6-phosphate isomerase	-1.91	-2.21	-2.06	Carbohydrate metabolism and transport
Psyr_1613	minC	septum site-determining protein MinC	-3.40	-3.70	-3.55	Cell division
Psyr_0454	bioA	adenosylmethionine-8-amino-7-oxononanoate aminotransferase apoenzyme	-2.56	-2.62	-2.59	Cofactor metabolism
Psyr_4687	bioB	biotin synthase	-2.87	-2.96	-2.91	Cofactor metabolism
Psyr_4683	bioD	dethiobiotin synthase	-3.30	-2.53	-2.92	Cofactor metabolism
Psyr_4686	bioF	8-amino-7-oxononanoate synthase	-2.89	-3.18	-3.03	Cofactor metabolism
Psyr_0951	moeB	UBA/THIF-type NAD/FAD binding fold:MoeZ/MoeB	-4.81	-6.07	-5.44	Cofactor metabolism
Psyr_2080	pabB	aminodeoxychorismate synthase, subunit I	-1.38	-2.65	-2.01	Cofactor metabolism
Psyr_4341	thiE	thiamine-phosphate diphosphorylase	-2.80	-3.71	-3.26	Cofactor metabolism
Psyr_4740	thiG	thiazole-phosphate synthase	-3.69	-3.98	-3.84	Cofactor metabolism
Psyr_4340		phosphomethylpyrimidine kinase, putative	-3.51	-4.07	-3.79	Cofactor metabolism
Psyr_0565		Protein of unknown function UPF0126	-2.35	-3.33	-2.84	Hypothetical
Psyr_0917	rfbA-2	ABC-2	-1.90	-2.53	-2.21	LPS synthesis and transport
Psyr_0918	rfbB-2	ABC transporter	-2.05	-2.37	-2.21	LPS synthesis and transport
Psyr_0259	envZ	Osmolarity sensor protein envZ	-2.29	-1.91	-2.10	Osmosensing & regulation
Psyr_3008	uppP	Undecaprenyl-diphosphatase	-2.95	-4.69	-3.82	Peptidoglycan/cell wall polymers
Psyr_0849	pssA-1	CDP-diacylglycerol--serine O-phosphatidyltransferase	-2.04	-2.32	-2.18	Phospholipid metabolism
Psyr_4091		8-oxo-dGTPase	-2.54	-2.47	-2.51	Replication and DNA repair
Psyr_1544		SirA-like protein	-3.94	-4.44	-4.19	

Table S4. Numbers of unique barcodes and median reads per gene in each of three replicate experiments and samples obtained from mid-log phase cultures following library outgrowth and before inoculation (time0) and from leaves pooled from 100 pots after growth in a particular habitat (sample). Unique barcodes were calculated as the total that mapped to the genome and had 3 or more reads in a given experiment. The total number of mapped barcodes in the library = 281,417. Technical (sequencing) replicates are listed separately ("a" and "b"), and share the same time0 reference sample. For an experiment to pass quality control, the median reads per gene in the sample must be ≥ 50 (7).

Experiment	Unique barcodes at time0	Unique barcodes in sample	% Recovery	Median reads/gene at time0	Median reads/gene in sample
KB_1	187,482	192,078	>100*	278	150
KB_2	197,089	226,857	>100*	378.5	251
Epiphytic_1a	196,894	191,648	97.3	279	167
Epiphytic_1b	196,894	182,055	92.5	279	148
Epiphytic_2a	216,700	177,679	82.0	381	175.5
Epiphytic_2b	216,700	173,567	80.1	381	164.5
Epiphytic_3a	230,482	201,171	87.3	453.5	248.5
Epiphytic_3b	230,482	196,807	85.4	453.5	211
Apoplast_1a	218,149	149,311	68.4	409.5	163
Apoplast_1b	218,149	151,211	69.3	409.5	155
Apoplast_2	214,346	156,416	73.0	390	160
Apoplast_3a	222,473	185,183	83.2	397	203
Apoplast_3b	222,473	187,289	84.2	397	216

* More unique barcodes sequenced at the end of an experiment indicates that additional unique barcodes were present but were not sequenced at the start of the experiment (time0).

Table S5. Genes with average fitness < -2 in epiphytic experiments. All genes represent those in which *t* < -3 in at least two experiments.

Locus	Name	Description	Epiphytic Fitness				Classification
			1	2	3	Average	
Psyr_4270	glyA	serine hydroxymethyltransferase	-2.35	-2.94	-3.42	-2.90	Amino acid metabolism and transport
Psyr_4369	proA	glutamate-5-semialdehyde dehydrogenase	-3.02	-2.42	-2.84	-2.76	Amino acid metabolism and transport
Psyr_0704	proB	glutamate 5-kinase	-2.91	-1.48	-2.12	-2.17	Amino acid metabolism and transport
Psyr_0557	serB	phosphoserine phosphatase	-2.16	-4.21	-3.27	-3.21	Amino acid metabolism and transport
Psyr_0033	trpA	tryptophan synthase, alpha chain	-3.26	-3.21	-4.03	-3.50	Amino acid metabolism and transport
Psyr_0034	trpB	tryptophan synthase, beta chain	-2.95	-3.04	-4.01	-3.33	Amino acid metabolism and transport
Psyr_4609	trpE	anthranilate synthase, component I	-3.60	-3.31	-3.96	-3.62	Amino acid metabolism and transport
Psyr_1663	trpF	phosphoribosylanthranilate isomerase	-3.54	-3.50	-4.59	-3.88	Amino acid metabolism and transport
Psyr_4852		D-3-phosphoglycerate dehydrogenase	-2.97	-3.51	-3.31	-3.26	Amino acid metabolism and transport
Psyr_2980	galU	UDP-glucose pyrophosphorylase	-2.64	-1.34	-2.07	-2.02	Carbohydrate metabolism and transport
Psyr_1613*	minC	septum site-determining protein MinC	-1.46	-2.27	-2.53	-2.09	Cell division
Psyr_0454*	bioA	adenosylmethionine-8-amino-7-oxononanoate aminotransferase apoenzyme	-2.43	-2.01	-2.35	-2.26	Cofactor metabolism
Psyr_4687*	bioB	biotin synthase	-2.03	-2.76	-2.05	-2.28	Cofactor metabolism
Psyr_4686*	bioF	8-amino-7-oxononanoate synthase	-1.94	-2.28	-2.18	-2.13	Cofactor metabolism
Psyr_3174	cysG	uroporphyrinogen-III C-methyltransferase / precorrin-2 dehydrogenase	-1.33	-2.70	-3.07	-2.37	Cofactor metabolism
Psyr_0846	ilvI	acetolactate synthase, large subunit	-2.34	-2.45	-2.35	-2.38	Cofactor metabolism
Psyr_0827	panC	pantothenate synthetase	-2.60	-4.00	-2.69	-3.10	Cofactor metabolism
Psyr_0532		conserved hypothetical protein	-3.40	-2.35	-1.73	-2.50	Hypothetical
Psyr_2461		Uncharacterized conserved protein UCP030820	-2.13	-1.79	-2.44	-2.12	Hypothetical
Psyr_0917*	rfbA-2	ABC-2	-3.05	-2.13	-2.62	-2.60	LPS synthesis and transport
Psyr_0918*	rfbB-2	ABC transporter	-2.83	-2.00	-2.05	-2.29	LPS synthesis and transport
Psyr_0411	gltB	glutamate synthase (NADPH) large subunit	-2.73	-1.65	-1.64	-2.01	Nitrogen metabolism

Psyr_1668	purF	amidophosphoribosyltransferase	-3.20	-3.21	-3.15	-3.18	Nucleotide metabolism and transport
Psyr_1269	purL	phosphoribosylformylglycinamidine synthase	-3.08	-2.52	-2.54	-2.71	Nucleotide metabolism and transport
Psyr_3008*	uppP	Undecaprenyl-diphosphatase	-1.86	-2.29	-3.01	-2.38	Peptidoglycan/cell wall polymers
Psyr_4512		putative phage-related protein	-2.24	-1.70	-2.38	-2.11	Phage & IS elements
Psyr_0377	mdoG	Periplasmic glucan biosynthesis protein, MdoG	-2.47	-2.48	-2.73	-2.56	Polysaccharide synthesis and regulation
Psyr_0378	mdoH	Glycosyl transferase, family 2	-2.38	-2.37	-3.05	-2.60	Polysaccharide synthesis and regulation
Psyr_1408	ruvC	Holliday junction endonuclease RuvC	-3.43	-2.74	-1.71	-2.63	Replication and DNA repair
Psyr_2462		Nitrite/sulfite reductase, hemoprotein beta-component, ferrodoxin-like:Nitrite and sulphite reductase 4Fe-4S region	-2.60	-3.62	-3.36	-3.19	Sulfur metabolism and transport
Psyr_0529		Glycosyl transferase, group 1	-3.59	-3.11	-2.88	-3.19	

* These genes also have a fitness of < -2 and *t* < -3 for growth in KB relative to the input library.

Table S6. Genes with average fitness < -2 in apoplastic experiments. All genes represent those in which *t* < -3 in at least two experiments.

Locus	Name	Description	Apoplastic Fitness				Classification
			1	2	3	Average	
Psyr_4270	glyA	serine hydroxymethyltransferase	-5.00	-5.34	-5.01	-5.11	Amino acid metabolism and transport
Psyr_4897	hisB	imidazoleglycerol-phosphate dehydratase	-3.76	-3.42	-2.69	-3.29	Amino acid metabolism and transport
Psyr_4132	hisC	histidinol phosphate aminotransferase apoenzyme	-1.90	-3.58	-2.57	-2.69	Amino acid metabolism and transport
Psyr_4133	hisD	histidinol dehydrogenase	-2.55	-3.66	-2.79	-3.00	Amino acid metabolism and transport
Psyr_4134	hisG	ATP phosphoribosyltransferase (homohexameric)	-2.51	-3.37	-2.40	-2.76	Amino acid metabolism and transport
Psyr_4896	hisH	imidazole glycerol phosphate synthase subunit hisH	-2.02	-2.56	-1.96	-2.18	Amino acid metabolism and transport
Psyr_1257	leuA	2-isopropylmalate synthase	-2.16	-2.56	-1.95	-2.22	Amino acid metabolism and transport
Psyr_1985	leuB	3-isopropylmalate dehydrogenase	-4.35	-3.99	-3.82	-4.05	Amino acid metabolism and transport
Psyr_1983	leuC	3-isopropylmalate dehydratase, large subunit	-4.30	-4.82	-3.35	-4.16	Amino acid metabolism and transport
Psyr_0473	metW	Methionine biosynthesis MetW	-4.66	-4.25	-3.38	-4.10	Amino acid metabolism and transport
Psyr_0474	metX	homoserine O-acetyltransferase	-4.04	-3.02	-3.97	-3.68	Amino acid metabolism and transport
Psyr_1669	metZ	O-succinylhomoserine sulfhydrylase	-4.14	-4.63	-3.93	-4.23	Amino acid metabolism and transport
Psyr_4369	proA	glutamate-5-semialdehyde dehydrogenase	-3.72	-2.12	-3.15	-3.00	Amino acid metabolism and transport
Psyr_0704	proB	glutamate 5-kinase	-3.24	-3.19	-1.98	-2.80	Amino acid metabolism and transport
Psyr_0557	serB	phosphoserine phosphatase	-2.88	-2.37	-1.64	-2.30	Amino acid metabolism and transport
Psyr_0033	trpA	tryptophan synthase, alpha chain	-2.89	-3.23	-1.87	-2.66	Amino acid metabolism and transport
Psyr_0034	trpB	tryptophan synthase, beta chain	-4.03	-3.75	-0.24	-2.67	Amino acid metabolism and transport
Psyr_4580	trpD	anthranilate phosphoribosyltransferase	-4.47	-4.70	-4.26	-4.47	Amino acid metabolism and transport
Psyr_4609	trpE	anthranilate synthase,	-4.87	-4.58	-4.10	-4.51	Amino acid

		component I					metabolism and transport
Psyr_1663	trpF	phosphoribosylanthranilate isomerase	-3.43	-3.93	-3.64	-3.67	Amino acid metabolism and transport
Psyr_4852		D-3-phosphoglycerate dehydrogenase	-3.06	-3.30	-2.49	-2.95	Amino acid metabolism and transport
Psyr_4894		1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase	-1.87	-3.00	-2.76	-2.54	Amino acid metabolism and transport
Psyr_2980	galU	UDP-glucose pyrophosphorylase	-2.10	-2.53	-2.28	-2.30	Carbohydrate metabolism and transport
Psyr_0826*	pgi	glucose-6-phosphate isomerase	-2.87	-2.96	-3.46	-3.10	Carbohydrate metabolism and transport
Psyr_3179	ftsK	DNA translocase FtsK	-2.86	-2.53	-2.23	-2.54	Cell division
Psyr_1613*	minC	septum site-determining protein MinC	-3.94	-3.75	-3.68	-3.79	Cell division
Psyr_4194	dnaJ-1	DnaJ central region:Heat shock protein DnaJ, N-terminal:Chaperone DnaJ, C-terminal	-1.85	-2.30	-1.87	-2.01	Chaperones/Heat shock proteins
Psyr_0454*	bioA	adenosylmethionine-8-amino-7-oxononanoate aminotransferase apoenzyme	-2.32	-2.17	-2.07	-2.19	Cofactor metabolism
Psyr_4687*	bioB	biotin synthase	-2.83	-2.85	-2.88	-2.85	Cofactor metabolism
Psyr_4683*	bioD	dethiobiotin synthase	-2.99	-2.49	-3.21	-2.90	Cofactor metabolism
Psyr_4686*	bioF	8-amino-7-oxononanoate synthase	-2.54	-2.81	-2.53	-2.63	Cofactor metabolism
Psyr_0848	ilvC	ketol-acid reductoisomerase	-2.91	-3.88	-2.11	-2.97	Cofactor metabolism
Psyr_0469	ilvD	dihydroxyacid dehydratase	-3.70	-4.02	-2.11	-3.28	Cofactor metabolism
Psyr_0847	ilvH	acetolactate synthase, small subunit	-2.85	-2.72	-1.72	-2.43	Cofactor metabolism
Psyr_0846	ilvI	acetolactate synthase, large subunit	-3.29	-3.70	-2.33	-3.11	Cofactor metabolism
Psyr_4341*	thiE	thiamine-phosphate diphosphorylase	-1.94	-2.69	-2.91	-2.51	Cofactor metabolism
Psyr_4740*	thiG	thiazole-phosphate synthase	-1.93	-2.18	-3.84	-2.65	Cofactor metabolism
Psyr_4340*		phosphomethylpyrimidine kinase, putative	-2.01	-2.79	-3.76	-2.85	Cofactor metabolism
Psyr_0167		hypothetical protein	-2.86	-3.64	-2.41	-2.97	Hypothetical
Psyr_1614	htrB	lipid A biosynthesis acyltransferase	-4.30	-4.58	-2.57	-3.82	LPS synthesis and transport
Psyr_0917*	rfbA-2	ABC-2	-2.43	-3.24	-3.27	-2.98	LPS synthesis and transport
Psyr_0918*	rfbB-2	ABC transporter	-2.08	-2.40	-3.38	-2.62	LPS synthesis and transport
Psyr_0014		lipid A biosynthesis acyltransferase	-3.14	-2.06	-2.81	-2.67	LPS synthesis and transport
Psyr_1668	purF	amidophosphoribosyltransferase	-3.64	-3.27	-4.53	-3.81	Nucleotide metabolism and transport
Psyr_1269	purL	phosphoribosylformylglycinamidi	-3.19	-4.03	-4.07	-3.76	Nucleotide

		ne synthase					metabolism and transport
Psyr_3008*	uppP	Undecaprenyl-diphosphatase	-3.42	-3.44	-3.36	-3.41	Peptidoglycan/cell wall polymers
Psyr_2613	syrD	Cyclic peptide transporter	-2.77	-2.88	-1.70	-2.45	Phytotoxin synthesis and transport
Psyr_0219	algC	phosphomannomutase	-3.35	-2.67	-3.77	-3.26	Polysaccharide synthesis and regulation
Psyr_1056	algL	Poly(beta-D-mannuronate) lyase	-3.02	-2.82	-4.29	-3.38	Polysaccharide synthesis and regulation
Psyr_0377	mdoG	Periplasmic glucan biosynthesis protein, MdoG	-3.27	-4.42	-3.49	-3.73	Polysaccharide synthesis and regulation
Psyr_0378	mdoH	Glycosyl transferase, family 2	-3.83	-3.70	-3.89	-3.81	Polysaccharide synthesis and regulation
Psyr_1350	mucP	site-2 protease, Metallo peptidase, MEROPS family M50B	-2.71	-3.14	-3.11	-2.99	Polysaccharide synthesis and regulation
Psyr_1748	clpX	ATP-dependent Clp protease ATP-binding subunit ClpX	-2.97	-2.42	-3.29	-2.90	Proteases
Psyr_1410	ruvB	Holliday junction DNA helicase RuvB	-1.88	-2.79	-2.74	-2.47	Replication and DNA repair
Psyr_0919		Chromosome segregation ATPase-like protein	-2.02	-1.91	-2.49	-2.14	Replication and DNA repair
Psyr_3958	algU	RNA polymerase, sigma-24 subunit, RpoE	-2.78	-2.67	-3.31	-2.92	Transcription - Sigma factor
Psyr_4408	retS	Response regulator receiver:ATP-binding region, ATPase-like:Histidine kinase A, N-terminal:Histidine kinase:Histidine kinase	-2.50	-2.32	-2.71	-2.51	Transcriptional regulation
Psyr_3637	wbpL	Glycosyl transferase, family 4	-2.50	-2.04	-1.95	-2.16	
Psyr_0936	wbpY	Glycosyl transferase, group 1	-1.90	-1.68	-2.61	-2.06	
Psyr_0914	wbpZ	Glycosyl transferase, group 1	-2.22	-1.78	-2.26	-2.09	
Psyr_0529		Glycosyl transferase, group 1	-3.78	-2.89	-3.69	-3.45	
Psyr_0915		NAD-dependent epimerase/dehydratase	-3.09	-3.05	-3.44	-3.20	
Psyr_1544*		SirA-like protein	-1.67	-2.64	-3.01	-2.44	
Psyr_0920		Glycosyl transferase, group 1	-2.69	-2.34	-2.24	-2.42	
Psyr_4130		Peptidase S1, chymotrypsin:PDZ/DHR/GLGF	-2.51	-2.20	-2.08	-2.26	

* These genes also fulfill average fitness < -2 and *t* < -3 for growth in KB relative to the input library.

Regarding *wbpYZ*: in the original gene metadata these genes are annotated as "*wpbY*" and "*wpbZ*". This is likely a typo, as *wbp* genes are involved in O-antigen biosynthesis in *P. aeruginosa*, and "*wpb*" genes could not be identified in *P. syringae* or other Pseudomonads.

Table S7. Functional categories that are enriched among genes important for fitness *in planta*, based on a hypergeometric test. For this analysis, we only included genes with average fitness < -2 in the *in planta* habitats. The KB data are shown for comparison. Functional categories were assigned based on the previously annotated genomic metadata (4).

Category	**Category size (total)**	**King's B**	**Epiphytic**	**Apoplastic**
		p-value		
Amino acid metabolism and transport	210	0.329	3.294E-08	5.127E-16
Polysaccharide synthesis and regulation	49	0.241	0.011	3.332E-04
Nucleotide metabolism and transport	48	0.029	7.801E-05	2.244E-03
Type III secretion system	42	0.211	0.333	8.653E-03
Phytotoxin synthesis and transport	24	0.126	0.206	0.01085

Table S8. Genes with average fitness < -1 but > -2 in King's B (A), epiphytic (B) and apoplastic experiments (C). All genes represent those in which *t* < -2.5 in at least two experiments.

A.

Locus	Name	Description	Fitness in King's B			Classification
			1	2	Average	
Psyr_2855	metE	methionine synthase (B12-independent)	-1.70	-1.34	-1.52	Amino acid metabolism and transport
Psyr_2980	galU	UDP-glucose pyrophosphorylase	-1.20	-1.68	-1.44	Carbohydrate metabolism and transport
Psyr_1751	ppiD	PpiC-type peptidyl-prolyl cis-trans isomerase	-1.44	-1.17	-1.31	Chaperones/Heat shock proteins
Psyr_1650	pabC	aminodeoxychorismate lyase apoprotein	-1.66	-2.18	-1.92	Cofactor metabolism
Psyr_0487	gshB	glutathione synthase	-1.81	-1.27	-1.54	Glutathione metabolism
Psyr_0475		Protein of unknown function YGGT	-2.19	-1.37	-1.78	Hypothetical
Psyr_3581		ribosomal large subunit pseudouridine synthase B	-1.31	-1.08	-1.19	Nucleotide metabolism and transport
Psyr_2854		conserved hypothetical protein	-1.80	-1.76	-1.78	Phage & IS elements
Psyr_0377	mdoG	Periplasmic glucan biosynthesis protein, MdoG	-1.39	-1.62	-1.50	Polysaccharide synthesis and regulation
Psyr_0378	mdoH	Glycosyl transferase, family 2	-0.81	-1.27	-1.04	Polysaccharide synthesis and regulation
Psyr_1748	clpX	ATP-dependent Clp protease ATP-binding subunit ClpX	-1.09	-1.55	-1.32	Proteases
Psyr_0574	hflK	protease FtsH subunit HflK	-1.51	-0.81	-1.16	Proteases
Psyr_1749	lon-1	ATP-dependent proteinase, Serine peptidase, MEROPS family S16	-0.97	-1.09	-1.03	Proteases
Psyr_1410	ruvB	Holliday junction DNA helicase RuvB	-1.35	-1.33	-1.34	Replication and DNA repair
Psyr_1408	ruvC	Holliday junction endonuclease RuvC	-1.57	-1.28	-1.42	Replication and DNA repair
Psyr_4408	retS	Response regulator receiver:ATP-binding region, ATPase-like:Histidine kinase A, N-terminal:Histidine kinase:Histidine kinase	-1.28	-0.92	-1.10	Transcriptional regulation
Psyr_5133	trmE	tRNA modification GTPase trmE	-0.92	-1.66	-1.29	Translation
Psyr_1935	hcp-3	Protein of unknown function DUF796	-1.47	-0.60	-1.04	Type VI secretion system
Psyr_3954	lepA	GTP-binding protein LepA	-1.17	-1.56	-1.37	
Psyr_0529		Glycosyl transferase,	-1.57	-1.48	-1.52	

		group 1				
Psyr_4622		Nucleotidyl transferase	-0.98	-1.85	-1.42	
Psyr_4424		Propeptide, PepSY amd peptidase M4:PepSY-associated TM helix	-0.91	-1.20	-1.05	

B.

Locus	Name	Description	Epiphytic Fitness				Classification
			1	2	3	Average	
Psyr_0025	aroE	shikimate dehydrogenase	-1.32	-1.36	-1.06	-1.25	Amino acid metabolism and transport
Psyr_4132	hisC	histidinol phosphate aminotransferase apoenzyme	-1.76	-1.67	-1.17	-1.54	Amino acid metabolism and transport
Psyr_4133	hisD	histidinol dehydrogenase	-1.87	-0.89	-1.03	-1.27	Amino acid metabolism and transport
Psyr_4134	hisG	ATP phosphoribosyltransferase (homohexameric)	-1.63	-1.11	-0.84	-1.19	Amino acid metabolism and transport
Psyr_4896	hisH	imidazole glycerol phosphate synthase subunit hisH	-2.68	-1.93	-1.20	-1.94	Amino acid metabolism and transport
Psyr_1985	leuB	3-isopropylmalate dehydrogenase	-1.58	-1.19	-0.90	-1.23	Amino acid metabolism and transport
Psyr_1983	leuC	3-isopropylmalate dehydratase, large subunit	-1.53	-2.03	-0.41	-1.33	Amino acid metabolism and transport
Psyr_4894		1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase	-2.42	-1.35	-1.22	-1.66	Amino acid metabolism and transport
Psyr_0916	gmd	GDP-mannose 4,6-dehydratase	-1.64	-0.61	-0.77	-1.01	Carbohydrate metabolism and transport
Psyr_0826	pgi	glucose-6-phosphate isomerase	-2.08	-1.05	-2.55	-1.89	Carbohydrate metabolism and transport
Psyr_4842		Phosphoenolpyruvate-protein phosphotransf	-1.67	-1.49	-1.13	-1.43	Carbohydrate metabolism and transport

		erase					
Psyr_3179	ftsK	DNA translocase FtsK	-1.98	-1.15	-1.90	-1.68	Cell division
Psyr_0848	ilvC	ketol-acid reductoisomerase	-1.11	-1.40	-1.62	-1.38	Cofactor metabolism
Psyr_0469	ilvD	dihydroxyacid dehydratase	-1.62	-2.48	-1.64	-1.91	Cofactor metabolism
Psyr_0847	ilvH	acetolactate synthase, small subunit	-2.09	-2.01	-1.90	-2.00	Cofactor metabolism
Psyr_1120	zwf-1	glucose-6-phosphate 1-dehydrogenase	-1.09	-1.37	-0.60	-1.02	Energy generation
Psyr_0983		Protein of unknown function DUF159	-1.93	-1.43	-1.64	-1.67	Hypothetical
Psyr_0923		hypothetical protein	-1.64	-1.28	-1.43	-1.45	Hypothetical
Psyr_0534		membrane protein, putative	-1.70	-0.76	-1.11	-1.19	Hypothetical
Psyr_3805		hypothetical protein	-2.26	0.57	-2.54	-1.41	Hypothetical
Psyr_3691		conserved hypothetical protein	-1.53	-0.22	-1.32	-1.03	Hypothetical
Psyr_0412	gltD	glutamate synthase (NADPH) small subunit	-1.85	-0.65	-0.78	-1.09	Nitrogen metabolism
Psyr_4822	ntrC	Helix-turn-helix, Fis-type:Nitrogen regulation protein NR(I)	-1.80	-1.74	-0.61	-1.38	Nitrogen metabolism
Psyr_0294	ppx	Exopolyphosphatase	-0.87	-0.78	-1.58	-1.08	Nucleotide metabolism and transport
Psyr_3581		ribosomal large subunit pseudouridine synthase B	-0.61	-1.44	-1.57	-1.21	Nucleotide metabolism and transport
Psyr_0976	mqo	Malate:quinone-oxidoreductase	-1.89	-1.55	-1.62	-1.69	Organic acid metabolism and transport
Psyr_0630	mpl	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase	-1.62	-0.82	-1.23	-1.22	Peptidoglycan/ cell wall polymers
Psyr_3281	nagZ	Glycoside hydrolase, family 3, N-	-2.33	-0.80	-2.37	-1.83	Peptidoglycan/ cell wall polymers

		terminal					
Psyr_0402	ponA	Peptidoglycan glycosyltransferase	-1.20	-0.78	-1.17	-1.05	Peptidoglycan/ cell wall polymers
Psyr_4158	eftA	conserved hypothetical protein	-1.85	-0.52	-1.32	-1.23	Plant-associated proteins
Psyr_3636	wbpM	Polysaccharide biosynthesis protein CapD	-1.56	-0.76	-1.07	-1.13	Polysaccharide synthesis and regulation
Psyr_4887	ctpA	Peptidase S41A, C-terminal protease	-2.42	-1.64	-0.98	-1.68	Post-translational modification
Psyr_1747	clpP	ATP-dependent Clp protease proteolytic subunit ClpP	-0.95	-1.53	-1.16	-1.21	Proteases
Psyr_1748	clpX	ATP-dependent Clp protease ATP-binding subunit ClpX	-1.47	-1.27	-1.06	-1.27	Proteases
Psyr_1378	recA	RecA protein	-2.01	-0.97	-2.41	-1.80	Replication and DNA repair
Psyr_0201	recG	ATP-dependent DNA helicase RecG	-1.50	-0.58	-0.99	-1.02	Replication and DNA repair
Psyr_1410	ruvB	Holliday junction DNA helicase RuvB	-1.65	-1.38	-2.38	-1.80	Replication and DNA repair
Psyr_5065	uvrD	ATP-dependent DNA helicase UvrD	-1.84	-1.41	-2.30	-1.85	Replication and DNA repair
Psyr_0919		Chromosome segregation ATPase-like protein	-1.66	-1.05	-1.16	-1.29	Replication and DNA repair
Psyr_0832	cbrA-1	Two-component sensor kinase CbrA	-1.28	-1.65	-1.35	-1.43	Signal transduction mechanisms
Psyr_0811	terC	Integral membrane protein TerC	-1.11	-0.74	-1.46	-1.10	Stress resistance
Psyr_4128	cysD	sulfate adenylyltransferase subunit 2	-1.28	-1.07	-0.83	-1.06	Sulfur metabolism and transport
Psyr_4126	cysNC	adenylylsulfate kinase / sulfate adenylyltransferase subunit 1	-1.08	-1.15	-1.40	-1.21	Sulfur metabolism and transport
Psyr_4408	retS	Response regulator	-2.05	-1.39	-1.10	-1.51	Transcriptional regulation

		receiver:ATP-binding region, ATPase-like:Histidine kinase A, N-terminal:Histidine kinase:Histidine kinase					
Psyr_5133	trmE	tRNA modification GTPase trmE	-0.86	-1.29	-1.16	-1.10	Translation
Psyr_4567	anmK	Protein of unknown function UPF0075	-1.22	-0.90	-1.17	-1.10	
Psyr_4362	rlpA-2	Rare lipoprotein A	-2.16	-1.93	-1.67	-1.92	
Psyr_3637	wbpL	Glycosyl transferase, family 4	-1.68	-1.09	-1.04	-1.27	
Psyr_0936	wpbY	Glycosyl transferase, group 1	-1.77	-0.52	-0.87	-1.06	
Psyr_0914	wpbZ	Glycosyl transferase, group 1	-1.65	-0.78	-0.83	-1.09	
Psyr_0920		Glycosyl transferase, group 1	-1.78	-0.95	-1.05	-1.26	
Psyr_0947		TPR repeat protein:TPR repeat protein	-1.54	-1.02	-1.08	-1.21	
Psyr_1251		quinoprotein	-1.04	-1.55	-1.03	-1.21	
Psyr_0915		NAD-dependent epimerase/dehydratase	-1.63	-0.97	-0.92	-1.17	
Psyr_4623		Aminoglycoside phosphotransferase	-1.56	-0.96	-0.83	-1.12	
Psyr_4844		HAD-superfamily hydrolase, subfamily IB (PSPase-like):HAD-superfamily subfamily IB hydrolase, hypothetical 2	-1.60	-0.97	-0.66	-1.08	
Psyr_4078		AmpG-related permease	-1.32	-0.74	-1.02	-1.03	
Psyr_4622		Nucleotidyl transferase	-1.65	-1.47	-0.93	-1.35	
Psyr_1544		SirA-like protein	-1.92	-1.54	0.22	-1.08	

C.

Locus	Name	Description	Apoplastic Fitness				Classification
			1	2	3	Average	
Psyr_0385	hisE	phosphoribosyl-ATP pyrophosphatase	-2.30	-2.21	-0.92	-1.81	Amino acid metabolism and transport
Psyr_1109	edd	6-phosphogluconate dehydratase	-0.95	-1.86	-0.69	-1.17	Carbohydrate metabolism and transport
Psyr_0916	gmd	GDP-mannose 4,6-dehydratase	-1.06	-1.38	-1.58	-1.34	Carbohydrate metabolism and transport
Psyr_0758	scrB	beta-fructofuranosidase	-1.44	-2.02	-1.52	-1.66	Carbohydrate metabolism and transport
Psyr_1914	talB	transaldolase	-0.96	-1.82	-0.67	-1.15	Carbohydrate metabolism and transport
Psyr_3676	cobQ	adenosylcobyric acid synthase (glutamine-hydrolysing)	-2.19	-0.79	-1.22	-1.40	Cofactor metabolism
Psyr_3675	cobU	adenosylcobinamide kinase	-3.24	-2.59	0.44	-1.80	Cofactor metabolism
Psyr_3174	cysG	uroporphyrinogen-III C-methyltransferase / precorrin-2 dehydrogenase	-1.89	-1.78	-0.39	-1.35	Cofactor metabolism
Psyr_1542	nadA	quinolinate synthetase	-1.65	-1.15	-0.67	-1.16	Cofactor metabolism
Psyr_5130		chromosome segregation ATPase	-0.91	-1.18	-1.18	-1.09	Cofactor metabolism
Psyr_0923		hypothetical protein	-1.67	-1.56	-2.34	-1.86	Hypothetical
Psyr_5067		conserved hypothetical protein	-2.92	-1.07	-1.53	-1.84	Hypothetical
Psyr_0534		membrane protein, putative	-1.26	-2.07	-2.00	-1.78	Hypothetical
Psyr_0532		conserved hypothetical protein	-1.83	-1.16	-1.68	-1.56	Hypothetical
Psyr_0533		conserved hypothetical protein	-1.46	-1.23	-0.57	-1.09	Hypothetical
Psyr_5053		asparaginase	-1.34	-0.72	-1.12	-1.06	Nitrogen metabolism
Psyr_3690	purN	formyltetrahydrofolate-dependent phosphoribosylglycinamide formyltransferase	-1.84	-1.13	-1.25	-1.41	Nucleotide metabolism and transport
Psyr_4018	purU	Formyltetrahydrofolate deformylase	-1.76	-1.39	-1.82	-1.66	Nucleotide metabolism and transport
Psyr_4044		Phospholipase D/Transphosphatidylase	-1.84	-1.04	-0.25	-1.04	Phospholipid metabolism
Psyr_2601	salA	regulatory protein, LuxR	-1.46	-1.14	-1.05	-1.21	Phytotoxin synthesis and transport
Psyr_4158	eftA	conserved hypothetical protein	-1.99	-0.99	-1.28	-1.42	Plant-associated proteins
Psyr_1055	algI	Membrane bound O-acyl transferase, MBOAT	-1.02	-1.00	-1.10	-1.04	Polysaccharide synthesis and regulation
Psyr_1054	algJ	alginate biosynthesis protein AlgJ	-1.50	-1.76	-0.94	-1.40	Polysaccharide synthesis and regulation
Psyr_3636	wbpM	Polysaccharide biosynthesis protein CapD	-1.88	-1.87	-1.56	-1.77	Polysaccharide synthesis and regulation
Psyr_1747	clpP	ATP-dependent Clp	-0.99	-2.55	-1.39	-1.64	Proteases

		protease proteolytic subunit ClpP					
Psyr_0574	hflK	protease FtsH subunit HflK	-0.89	-1.34	-0.81	-1.01	Proteases
Psyr_0201	recG	ATP-dependent DNA helicase RecG	-0.95	-1.36	-0.84	-1.05	Replication and DNA repair
Psyr_3287	topA	DNA topoisomerase I	-1.69	-1.49	-1.82	-1.67	Replication and DNA repair
Psyr_5065	uvrD	ATP-dependent DNA helicase UvrD	-1.52	-0.96	-1.01	-1.16	Replication and DNA repair
Psyr_4091		8-oxo-dGTPase	-1.52	-1.76	-1.46	-1.58	Replication and DNA repair
Psyr_0579	rnr	RNAse R	-1.79	-1.32	-0.67	-1.26	RNA degradation
Psyr_4843		NUDIX hydrolase	-0.87	-1.97	-0.99	-1.28	RNA degradation
Psyr_4882	secB	protein translocase subunit secB	-1.27	-0.69	-1.46	-1.14	Secretion/Efflux/Export
Psyr_4069	colS	ATP-binding region, ATPase-like:Histidine kinase, HAMP region:Histidine kinase A, N-terminal	-1.93	-0.79	-0.93	-1.22	Signal transduction mechanisms
Psyr_2077	cysB	regulatory protein, LysR:LysR, substrate-binding protein	-1.45	-1.17	-1.12	-1.24	Sulfur metabolism and transport
Psyr_3698	gacS	Response regulator receiver:ATP-binding region, ATPase-like:Histidine kinase, HAMP region:Histidine kinase A, N-terminal:Hpt	-1.47	-1.55	-1.43	-1.48	Transcriptional regulation
Psyr_5133	trmE	tRNA modification GTPase trmE	-0.74	-1.77	-1.29	-1.27	Translation
Psyr_1200	hrcC	outer-membrane type III secretion protein HrcC	-1.76	-1.15	-0.76	-1.22	Type III secretion system
Psyr_1213	hrcN	type III secretion cytoplasmic ATPase HrcN	-2.08	-1.78	-1.61	-1.82	Type III secretion system
Psyr_1208	hrcR	type III secretion protein HrcR	-1.93	-1.56	-1.00	-1.50	Type III secretion system
Psyr_1206	hrcT	type III secretion protein HrcT	-1.11	-1.22	-0.98	-1.10	Type III secretion system
Psyr_1205	hrcU	type III secretion protein HrcU	-1.61	-0.99	-0.92	-1.17	Type III secretion system
Psyr_1215	hrcV	Type III secretion protein HrcV	-1.25	-1.24	-1.23	-1.24	Type III secretion system
Psyr_1197	hrpE	type III secretion protein HrpE	-1.11	-2.74	-1.79	-1.88	Type III secretion system
Psyr_1216	hrpJ	type III secretion outer membrane protein PopN	-1.58	-1.48	-0.94	-1.34	Type III secretion system
Psyr_1218	hrpK1	type III helper protein HrpK1	-1.22	-1.50	-1.15	-1.29	Type III secretion system
Psyr_1211	hrpP	type III secretion protein HrpP	-2.00	-1.87	-1.87	-1.91	Type III secretion system
Psyr_1191	hrpS	type III transcriptional regulator HrpS	-1.62	-1.21	-0.94	-1.26	Type III secretion system
Psyr_5132	gidA	Glucose-inhibited division protein A subfamily	-1.25	-1.92	-2.65	-1.94	
Psyr_1588	rdgC	Putative exonuclease, RdgC	-2.81	-1.76	-0.91	-1.83	
Psyr_4362	rlpA-2	Rare lipoprotein A	-2.88	-0.85	-2.10	-1.95	
Psyr_1395		virulence	-1.40	-1.50	-1.84	-1.58	

Psyr_4566		Peptidase M23B	-2.14	-1.11	-1.34	-1.53	
Psyr_1417		TPR repeat protein	-1.06	-1.09	-0.89	-1.02	
Psyr_4844		HAD-superfamily hydrolase, subfamily IB (PSPase-like):HAD-superfamily subfamily IB hydrolase, hypothetical 2	-0.96	-1.16	-0.92	-1.01	

Strains and plasmids

Strains		
Organism	Description	Source
E. coli TOP10	For general cloning	Invitrogen
E. coli XL1-Blue	For general cloning	QB3 Macrolab
E. coli S17-1	Conjugation donor strain	(8)
E. coli WM3064	Strain APA752; barcoded *mariner* transposon vector (Kan^R) in *E. coli* conjugation strain	(7)
P. syringae B728a	Wild type strain (Rif^R)	(9)
P. syringae B728a	Whole genome barcoded *mariner* transposon library (Rif^R Kan^R)	This work
P. syringae B728a	Δ*hrpL* (Rif^R)	(10)
P. syringae B728a	Δ*trpA* (Rif^R Kan^R)	This work
P. syringae B728a	Δ*hisD* (Rif^R Kan^R)	This work
P. syringae B728a	Δ*syrP* (Rif^R Kan^R)	This work
P. syringae B728a	Δ*eftA* (Rif^R Kan^R)	This work
P. syringae B728a	Δ*Psyr_0532* (Rif^R Kan^R)	This work
P. syringae B728a	Δ*Psyr_0920* (Rif^R Kan^R)	This work
Geotrichum candidum Fr260	To assay for the production of syringomycin	Dennis Gross

Plasmids			
Plasmid name	Description	Antibiotic	Reference
pKD13	Source of kanamycin resistance	Kan	(11)
pT*sacB*	Suicide plasmid to introduce DNA into *P. syringae*	Tet	(12)
pT:0033-kan	To delete *trpA*, contains *Psyr_0033* flanking regions bordering kan^R cassette, inserted into SmaI site of pT*sacB*	Tet Kan	This work
pT:4133-kan	To delete *hisD*, contains *Psyr_4133* flanking regions bordering kan^R cassette, inserted into SmaI site of pT*sacB*	Tet Kan	This work
pT:2612-kan	To delete *syrP*, contains *Psyr_2612* flanking regions bordering kan^R cassette, inserted into SmaI site of pT*sacB*	Tet Kan	This work
pT:4158-kan	To delete *eftA*, contains *Psyr_4158* flanking regions bordering kan^R cassette, inserted into SmaI site of pT*sacB*	Tet Kan	This work
pT:0532-kan	To delete *Psyr_0532*, contains *Psyr_0532* flanking regions bordering kan^R cassette, inserted into SmaI site of pT*sacB*	Tet Kan	This work
pT:0920-kan	To delete *Psyr_0920*, contains *Psyr_0920* flanking regions bordering kan^R cassette, inserted into SmaI site of pT*sacB*	Tet Kan	This work

Primers

Bold sequence complements FRT-Kan sequence for splicing by overlap extension protocol. The primers used for TnSeq mapping and BarSeq amplification are described in (7).

Name	Sequence
FRT-KanF	GTGTAGGCTGGAGCTGCTTC
FRT-KanR	ATTCCGGGGATCCGTCGACC
0033 up F	TGATCGGCTGCCCTTATGTG
FRT 0033 up R	**GAAGCAGCTCCAGCCTACAC**AGCAAGACACAAGGGGTTCA
FRT 0033 dwn F	**GGTCGACGGATCCCCGGAAT**TTTGCATGTCTTTGTCGCCG
0033 dwn R	TGGTGTTAGACCTCAACCGC
4133 up F	CTTCCAGCAACGCCTGATGT
FRT 4133 up R	**GAAGCAGCTCCAGCCTACAC**TGAGCAAATTCTGGAGCCCT
FRT 4133 dwn F	**GGTCGACGGATCCCCGGAAT**GGGCCTCAATAATTGGCGGA
4133 dwn R	TGATCGACCGCATCTACCAC
4133 up F	CTTCCAGCAACGCCTGATGT
2612 up F	CACCCCAGATTTCCCAGACC
FRT 2612 up R	**GAAGCAGCTCCAGCCTACAC**GACCTCAGCCCTTCACATCC
FRT 2612 dwn F	**GGTCGACGGATCCCCGGAAT**TCCCGTTATCAAGCCAGGAC
2612 dwn R	TGGAGAATCCGAAATCCGCC
4158 up F	CAGGACTCGGAGATCATGCC
FRT 4158 up R	**GAAGCAGCTCCAGCCTACAC**CGCCTCATGGAGTACAGTGG
FRT 4158 dwn F	**GGTCGACGGATCCCCGGAAT**GGTGCAAAGAGCAGAATCGG
4158 dwn R	GTATCGACTCGCGGGAAACT
0532 up F	ACCTCGTCTCTGGCTGTTTC
FRT 0532 up R	**GAAGCAGCTCCAGCCTACAC**CAGTACTGCGCCTGCTGAAT
FRT 0532 dwn F	**GGTCGACGGATCCCCGGAAT**ATGATGGTATTCAGCGAAAACAG
0532 dwn R	TAATCCCGGCCACGACAAAG
0920 up F	GTACGCTGGAAGAATCGGGT
FRT 0920 up R	**GAAGCAGCTCCAGCCTACAC**TTTCCTTGCGCTCAAAAGCC
FRT 0920 dwn F	**GGTCGACGGATCCCCGGAAT**ACAGCCGATTTGAACCTGGG
0920 dwn R	ACTTCCATGCCAGAAGGTGG

Dataset S1. List of all *P. syringae* B728a genes with predicted orthologs (2) in *P. aeruginosa* PAO1, *P. simiae* WCS417, and *P. stutzeri* RCH2. Ortholog essential gene predictions are from (1, 5, 6).

References

1. Price MN, et al. (2018) Mutant phenotypes for thousands of bacterial genes of unknown function. *Nature* 557(7706):503–509.
2. Chen IMA, et al. (2019) IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Res* 47(D1):D666–D677.
3. Kanehisa M, Goto S (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* 28(1):27–30.
4. Yu X, et al. (2013) Transcriptional responses of *Pseudomonas syringae* to growth in epiphytic versus apoplastic leaf sites. *Proc Natl Acad Sci* 110(5):E425–E434.
5. Turner KH, Wessel AK, Palmer GC, Murray JL, Whiteley M (2015) Essential genome of *Pseudomonas aeruginosa* in cystic fibrosis sputum. *Proc Natl Acad Sci* 112(13):4110–4115.
6. Jacobs MA, et al. (2003) Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci* 100(24):14339–14344.
7. Wetmore KM, et al. (2015) Rapid quantification of mutant fitness in diverse bacteria by sequencing randomly bar-coded transposons. *MBio* 6(6):1–15.
8. Simon R, Priefer U, Pühler A (1983) A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in Gram negative bacteria. *Nat Biotechnol* 1:784–791.
9. Loper JE, Lindow SE (1987) Lack of evidence for *in situ* fluorescent pigment production by *Pseudomonas syringae* pv. *syringae* on bean leaf surfaces. *Phytopathology* 77:1449–1454.
10. Yu X, et al. (2014) Transcriptional analysis of the global regulatory networks active in *Pseudomonas syringae* during leaf colonization. *MBio* 5(5):e01683-14.
11. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci* 97(12):6640–6645.
12. Chen C, Malek AA, Wargo MJ, Hogan DA, Beattie GA (2010) The ATP-binding cassette transporter Cbc (choline/betaine/carnitine) recruits multiple substrate-binding proteins with strong specificity for distinct quaternary ammonium compounds. *Mol Microbiol* 75(1):29–45.