Supplementary Information for

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

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Other supplementary materials for this manuscript include the following:

Dataset S1

Supplementary data and figures

Fig. 3 Expanded.

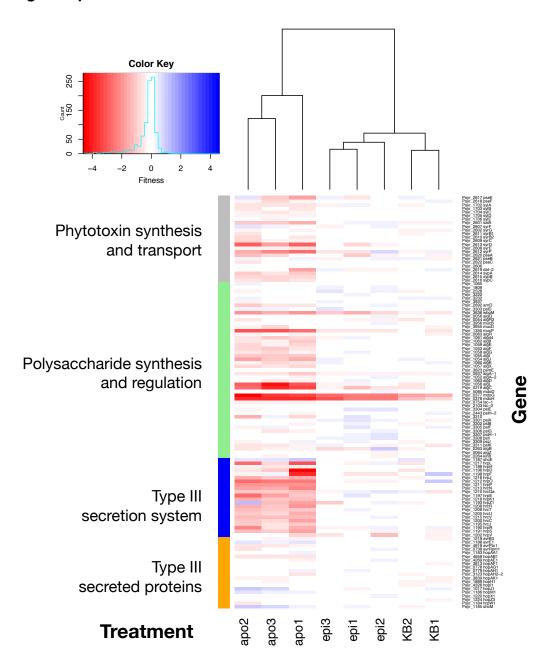
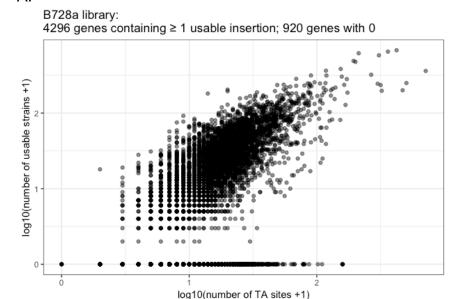
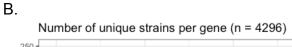


Fig. 3 Expanded. Fitness contributions of genes involved in phytotoxin synthesis and transport, the type III secretion system, and polysaccharide synthesis and regulation are required for apoplastic colonization. Gene fitness values are shown from 3 replicate epiphytic experiments (epi), 3 replicate 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.





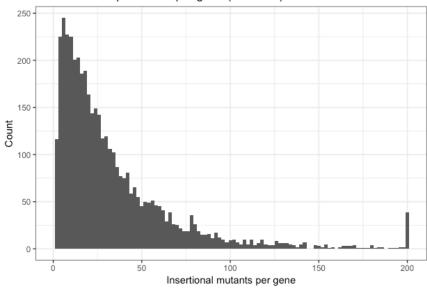


Fig. S1. The B728a mariner transposon library contains 281,417 mapped insertions, 169,826 of which lie within the central 10-90 % of a gene. Only these central insertions are used to calculate fitness. (A) The number of usable insertions for each gene is correlated with the number of TA dinucleotide 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.

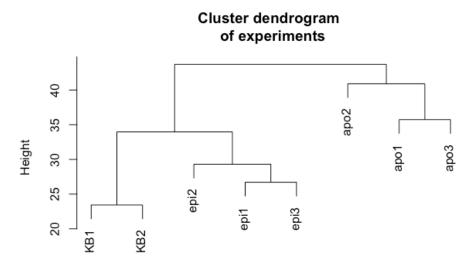
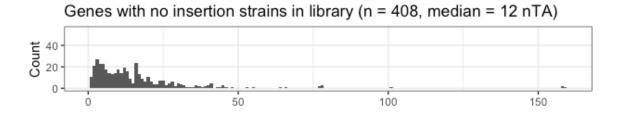
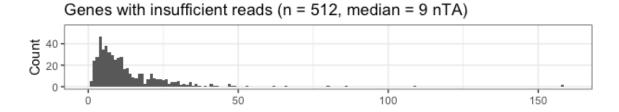


Fig. S2. Dendrogram of experiments, generated using *P. syringae* gene fitness values determined in experimental replicates of the three conditions tested. Rich medium King's B (KB) experiments cluster more closely with the epiphytic experiments (epi) than the apoplastic experiments (apo).

Fig. S3.





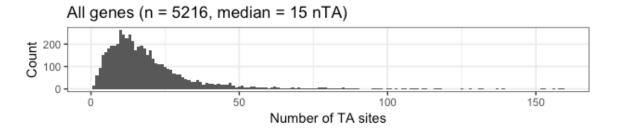


Fig. S3. Of the 920 genes for which their fitness contribution could not be calculated, 408 have no insertion strains in the library, while 512 have at least one insertion but an insufficient number of sequencing reads in time0 samples to calculate fitness. These 920 genes range in size from 73 bp to 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 10⁵ CFU/ml in 1 mM KPO₄ 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.

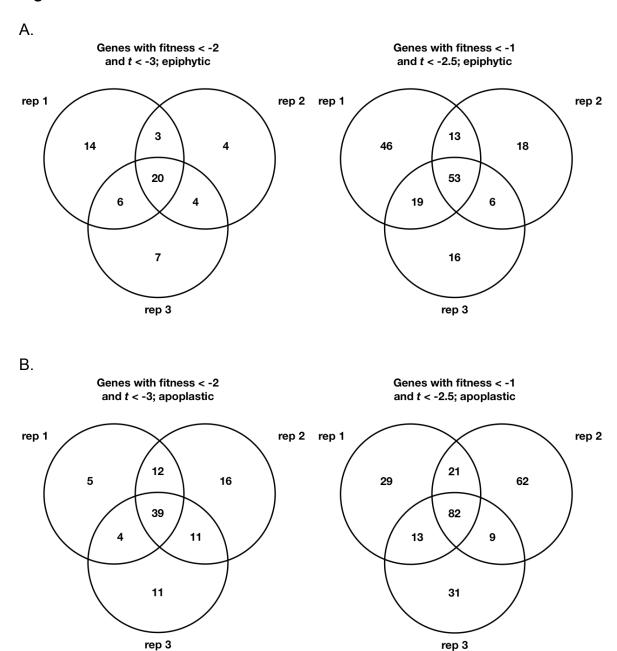
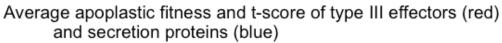


Fig. S5. Venn diagrams showing consistency in identifying genes contributing to epiphytic (A) and apoplastic growth (B) in different replicate experiments. Fitness contribution thresholds are fitness < -2, t < -3 (left) and fitness < -1, t < -2.5 (right).

Fig. S6.



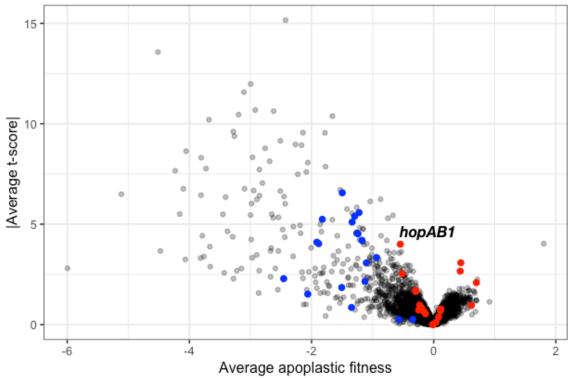
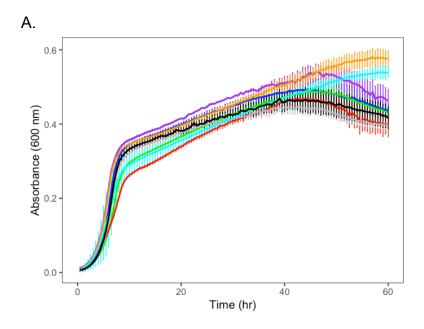


Fig. S6. Scatterplot of average apoplastic fitness and absolute average *t*-score for all genes, averaging values from three replicate experiments. Genes encoding type III secreted proteins are shown in red, with those encoding type III secretion system components and regulatory elements shown in blue. 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.



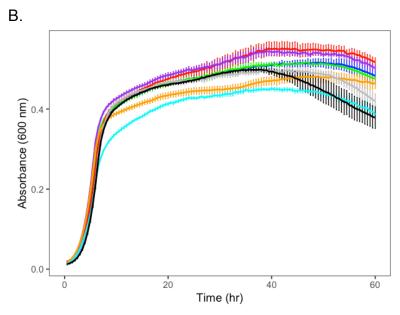
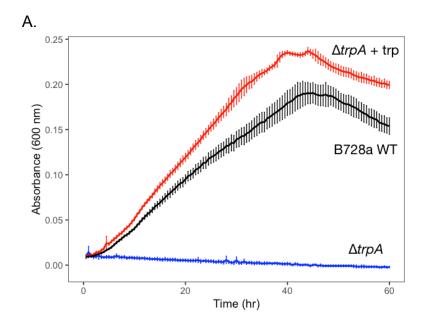


Fig. S7. Growth of deletion mutants in KB (A) and LB (B). Absorbance at 600 nm was measured in 4 to 5 replicate wells per strain. Strains shown are B728a WT (black), $\Delta syrP$ (blue), $\Delta hisD$ (red), $\Delta trpA$ (green), $\Delta hrpL$ (purple), $\Delta eftA$ (grey), $\Delta Psyr_0532$ (orange),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

Fig. S8.



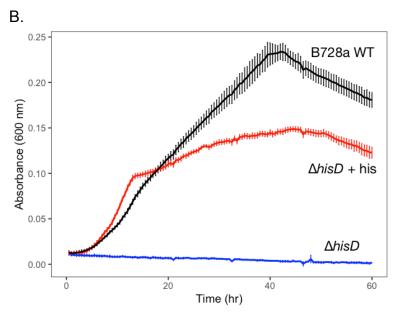


Fig. S8. Growth of B728a auxotrophic mutant strains $\Delta trpA$ (A) and $\Delta hisD$ (B) in M9 minimal medium supplemented by 0.2% glycerol as a carbon source. Auxotrophic deletion strains were complemented by the addition of 20.8 μ g/ml tryptophan or 62 μ g/ml histidine. Mean absorbance of 3 replicate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

Fig. S9.

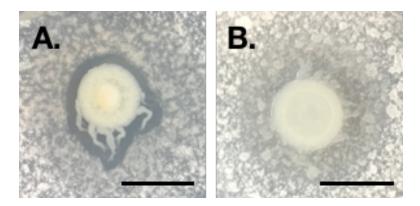


Fig. S9. Inhibition of *Geotrichum candidum* Fr260 growth by B728a WT (A) and $\triangle syrP$ (B). Scale bars are 10 mm in length.

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	B728a
	essential	nonessential
P. simiae essential	282	133
P. simiae nonessential	75	2070
No homolog in <i>P. simiae</i>	35	2393
Total	392	4596

C.

	B728a	B728a
	essential	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	n King's B		Classification
			1	2	Average	
						Amino acid
		anthranilate synthase,				metabolism and
Psyr_4581	trpG	component II	-2.47	-1.71	-2.09	transport
						Carbohydrate
		glucose-6-phosphate				metabolism and
Psyr_0826	pgi	isomerase	-1.91	-2.21	-2.06	transport
D 4040		septum site-determining	0.40	0.70	0.55	0 11 11 11
Psyr_1613	minC	protein MinC	-3.40	-3.70	-3.55	Cell division
		adenosylmethionine-8- amino-7-oxononanoate				
		amino-7-oxononanoate aminotransferase				Cofactor
Psyr 0454	bioA		-2.56	-2.62	-2.59	metabolism
PSyl_0454	DIOA	apoenzyme	-2.50	-2.02	-2.59	Cofactor
Psyr 4687	bioB	biotin synthase	-2.87	-2.96	-2.91	metabolism
1 3y1_ 1 007	DIOD	biotiii synthase	-2.01	-2.50	-2.51	Cofactor
Psyr 4683	bioD	dethiobiotin synthase	-3.30	-2.53	-2.92	metabolism
1 0y1_1000	DIOD	8-amino-7-oxononanoate	0.00	2.00	2.02	Cofactor
Psvr 4686	bioF	synthase	-2.89	-3.18	-3.03	metabolism
. cyccc	2.0.	UBA/THIF-type		00	0.00	
		NAD/FAD binding				Cofactor
Psyr 0951	moeB	fold:MoeZ/MoeB	-4.81	-6.07	-5.44	metabolism
, -		aminodeoxychorismate				Cofactor
Psyr_2080	pabB	synthase, subunit I	-1.38	-2.65	-2.01	metabolism
		thiamine-phosphate				Cofactor
Psyr_4341	thiE	diphosphorylase	-2.80	-3.71	-3.26	metabolism
		thiazole-phosphate				Cofactor
Psyr_4740	thiG	synthase	-3.69	-3.98	-3.84	metabolism
		phosphomethylpyrimidine				Cofactor
Psyr_4340		kinase, putative	-3.51	-4.07	-3.79	metabolism
Davis 0505		Protein of unknown	2.25	2.22	2.04	l lunathatiaal
Psyr_0565		function UPF0126	-2.35	-3.33	-2.84	Hypothetical LPS synthesis
Psyr 0917	rfbA-2	ABC-2	-1.90	-2.53	-2.21	and transport
PSyl_0917	110A-Z	ABC-2	-1.90	-2.55	-2.21	LPS synthesis
Psyr 0918	rfbB-2	ABC transporter	-2.05	-2.37	-2.21	and transport
1 3y1_0310	ווטט-ב	Osmolarity sensor	-2.00	-2.31	-2.21	Osmosensing &
Psyr_0259	envZ	protein envZ	-2.29	-1.91	-2.10	regulation
. 5,1_0200	52	Undecaprenyl-	0		20	Peptidoglycan/cell
Psyr 3008	uppP	diphosphatase	-2.95	-4.69	-3.82	wall polymers
		CDP-diacylglycerol				
		serine O-				Phospholipid
Psyr 0849	pssA-1	phosphatidyltransferase	-2.04	-2.32	-2.18	metabolism
						Replication and
Psyr_4091	<u> </u>	8-oxo-dGTPase	-2.54	-2.47	-2.51	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	Unique	%	Median	Median
•	barcodes at	barcodes in	Recovery	reads/gene at	reads/gene in
	time0	sample		time0	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	Epiphy	tic Fitnes	SS		Classification
			1	2	3	Average	
							Amino acid
							metabolism and
Psyr_4270	glyA	serine hydroxymethyltransferase	-2.35	-2.94	-3.42	-2.90	transport
							Amino acid
		glutamate-5-semialdehyde					metabolism and
Psyr_4369	proA	dehydrogenase	-3.02	-2.42	-2.84	-2.76	transport
							Amino acid
							metabolism and
Psyr_0704	proB	glutamate 5-kinase	-2.91	-1.48	-2.12	-2.17	transport
							Amino acid
D 0557	_		0.40	4.04	0.07	0.04	metabolism and
Psyr_0557	serB	phosphoserine phosphatase	-2.16	-4.21	-3.27	-3.21	transport
							Amino acid
D 0000	4 A	to out a de la constitución de la lacia	0.00	0.04	4.00	0.50	metabolism and
Psyr_0033	trpA	tryptophan synthase, alpha chain	-3.26	-3.21	-4.03	-3.50	transport
							Amino acid
Dovr. 0024	troD	tryptophon synthose hote chain	-2.95	-3.04	-4.01	-3.33	metabolism and
Psyr_0034	trpB	tryptophan synthase, beta chain	-2.95	-3.04	-4.01	-3.33	transport
		anthranilate synthese component					Amino acid metabolism and
Psyr_4609	trpE	anthranilate synthase, component	-3.60	-3.31	-3.96	-3.62	
PSyl_4609	up⊏	I	-3.00	-3.31	-3.90	-3.02	transport Amino acid
		phosphoribosylanthranilate					metabolism and
Psyr 1663	trpF	isomerase	-3.54	-3.50	-4.59	-3.88	transport
1 3y1_1000	прі	Isomerase	0.04	0.00	7.00	0.00	Amino acid
		D-3-phosphoglycerate					metabolism and
Psyr_4852		dehydrogenase	-2.97	-3.51	-3.31	-3.26	transport
1 0y1_1002		derrydregendee	2.07	0.01	0.01	0.20	Carbohydrate
							metabolism and
Psyr_2980	galU	UDP-glucose pyrophosphorylase	-2.64	-1.34	-2.07	-2.02	transport
	J	septum site-determining protein				-	
Psyr_1613*	minC	MinC	-1.46	-2.27	-2.53	-2.09	Cell division
, <u> </u>		adenosylmethionine-8-amino-7-					
		oxononanoate aminotransferase					Cofactor
Psyr_0454*	bioA	apoenzyme	-2.43	-2.01	-2.35	-2.26	metabolism
							Cofactor
Psyr_4687*	bioB	biotin synthase	-2.03	-2.76	-2.05	-2.28	metabolism
							Cofactor
Psyr_4686*	bioF	8-amino-7-oxononanoate synthase	-1.94	-2.28	-2.18	-2.13	metabolism
		uroporphyrinogen-III C-					
		methyltransferase / precorrin-2					Cofactor
Psyr_3174	cysG	dehydrogenase	-1.33	-2.70	-3.07	-2.37	metabolism
	l	acetolactate synthase, large			l _		Cofactor
Psyr_0846	ilvl	subunit	-2.34	-2.45	-2.35	-2.38	metabolism
							Cofactor
Psyr_0827	panC	pantothenate synthetase	-2.60	-4.00	-2.69	-3.10	metabolism
Psyr_0532		conserved hypothetical protein	-3.40	-2.35	-1.73	-2.50	Hypothetical
		Uncharacterized conserved protein			l		
Psyr_2461		UCP030820	-2.13	-1.79	-2.44	-2.12	Hypothetical
				٠			LPS synthesis
Psyr_0917*	rfbA-2	ABC-2	-3.05	-2.13	-2.62	-2.60	and transport
				0.55		0.00	LPS synthesis
Psyr_0918*	rfbB-2	ABC transporter	-2.83	-2.00	-2.05	-2.29	and transport
		glutamate synthase (NADPH)		4			Nitrogen
Psyr_0411	gltB	large subunit	-2.73	-1.65	-1.64	-2.01	metabolism

							Nucleotide metabolism and
Psyr_1668	purF	amidophosphoribosyltransferase	-3.20	-3.21	-3.15	-3.18	transport
· -							Nucleotide
Dovr. 1260	nurl	phosphoribosylformylglycinamidine	-3.08	-2.52	-2.54	-2.71	metabolism and
Psyr_1269	purL	synthase	-3.06	-2.52	-2.54	-2.71	transport
Psyr_3008*	uppP	Undecaprenyl-diphosphatase	-1.86	-2.29	-3.01	-2.38	Peptidoglycan/cell wall polymers
							Phage & IS
Psyr_4512		putative phage-related protein	-2.24	-1.70	-2.38	-2.11	elements
							Polysaccharide
		Periplasmic glucan biosynthesis					synthesis and
Psyr_0377	mdoG	protein, MdoG	-2.47	-2.48	-2.73	-2.56	regulation
							Polysaccharide
							synthesis and
Psyr_0378	mdoH	Glycosyl transferase, family 2	-2.38	-2.37	-3.05	-2.60	regulation
		Holliday junction endonuclease					Replication and
Psyr_1408	ruvC	RuvC	-3.43	-2.74	-1.71	-2.63	DNA repair
		Nitrite/sulfite reductase,					
		hemoprotein beta-component,					
		ferrodoxin-like:Nitrite and sulphite					Sulfur metabolism
Psyr_2462		reductase 4Fe-4S region	-2.60	-3.62	-3.36	-3.19	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	Apoplas	tic Fitnes	S		Classification
			1	2	3	Average	
							Amino acid
							metabolism and
Psyr_4270	glyA	serine hydroxymethyltransferase	-5.00	-5.34	-5.01	-5.11	transport
							Amino acid
		imidazoleglycerol-phosphate					metabolism and
Psyr 4897	hisB	dehydratase	-3.76	-3.42	-2.69	-3.29	transport
							Amino acid
		histidinol phosphate					metabolism and
Psyr_4132	hisC	aminotransferase apoenzyme	-1.90	-3.58	-2.57	-2.69	transport
							Amino acid
							metabolism and
Psyr_4133	hisD	histidinol dehydrogenase	-2.55	-3.66	-2.79	-3.00	transport
, <u> </u>		i i					Amino acid
		ATP phosphoribosyltransferase					metabolism and
Psyr_4134	hisG	(homohexameric)	-2.51	-3.37	-2.40	-2.76	transport
				0.0.			Amino acid
		imidazole glycerol phosphate					metabolism and
Psyr 4896	hisH	synthase subunit hisH	-2.02	-2.56	-1.96	-2.18	transport
· • • • • • • • • • • • • • • • • • • •							Amino acid
							metabolism and
Psyr_1257	leuA	2-isopropylmalate synthase	-2.16	-2.56	-1.95	-2.22	transport
1 3y1_1201	icurt	2 isopropyimalate synthase	2.10	2.00	1.55	2.22	Amino acid
		3-isopropylmalate					metabolism and
Psyr_1985	leuB	dehydrogenase	-4.35	-3.99	-3.82	-4.05	transport
1 3y1_1303	ICUD	denydrogenase	-4.00	-0.00	-3.02	-4.00	Amino acid
		3-isopropylmalate dehydratase,					metabolism and
Psyr_1983	leuC	large subunit	-4.30	-4.82	-3.35	-4.16	transport
F Syl_ 1903	ieuc	large suburiit	-4.50	-4.02	-3.33	-4.10	Amino acid
							metabolism and
Psyr_0473	metW	Methionine biosynthesis MetW	-4.66	-4.25	-3.38	-4.10	transport
1 3y1_0473	IIICLVV	Wethornine blosynthesis Wetvv	-4.00	-4.23	-3.30	-4.10	Amino acid
							metabolism and
Psyr_0474	metX	homoserine O-acetyltransferase	-4.04	-3.02	-3.97	-3.68	transport
1 3y1_0474	IIICLX	Homosenne O-acetymansierase	-4.04	-3.02	-3.31	-5.00	Amino acid
		O-succinylhomoserine					metabolism and
Psyr 1669	metZ	sulfhydrylase	-4.14	-4.63	-3.93	-4.23	transport
1 3y1_1009	IIICLZ	Sulliyuryiase	-4.14	-4.03	-3.93	-4.23	Amino acid
		glutamate-5-semialdehyde					metabolism and
Psyr_4369	proA	dehydrogenase	-3.72	-2.12	-3.15	-3.00	transport
1 3y1_4309	pion	denydrogenase	-5.12	-2.12	-3.13	-5.00	Amino acid
							metabolism and
Psyr 0704	proB	glutamate 5-kinase	-3.24	-3.19	-1.98	-2.80	transport
. 3y1_070 4	PIOD	giatamate o kindoe	0.27	0.10	1.00	2.00	Amino acid
							metabolism and
Psyr 0557	serB	phosphoserine phosphatase	-2.88	-2.37	-1.64	-2.30	
1 3y1_0007	SCID	phosphoseine phosphatase	-2.00	-2.31	-1.04	-2.50	transport Amino acid
		tryptophan synthese sinhs					metabolism and
Psyr_0033	trpA	tryptophan synthase, alpha chain	-2.89	-3.23	-1.87	-2.66	
1-8y1_0033	прА	Giaiff	-2.09	-5.23	-1.01	-2.00	transport Amino acid
Dov. 0004	tro D	truntonbon overtheses bets statis	4.00	2.75	0.04	2.67	metabolism and
Psyr_0034	trpB	tryptophan synthase, beta chain	-4.03	-3.75	-0.24	-2.67	transport
		anthun vilata					Amino acid
Dev 4500	4	anthranilate	4 47	4 70	4.00	4.47	metabolism and
Psyr_4580	trpD	phosphoribosyltransferase	-4.47	-4.70	-4.26	-4.47	transport
Psyr_4609	trpE	anthranilate synthase,	-4.87	-4.58	-4.10	-4.51	Amino acid

		component I					metabolism and
			ļ				transport
							Amino acid
		phosphoribosylanthranilate					metabolism and
Psyr_1663	trpF	isomerase	-3.43	-3.93	-3.64	-3.67	transport
. cyccc	р.		00	0.00	0.0.	0.0.	Amino acid
		D-3-phosphoglycerate					metabolism and
Psyr 4852		dehydrogenase	-3.06	-3.30	-2.49	-2.95	transport
FSyl_4002		1-(5-phosphoribosyl)-5-[(5-	-3.00	-3.30	-2.49	-2.90	transport
							A mains a said
		phosphoribosylamino)methylide					Amino acid
		neamino] imidazole-4-					metabolism and
Psyr_4894		carboxamide isomerase	-1.87	-3.00	-2.76	-2.54	transport
							Carbohydrate
		UDP-glucose					metabolism and
Psyr_2980	galU	pyrophosphorylase	-2.10	-2.53	-2.28	-2.30	transport
							Carbohydrate
							metabolism and
Psyr_0826*	pgi	glucose-6-phosphate isomerase	-2.87	-2.96	-3.46	-3.10	transport
Psyr_3179	ftsK	DNA translocase FtsK	-2.86	-2.53	-2.23	-2.54	Cell division
1 3y1_0170	11311	septum site-determining protein	2.00	2.00	2.20	2.04	Cell division
Psyr_1613*	minC	MinC	-3.94	-3.75	-3.68	-3.79	Cell division
PSyl_1013	mine		-3.94	-3.75	-3.00	-3.79	Cell division
		DnaJ central region:Heat shock					
		protein DnaJ, N-					
	dnaJ-	terminal:Chaperone DnaJ, C-					Chaperones/Heat
Psyr_4194	1	terminal	-1.85	-2.30	-1.87	-2.01	shock proteins
		adenosylmethionine-8-amino-7-					
		oxononanoate aminotransferase					Cofactor
Psyr 0454*	bioA	apoenzyme	-2.32	-2.17	-2.07	-2.19	metabolism
		1					Cofactor
Psyr 4687*	bioB	biotin synthase	-2.83	-2.85	-2.88	-2.85	metabolism
. cyco.	DIOD	Distin Synthass	2.00	2.00	2.00	2.00	Cofactor
Psyr_4683*	bioD	dethiobiotin synthase	-2.99	-2.49	-3.21	-2.90	metabolism
F 5 y 1 _ 4 0 0 3	טוטט	8-amino-7-oxononanoate	-2.99	-2.49	-3.21	-2.90	Cofactor
Dav. 4000*	h:aF		2.54	2.04	0.50	2.02	
Psyr_4686*	bioF	synthase	-2.54	-2.81	-2.53	-2.63	metabolism
D 0040			0.04	0.00	0.44	0.07	Cofactor
Psyr_0848	ilvC	ketol-acid reductoisomerase	-2.91	-3.88	-2.11	-2.97	metabolism
							Cofactor
Psyr_0469	ilvD	dihydroxyacid dehydratase	-3.70	-4.02	-2.11	-3.28	metabolism
		acetolactate synthase, small					Cofactor
Psyr_0847	ilvH	subunit	-2.85	-2.72	-1.72	-2.43	metabolism
		acetolactate synthase, large					Cofactor
Psyr_0846	ilvl	subunit	-3.29	-3.70	-2.33	-3.11	metabolism
		thiamine-phosphate					Cofactor
Psyr 4341*	thiE	diphosphorylase	-1.94	-2.69	-2.91	-2.51	metabolism
. oyı_+o+1		a.p.100p1101y1000	1.04	2.00	2.01	2.01	Cofactor
Psyr 4740*	thiG	thiazole-phosphate synthase	-1.93	-2.18	-3.84	-2.65	metabolism
PSyl_4/40	แแน		-1.93	-2.10	-3.04	-2.00	
D 1010:		phosphomethylpyrimidine	0.04	0.70	0 =0	0.05	Cofactor
Psyr_4340*		kinase, putative	-2.01	-2.79	-3.76	-2.85	metabolism
Psyr_0167		hypothetical protein	-2.86	-3.64	-2.41	-2.97	Hypothetical
		lipid A biosynthesis					LPS synthesis and
Psyr_1614	htrB	acyltransferase	-4.30	-4.58	-2.57	-3.82	transport
							LPS synthesis and
Psyr_0917*	rfbA-2	ABC-2	-2.43	-3.24	-3.27	-2.98	transport
	T						LPS synthesis and
Psyr 0918*	rfbB-2	ABC transporter	-2.08	-2.40	-3.38	-2.62	transport
1 3y1_U310	HVD-Z		-2.00	-2.40	-5.50	-2.02	
Dover 0044		lipid A biosynthesis	244	2.00	2.04	2.67	LPS synthesis and
Psyr_0014		acyltransferase	-3.14	-2.06	-2.81	-2.67	transport
							Nucleotide
							metabolism and
D 4000	I	I amidanhaanharihaayltranafaraaa	-3.64	-3.27	-4.53	-3.81	Ltranapart
Psyr_1668 Psyr_1269	purF purL	amidophosphoribosyltransferase phosphoribosylformylglycinamidi	-3.19	-4.03	-4.07	-3.76	transport Nucleotide

	1	ne synthase	1		1		metabolism and
		ne syntnase					
							transport
Day # 2000*		Linda company din base batasa	2.42	244	2.20	2.44	Peptidoglycan/cell
Psyr_3008*	uppP	Undecaprenyl-diphosphatase	-3.42	-3.44	-3.36	-3.41	wall polymers
							Phytotoxin
D 0040	D	Overlie we wild a two we will a	0.77	0.00	4.70	0.45	synthesis and
Psyr_2613	syrD	Cyclic peptide transporter	-2.77	-2.88	-1.70	-2.45	transport
							Polysaccharide
Day # 0040	ala:C		2.25	0.07	2.77	2.20	synthesis and
Psyr_0219	algC	phosphomannomutase	-3.35	-2.67	-3.77	-3.26	regulation
							Polysaccharide
Day # 4050	اماما	Deliv/hata D. manning mata) hisaa	2.00	2.00	4.00	2.20	synthesis and
Psyr_1056	algL	Poly(beta-D-mannuronate) lyase	-3.02	-2.82	-4.29	-3.38	regulation
		Davinlancia alunga biga wathania					Polysaccharide
Day # 0277	d-O	Periplasmic glucan biosynthesis	2.07	4.40	2.40	2.72	synthesis and
Psyr_0377	mdoG	protein, MdoG	-3.27	-4.42	-3.49	-3.73	regulation
							Polysaccharide
Dover 0270	mdall	Change transferage family 2	-3.83	-3.70	-3.89	-3.81	synthesis and
Psyr_0378	mdoH	Glycosyl transferase, family 2 site-2 protease, Metallo	-3.63	-3.70	-3.09	-3.61	regulation
							Polysaccharide
Psyr 1350	mucP	peptidase, MEROPS family	2 71	-3.14	-3.11	-2.99	synthesis and regulation
PSyl_1330	muce	M50B ATP-dependent Clp protease	-2.71	-3.14	-3.11	-2.99	regulation
Psyr_1748	clpX	ATP-binding subunit ClpX	-2.97	-2.42	-3.29	-2.90	Proteases
F 5 y 1 / 40	СІРХ	Holliday junction DNA helicase	-2.91	-2.42	-3.29	-2.90	Replication and
Psyr_1410	ruvB	RuvB	-1.88	-2.79	-2.74	-2.47	DNA repair
1 3yi_1410	TUVD	Chromosome segregation	-1.00	-2.13	-2.14	-2.41	Replication and
Psyr_0919		ATPase-like protein	-2.02	-1.91	-2.49	-2.14	DNA repair
1 3y1_0313		RNA polymerase, sigma-24	-2.02	-1.91	-2.43	-2.14	Transcription -
Psyr 3958	algU	subunit, RpoE	-2.78	-2.67	-3.31	-2.92	Sigma factor
F 5 91_3930	aigu	Response regulator	-2.70	-2.07	-3.31	-2.92	Sigina factor
		receiver:ATP-binding region,					
		ATPase-like:Histidine kinase A,					
		N-terminal:Histidine					Transcriptional
Psyr 4408	retS	kinase:Histidine kinase	-2.50	-2.32	-2.71	-2.51	regulation
Psyr 3637	wbpL	Glycosyl transferase, family 4	-2.50	-2.04	-1.95	-2.16	rogulation
Psyr_0936	wbpY	Glycosyl transferase, group 1	-1.90	-1.68	-2.61	-2.06	
Psyr 0914	wbpT	Glycosyl transferase, group 1	-2.22	-1.78	-2.26	-2.09	
Psyr_0529	vv.op.	Glycosyl transferase, group 1	-3.78	-2.89	-3.69	-3.45	
1 3y1_0029		NAD-dependent	0.70	2.00	0.00	3.40	
Psyr 0915		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	-2.44	
1 3y1_0320		Peptidase S1,	-2.03	-2.04	-4.4	-2.72	
Psyr 4130		chymotrypsin:PDZ/DHR/GLGF	-2.51	-2.20	-2.08	-2.26	
i⁻5yi <u>4</u> 130	L	GHYHIOU YPSHLEDZ/DFR/GLGF	-2.31	-2.20	-2.00	-2.20	

^{*} 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 i	in King's B		Classification
			1	2	Average	
						Amino acid
		methionine synthase				metabolism and
Psyr_2855	metE	(B12-independent)	-1.70	-1.34	-1.52	transport
		UDP-glucose				Carbohydrate metabolism and
Psyr_2980	galU	pyrophosphorylase	-1.20	-1.68	-1.44	transport
1 cy:2ccc	gaio	PpiC-type peptidyl-	1.20	1.00		панороге
		prolyl cis-trans				Chaperones/Heat
Psyr_1751	ppiD	isomerase	-1.44	-1.17	-1.31	shock proteins
5 4050		aminodeoxychorismate	4.00	0.40	4.00	Cofactor
Psyr_1650	pabC	lyase apoprotein	-1.66	-2.18	-1.92	metabolism
Psyr_0487	gshB	glutathione synthase	-1.81	-1.27	-1.54	Glutathione metabolism
1 3y1_0407	ysnb	Protein of unknown	-1.01	-1.21	-1.54	metabolism
Psyr_0475		function YGGT	-2.19	-1.37	-1.78	Hypothetical
· -		ribosomal large				Nucleotide
		subunit pseudouridine	1		1	metabolism and
Psyr_3581		synthase B	-1.31	-1.08	-1.19	transport
Dovr. 2954		conserved hypothetical	1 00	1 76	1 70	Phage & IS
Psyr_2854		protein Periplasmic glucan	-1.80	-1.76	-1.78	elements Polysaccharide
		biosynthesis protein,				synthesis and
Psyr_0377	mdoG	MdoG	-1.39	-1.62	-1.50	regulation
						Polysaccharide
		Glycosyl transferase,				synthesis and
Psyr_0378	mdoH	family 2	-0.81	-1.27	-1.04	regulation
		ATP-dependent Clp protease ATP-binding				
Psyr_1748	clpX	subunit ClpX	-1.09	-1.55	-1.32	Proteases
1 cy17 10	O.p.r.	protease FtsH subunit	1.00	1.00	1.02	110104000
Psyr_0574	hflK	HflK	-1.51	-0.81	-1.16	Proteases
		ATP-dependent				
		proteinase, Serine				
Psyr_1749	lon-1	peptidase, MEROPS family S16	-0.97	-1.09	-1.03	Proteases
1 3y1_1749	1011-1	Holliday junction DNA	-0.31	-1.09	-1.03	Replication and
Psyr_1410	ruvB	helicase RuvB	-1.35	-1.33	-1.34	DNA repair
		Holliday junction				Replication and
Psyr_1408	ruvC	endonuclease RuvC	-1.57	-1.28	-1.42	DNA repair
		Response regulator				
		receiver:ATP-binding				
		region, ATPase- like:Histidine kinase A,				
		N-terminal:Histidine				Transcriptional
Psyr_4408	retS	kinase:Histidine kinase	-1.28	-0.92	-1.10	regulation
		tRNA modification				_
Psyr_5133	trmE	GTPase trmE	-0.92	-1.66	-1.29	Translation
Davis 4005	h = = 0	Protein of unknown	4.47	0.00	1.04	Type VI secretion
Psyr_1935	hcp-3	function DUF796 GTP-binding protein	-1.47	-0.60	-1.04	system
Psyr_3954	lepA	LepA	-1.17	-1.56	-1.37	
Psyr 0529		Glycosyl transferase,	-1.57	-1.48	-1.52	
1.9A1_0058		Giycosyi ilalisielase,	-1.01	-1.40	-1.02	

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

В.

В.	1	1					Ta: :::::::::::::::::::::::::::::::::::
Locus	Name	Description	Epiphyti	c Fitness	ı	1	Classification
			1	2	3	Average	
		shikimate					Amino acid
		dehydrogenas					metabolism
Psyr_0025	aroE	е	-1.32	-1.36	-1.06	-1.25	and transport
		histidinol					
		phosphate					
		aminotransfer					Amino acid
D 4400	h:-0	ase	4.70	4.07	4 4 7	4.54	metabolism
Psyr_4132	hisC	apoenzyme histidinol	-1.76	-1.67	-1.17	-1.54	and transport Amino acid
		dehydrogenas					metabolism
Psyr 4133	hisD	e	-1.87	-0.89	-1.03	-1.27	and transport
1 3y1_4133	III3D	ATP	-1.01	-0.09	-1.03	-1.21	and transport
		phosphoribosy					
		Itransferase					Amino acid
		(homohexame					metabolism
Psyr 4134	hisG	ric)	-1.63	-1.11	-0.84	-1.19	and transport
		imidazole					
		glycerol]				
		phosphate					Amino acid
		synthase					metabolism
Psyr_4896	hisH	subunit hisH	-2.68	-1.93	-1.20	-1.94	and transport
		3-					
		isopropylmalat					A main a said
		e dehydrogenas					Amino acid metabolism
Psyr_1985	leuB	e	-1.58	-1.19	-0.90	-1.23	and transport
1 3y1_1300	ICUD	3-	1.00	1.10	0.50	1.20	and transport
		isopropylmalat					
		е					Amino acid
		dehydratase,					metabolism
Psyr_1983	leuC	large subunit	-1.53	-2.03	-0.41	-1.33	and transport
		1-(5-					
		phosphoribosy					
		I)-5-[(5-					
		phosphoribosy					
		lamino)methyli					
		deneamino]					Amino ooid
		imidazole-4-					Amino acid
Psyr 4894		carboxamide isomerase	-2.42	-1.35	-1.22	-1.66	metabolism and transport
1 Syl_4094		GDP-	-2.42	-1.55	-1.22	-1.00	Carbohydrate
		mannose 4,6-					metabolism
Psyr 0916	gmd	dehydratase	-1.64	-0.61	-0.77	-1.01	and transport
	J	glucose-6-	1	1			Carbohydrate
		phosphate					metabolism
Psyr_0826	pgi	isomerase	-2.08	-1.05	-2.55	-1.89	and transport
		Phosphoenolp					
		yruvate-]				Carbohydrate
		protein					metabolism
Psyr_4842		phosphotransf	-1.67	-1.49	-1.13	-1.43	and transport

		Larana					
		erase DNA					
		translocase					
Psyr_3179	ftsK	FtsK	-1.98	-1.15	-1.90	-1.68	Cell division
1 0)1_0110	Itort	ketol-acid	1.00	10	1.00	1.00	Con dividion
		reductoisomer					Cofactor
Psyr_0848	ilvC	ase	-1.11	-1.40	-1.62	-1.38	metabolism
		dihydroxyacid					Cofactor
Psyr_0469	ilvD	dehydratase	-1.62	-2.48	-1.64	-1.91	metabolism
		acetolactate					
	l	synthase,		0.04	4.00		Cofactor
Psyr_0847	ilvH	small subunit	-2.09	-2.01	-1.90	-2.00	metabolism
		glucose-6-					
		phosphate 1- dehydrogenas					Energy
Psyr_1120	zwf-1	e	-1.09	-1.37	-0.60	-1.02	generation
1 3y1_1120	ZVVII	Protein of	1.00	1.07	0.00	1.02	generation
		unknown					
		function					
Psyr_0983		DUF159	-1.93	-1.43	-1.64	-1.67	Hypothetical
		hypothetical					
Psyr_0923		protein	-1.64	-1.28	-1.43	-1.45	Hypothetical
		membrane					
D 0504		protein,	4.70	0.70		4.40	
Psyr_0534		putative	-1.70	-0.76	-1.11	-1.19	Hypothetical
Psyr_3805		hypothetical protein	-2.26	0.57	-2.54	-1.41	Hypothotical
PSyl_3603	1	conserved	-2.20	0.57	-2.54	-1.41	Hypothetical
		hypothetical					
Psyr 3691		protein	-1.53	-0.22	-1.32	-1.03	Hypothetical
. cyccc.		glutamate		0			pourousus
		synthase					
		(NADPH)					Nitrogen
Psyr_0412	gltD	small subunit	-1.85	-0.65	-0.78	-1.09	metabolism
		Helix-turn-					
		helix, Fis-					
		type:Nitrogen					NII dana ara ara
Psyr 4822	ntrC	regulation protein NR(I)	-1.80	-1.74	-0.61	-1.38	Nitrogen metabolism
F 5 y 1 _ 4 0 2 2	TILLO	protein NK(i)	-1.00	-1./4	-0.01	-1.30	Nucleotide
		Exopolyphosp					metabolism
Psyr_0294	ррх	hatase	-0.87	-0.78	-1.58	-1.08	and transport
· -		ribosomal					•
		large subunit					Nucleotide
		pseudouridine					metabolism
Psyr_3581	1	synthase B	-0.61	-1.44	-1.57	-1.21	and transport
		Malate:quinon					
		e-					Organic acid
Psyr 0976	maa	oxidoreductas	1 00	1 55	1.60	-1.69	metabolism
-5yi_09/6	mqo	UDP-N-	-1.89	-1.55	-1.62	-1.09	and transport
	1	acetylmuramat					
	1	e:L-alanyl-					
	1	gamma-D-					
		glutamyl-					
		meso-					Peptidoglycan/
	1	diaminopimela					cell wall
Psyr_0630	mpl	te ligase	-1.62	-0.82	-1.23	-1.22	polymers
		Glycoside					Peptidoglycan/
Dev. 0004		hydrolase,	0.00	0.00	0.07	4.00	cell wall
Psyr_3281	nagZ	family 3, N-	-2.33	-0.80	-2.37	-1.83	polymers

		terminal				1	
		Peptidoglycan					Peptidoglycan/
		glycosyltransf					cell wall
Psyr_0402	ponA	erase	-1.20	-0.78	-1.17	-1.05	polymers
1 3y1_0+02	ponA	conserved	-1.20	-0.70	-1.17	-1.05	Plant-
		hypothetical					associated
Psyr 4158	eftA	protein	-1.85	-0.52	-1.32	-1.23	proteins
1 3y1_ 1 100	CILA	Polysaccharid	-1.00	-0.02	-1.02	-1.20	Polysaccharid
		e biosynthesis					e synthesis
Psyr 3636	wbpM	protein CapD	-1.56	-0.76	-1.07	-1.13	and regulation
1 3y1_0000	Wopivi	Peptidase	1.00	0.70	1.07	1.10	and regulation
		S41A, C-					Post-
		terminal					translational
Psyr 4887	ctpA	protease	-2.42	-1.64	-0.98	-1.68	modification
1 0y1 <u>_</u> 1007	Otp/ t	ATP-		1.01	0.00	1.00	modification
		dependent Clp					
		protease					
		proteolytic					
Psyr 1747	clpP	subunit ClpP	-0.95	-1.53	-1.16	-1.21	Proteases
- <u></u>		ATP-		1	1	1	
		dependent Clp		1			
		protease ATP-					
		binding		1			
Psyr_1748	clpX	subunit ClpX	-1.47	-1.27	-1.06	-1.27	Proteases
	•	,					Replication
							and DNA
Psyr_1378	recA	RecA protein	-2.01	-0.97	-2.41	-1.80	repair
		ATP-					•
		dependent					Replication
		DNA helicase					and DNA
Psyr_0201	recG	RecG	-1.50	-0.58	-0.99	-1.02	repair
		Holliday					Replication
		junction DNA					and DNA
Psyr_1410	ruvB	helicase RuvB	-1.65	-1.38	-2.38	-1.80	repair
		ATP-					
		dependent					Replication
		DNA helicase					and DNA
Psyr_5065	uvrD	UvrD	-1.84	-1.41	-2.30	-1.85	repair
		Chromosome					
		segregation					Replication
		ATPase-like					and DNA
Psyr_0919		protein	-1.66	-1.05	-1.16	-1.29	repair
		Two-					
		component					Signal
D 0000	,	sensor kinase	4.00	1.0-	4.0-	1	transduction
Psyr_0832	cbrA-1	CbrA	-1.28	-1.65	-1.35	-1.43	mechanisms
		Integral		1			
D 0044	4	membrane		0.74	4 40	1 440	Stress
Psyr_0811	terC	protein TerC	-1.11	-0.74	-1.46	-1.10	resistance
		sulfate					O. J.
		adenylyltransf					Sulfur
Dovr 4400	oveD.	erase subunit	1.00	1.07	0.00	1.00	metabolism
Psyr_4128	cysD	2	-1.28	-1.07	-0.83	-1.06	and transport
		adenylylsulfat					
		e kinase /					
		sulfate					Cultur
		adenylyltransf					Sulfur
Devr 4126	OVONIC	erase subunit	1 00	1 15	1 40	1 21	metabolism
Psyr_4126	cysNC		-1.08	-1.15	-1.40	-1.21	and transport
Psyr 4408	retS	Response	2.05	1 20	1 10	1 51	Transcriptional
	11619	regulator	-2.05	-1.39	-1.10	-1.51	regulation

		1		1	1	1	1
		receiver:ATP-					
		binding region,					
		ATPase-					
		like:Histidine					
		kinase A, N-					
		terminal:Histidi					
		ne					
		kinase:Histidin e kinase					
		tRNA					
		modification					
Dover 5122	trmE	GTPase trmE	-0.86	-1.29	1 16	1 10	Translation
Psyr_5133	uiiii	Protein of	-0.00	-1.29	-1.16	-1.10	Translation
		unknown					
D 4507	16	function	4.00	0.00	4 4 7	4.40	
Psyr_4567	anmK	UPF0075	-1.22	-0.90	-1.17	-1.10	
D 4000		Rare	0.40	4.00	4.0=	4.00	
Psyr_4362	rlpA-2	lipoprotein A	-2.16	-1.93	-1.67	-1.92	
		Glycosyl					
		transferase,					
Psyr_3637	wbpL	family 4	-1.68	-1.09	-1.04	-1.27	
		Glycosyl					
		transferase,					
Psyr_0936	wpbY	group 1	-1.77	-0.52	-0.87	-1.06	
		Glycosyl					
		transferase,					
Psyr_0914	wpbZ	group 1	-1.65	-0.78	-0.83	-1.09	
	<u> </u>	Glycosyl					
		transferase,					
Psyr 0920		group 1	-1.78	-0.95	-1.05	-1.26	
		TPR repeat			11100		
		protein:TPR					
Psyr_0947		repeat protein	-1.54	-1.02	-1.08	-1.21	
	†						
Psyr_1251		quinoprotein	-1.04	-1.55	-1.03	-1.21	
		NAD-					
		dependent					
		epimerase/de					
Psyr_0915		hydratase	-1.63	-0.97	-0.92	-1.17	
		Aminoglycosid					
		е					
		phosphotransf					
Psyr_4623		erase	-1.56	-0.96	-0.83	-1.12	
		HAD-					
		superfamily					
		hydrolase,					
		subfamily IB					
		(PSPase-					
		like):HAD-					
		superfamily					
		subfamily IB					
		hydrolase,					
Psyr 4844		hypothetical 2	-1.60	-0.97	-0.66	-1.08	
. 5,1_1517	1	AmpG-related	1.55	3.07	0.00	1.00	
Psyr 4078		permease	-1.32	-0.74	-1.02	-1.03	
1 3y1_4010		Nucleotidyl	-1.04	-0.74	-1.02	-1.00	
Psyr 4622		transferase	-1.65	-1.47	-0.93	-1.35	
F391_4022		SirA-like	-1.00	-1.4/	-0.93	-1.30	
Dove 1511			1.00	1 = 1	0.22	1.00	
Psyr_1544	1	protein	-1.92	-1.54	0.22	-1.08	

Locus	Name	Description	Apoplastic Fitness				Classification
			1	2	3	Average	
							Amino acid
		phosphoribosyl-ATP		0.04		4.04	metabolism and
Psyr_0385	hisE	pyrophosphatase	-2.30	-2.21	-0.92	-1.81	transport Carbohydrate
		6-phosphogluconate					metabolism and
Psyr_1109	edd	dehydratase	-0.95	-1.86	-0.69	-1.17	transport
		, , , , , , , , , , , , , , , , , , , ,					Carbohydrate
		GDP-mannose 4,6-					metabolism and
Psyr_0916	gmd	dehydratase	-1.06	-1.38	-1.58	-1.34	transport
							Carbohydrate
Psyr_0758	scrB	beta-fructofuranosidase	-1.44	-2.02	-1.52	-1.66	metabolism and transport
1 3y1_0/30	3010	beta-iructorurariosidase	-1.44	-2.02	-1.52	-1.00	Carbohydrate
							metabolism and
Psyr_1914	talB	transaldolase	-0.96	-1.82	-0.67	-1.15	transport
		adenosylcobyric acid					
D 0070		synthase (glutamine-	0.40	0.70	4.00	4.40	0-ft
Psyr_3676	cobQ	hydrolysing) adenosylcobinamide	-2.19	-0.79	-1.22	-1.40	Cofactor metabolism
Psyr_3675	cobU	kinase	-3.24	-2.59	0.44	-1.80	Cofactor metabolism
1 3y1_0070	CODO	uroporphyrinogen-III C-	0.24	2.00	0.44	1.00	Coldctol Illetabolishi
		methyltransferase /					
Psyr_3174	cysG	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
D 5400		chromosome segregation	0.04	4.40	4.40	4.00	
Psyr_5130		ATPase	-0.91	-1.18	-1.18	-1.09	Cofactor metabolism
Psyr_0923		hypothetical protein	-1.67	-1.56	-2.34	-1.86	Hypothetical
Dovr. 5067		conserved hypothetical protein	-2.92	1.07	1 52	1 0/	Llypothotical
Psyr_5067		membrane protein,	-2.92	-1.07	-1.53	-1.84	Hypothetical
Psyr 0534		putative	-1.26	-2.07	-2.00	-1.78	Hypothetical
		conserved hypothetical					,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
Psyr_0532		protein	-1.83	-1.16	-1.68	-1.56	Hypothetical
		conserved hypothetical					
Psyr_0533		protein	-1.46	-1.23	-0.57	-1.09	Hypothetical
Psyr_5053		asparaginase	-1.34	-0.72	-1.12	-1.06	Nitrogen metabolism
		formyltetrahydrofolate-					
		dependent phosphoribosylglycinamide					Nucleotide metabolism
Psyr_3690	purN	formyltransferase	-1.84	-1.13	-1.25	-1.41	and transport
1 01000	pani	Formyltetrahydrofolate	1.01	11.10	1.20		Nucleotide metabolism
Psyr_4018	purU	deformylase	-1.76	-1.39	-1.82	-1.66	and transport
		Phospholipase					Phospholipid
Psyr_4044		D/Transphosphatidylase	-1.84	-1.04	-0.25	-1.04	metabolism
Dovr 2601	cal^	regulatory pretein LuyP	-1.46	1 14	1.05	1 21	Phytotoxin synthesis
Psyr_2601	salA	regulatory protein, LuxR conserved hypothetical	-1.40	-1.14	-1.05	-1.21	and transport Plant-associated
Psyr_4158	eftA	protein	-1.99	-0.99	-1.28	-1.42	proteins
		p	1.55	3.30	15		Polysaccharide
		Membrane bound O-acyl					synthesis and
Psyr_1055	algl	transferase, MBOAT	-1.02	-1.00	-1.10	-1.04	regulation
		alainata biana d					Polysaccharide
Devr 1054	alc I	alginate biosynthesis	-1.50	1 76	-0.94	1 40	synthesis and
Psyr_1054	algJ	protein AlgJ	-1.50	-1.76	-0.94	-1.40	regulation Polysaccharide
		Polysaccharide					synthesis and
Psyr_3636	wbpM	biosynthesis protein CapD	-1.88	-1.87	-1.56	-1.77	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
1 3y1_007+	Timex	ATP-dependent DNA	0.00	1.04	0.01	1.01	Replication and DNA
Psyr 0201	recG	helicase RecG	-0.95	-1.36	-0.84	-1.05	repair
1 3y1_0201	1000	Ticiicase Neces	0.55	1.00	0.04	1.00	Replication and DNA
Psyr_3287	topA	DNA topoisomerase I	-1.69	-1.49	-1.82	-1.67	repair
. cyczc.	top/ t	ATP-dependent DNA	1.00		1.02	1.07	Replication and DNA
Psyr 5065	uvrD	helicase UvrD	-1.52	-0.96	-1.01	-1.16	repair
· • • • • • • • • • • • • • • • • • • •					1171	1	Replication and DNA
Psyr 4091		8-oxo-dGTPase	-1.52	-1.76	-1.46	-1.58	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
PSyl_4043			-0.07	-1.97	-0.99	-1.20	RIVA degradation
Dov. 4000	a a a D	protein translocase subunit	1 27	0.60	1.46	1 1 1	Coarotion/Efflux/Export
Psyr_4882	secB	secB	-1.27	-0.69	-1.46	-1.14	Secretion/Efflux/Export
		ATP-binding region, ATPase-like:Histidine					
		kinase, HAMP					Signal transduction
Dovr 4060	200	region:Histidine kinase A,	1.02	0.70	-0.93	1 22	mechanisms
Psyr_4069	colS	N-terminal	-1.93	-0.79	-0.93	-1.22	mechanisms
		regulatory protein,					Cultius se etab aliese and
Dov. 2077	avaD	LysR:LysR, substrate-	1 15	1 17	1 12	-1.24	Sulfur metabolism and
Psyr_2077	cysB	binding protein	-1.45	-1.17	-1.12	-1.24	transport
		Response regulator					
		receiver:ATP-binding					
		region, ATPase-					
		like:Histidine kinase,					Tueseesiatieseel
D 0000	0	HAMP region:Histidine	4 47	4.55	4.40	4.40	Transcriptional
Psyr_3698	gacS	kinase A, N-terminal:Hpt	-1.47	-1.55	-1.43	-1.48	regulation
Dov. F122	trm-	tRNA modification GTPase	0.74	1 77	1.20	1.07	Translation
Psyr_5133	trmE	trmE	-0.74	-1.77	-1.29	-1.27	Translation
Davis 1000	h.a.C	outer-membrane type III	4.70	4 45	0.70	4.00	Type III secretion
Psyr_1200	hrcC	secretion protein HrcC	-1.76	-1.15	-0.76	-1.22	system
D 4040	la N I	type III secretion	0.00	4 70	4.04	4.00	Type III secretion
Psyr_1213	hrcN	cytoplasmic ATPase HrcN	-2.08	-1.78	-1.61	-1.82	system
Davis 4000	h na D	type III secretion protein	4.00	4.50	4.00	4.50	Type III secretion
Psyr_1208	hrcR	HrcR	-1.93	-1.56	-1.00	-1.50	system
Davis 1000	h na T	type III secretion protein	444	4.00	0.00	1.10	Type III secretion
Psyr_1206	hrcT	HrcT	-1.11	-1.22	-0.98	-1.10	system
Davis 4005	la mal I	type III secretion protein	4.04	0.00	0.00	4 47	Type III secretion
Psyr_1205	hrcU	HrcU	-1.61	-0.99	-0.92	-1.17	system
Day: 1015	la ma\/	Type III secretion protein	4.05	4.04	4.00	4.04	Type III secretion
Psyr_1215	hrcV	type III secretion protein	-1.25	-1.24	-1.23	-1.24	system Type III secretion
Davis 4407	h	1 - 1	444	0.74	4.70	4.00	, ·
Psyr_1197	hrpE	HrpE	-1.11	-2.74	-1.79	-1.88	system
D 4040	la anna d	type III secretion outer	4.50	4 40	0.04	4.04	Type III secretion
Psyr_1216	hrpJ	membrane protein PopN	-1.58	-1.48	-0.94	-1.34	system
D 4040	l 1/4	type III helper protein	4.00	4.50	4.45	4.00	Type III secretion
Psyr_1218	hrpK1	HrpK1	-1.22	-1.50	-1.15	-1.29	system
D 4044	h D	type III secretion protein	0.00	4.07	4.07	4.04	Type III secretion
Psyr_1211	hrpP	HrpP	-2.00	-1.87	-1.87	-1.91	system
5 4404		type III transcriptional	4.00	4.04	0.04	4.00	Type III secretion
Psyr_1191	hrpS	regulator HrpS	-1.62	-1.21	-0.94	-1.26	system
		Glucose-inhibited division		4	0.5-	1	
Psyr_5132	gidA	protein A subfamily	-1.25	-1.92	-2.65	-1.94	
		Putative exonuclease,				1	
Psyr_1588	rdgC	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	1	virulence	-1.40	-1.50	-1.84	-1.58	
		virulerice	-1.40	-1.50	1.04	-1.00	

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	
	HAD-superfamily					
	hydrolase, subfamily IB					
	(PSPase-like):HAD-					
	superfamily subfamily IB					
Psyr_4844	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	(7)
	transposon vector (Kan ^R) in <i>E. coli</i>	
	conjugation strain	
P. syringae B728a	Wild type strain (Rif ^R)	(9)
P. syringae B728a	Whole genome barcoded <i>mariner</i>	This work
	transposon library (Rif ^R Kan ^R)	
P. syringae B728a	$\Delta hrpL$ (Rif ^R)	(10)
P. syringae B728a	$\Delta trpA$ (Rif ^R Kan ^R)	This work
P. syringae B728a	∆hisD (Rif ^R Kan ^R)	This work
P. syringae B728a	∆s <i>yrP</i> (Rif ^R Kan ^R)	This work
P. syringae B728a	∆ <i>eftA</i> (Rif ^R Kan ^R)	This work
P. syringae B728a	∆ <i>Psyr_05</i> 32 (Rif ^R Kan ^R)	This work
P. syringae B728a	∆ <i>Psyr_</i> 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 <i>sacB</i>	Suicide plasmid to introduce DNA into <i>P. syringae</i>	Tet	(12)
pT:0033-kan	To delete <i>trpA</i> , contains <i>Psyr_0033</i> flanking regions bordering kan ^R cassette, inserted into Smal site of pTsacB	Tet Kan	This work
pT:4133-kan	To delete <i>hisD</i> , contains <i>Psyr_4133</i> flanking regions bordering kan ^R cassette, inserted into Smal site of pTsacB	Tet Kan	This work
pT:2612-kan	To delete <i>syrP</i> , contains <i>Psyr_2612</i> flanking regions bordering kan ^R cassette, inserted into Smal site of pT <i>sacB</i>	Tet Kan	This work
pT:4158-kan	To delete <i>eftA</i> , contains <i>Psyr_4158</i> flanking regions bordering kan ^R cassette, inserted into Smal site of pTsacB	Tet Kan	This work
pT:0532-kan	To delete <i>Psyr_0532</i> , contains <i>Psyr_0532</i> flanking regions bordering kan ^R cassette, inserted into Smal site of pTsacB	Tet Kan	This work
pT:0920-kan	To delete <i>Psyr_0920</i> , contains <i>Psyr_0920</i> flanking regions bordering kan ^R cassette, inserted into Smal site of pTsacB	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	GAAGCAGCTCCAGCCTACACAGCAAGACACAAGGGGTTCA
FRT 0033 dwn F	GGTCGACGGATCCCCGGAATTTTGCATGTCTTTGTCGCCG
0033 dwn R	TGGTGTTAGACCTCAACCGC
4133 up F	CTTCCAGCAACGCCTGATGT
FRT 4133 up R	GAAGCAGCTCCAGCCTACACTGAGCAAATTCTGGAGCCCT
FRT 4133 dwn F	GGTCGACGGATCCCCGGAATGGGCCTCAATAATTGGCGGA
4133 dwn R	TGATCGACCGCATCTACCAC
4133 up F	CTTCCAGCAACGCCTGATGT
2612 up F	CACCCAGATTTCCCAGACC
FRT 2612 up R	GAAGCAGCTCCAGCCTACACGACCTCAGCCCTTCACATCC
FRT 2612 dwn F	GGTCGACGGATCCCCGGAATTCCCGTTATCAAGCCAGGAC
2612 dwn R	TGGAGAATCCGAAATCCGCC
4158 up F	CAGGACTCGGAGATCATGCC
FRT 4158 up R	GAAGCAGCTCCAGCCTACACCGCCTCATGGAGTACAGTGG
FRT 4158 dwn F	GGTCGACGGATCCCCGGAATGGTGCAAAGAGCAGAATCGG
4158 dwn R	GTATCGACTCGCGGGAAACT
0532 up F	ACCTCGTCTCTGGCTGTTTC
FRT 0532 up R	GAAGCAGCTCCAGCCTACACCAGTACTGCGCCTGCTGAAT
FRT 0532 dwn F	GGTCGACGGATCCCCGGAATATGATGGTATTCAGCGAAAACAG
0532 dwn R	TAATCCCGGCCACGACAAAG
0920 up F	GTACGCTGGAAGAATCGGGT
FRT 0920 up R	GAAGCAGCTCCAGCCTACACTTTCCTTGCGCTCAAAAGCC
FRT 0920 dwn F	GGTCGACGGATCCCCGGAATACAGCCGATTTGAACCTGGG
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).

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