SUPPLEMENTARY INFORMATION

Title: The transcription factors ActR and SoxR differentially affect the phenazine tolerance of

Agrobacterium tumefaciens

Authors: Elena K. Perry, Dianne K. Newman

List of contents:

- <u>Table S1</u> PYO-sensitive transposon insertion mutants identified in this study.
- Table S2 Top 15 genes (by fold change) upregulated by PYO in WT.
- <u>Table S3</u> Genes that were expressed at least 2-fold more highly in $\Delta actR$ than in WT only upon PYO treatment.
- <u>Table S4</u> Genes that were statistically significantly upregulated in $\triangle actR$ vs. WT regardless of PYO treatment.
- <u>Table S5</u> Genes that were statistically significantly downregulated in $\triangle actR$ vs. WT regardless of PYO treatment.
- Table S6 -Top six most highly expressed non-ribosomal genes in A. tumefaciens NT1 WT upon
treatment with $100 \mu M$ PYO.
- Figure S1 Supplemental data from growth-based PYO sensitivity assays.
- <u>Figure S2</u> Structures and standard reduction potentials (E_0') of the redox-active molecules tested in this study.
- <u>Figure S3</u> Supplemental gradient plate assays.
- Figure S4 Supplemental qRT-PCR data for SoxR regulon candidates and *cydA*.
- <u>Figure S5</u> Loss of ActR does not significantly alter the NADH/NAD+ ratio or PYO-mediated cyanide-insensitive oxygen consumption rate.
- Figure S6 Inhibition of ATP synthesis does not necessarily correlate with PYO sensitivity.
- <u>Figure S7</u> EPR spectra and DHE oxidation in the presence of PYO.
- <u>Table S7</u> Primers used in this study.
- Table S8 Strains and plasmids used in this study.

Mutant	Transposon Insertion Site	Gene Product
soxR	<i>soxR</i> , bp 6-7	transcriptional regulator
actS	<i>actS</i> , bp 1007-1008	two-component sensor kinase
rpoH	<i>rpoH</i> , bp 87-88	RNA polymerase factor sigma-32
Atu1426	Atu1426, bp 170-171	enolase/phosphoenolpyruvate hydratase
Atu3738	Atu3738, bp 11-12	potassium/proton antiporter
pykA	<i>pykA</i> , bp 1129-1130	pyruvate kinase
Atu2584	Atu2584, bp 548-549	hypothetical protein with high sequence similarity to rhizobial NodB-like proteins that modify cell wall polysaccharides
Atu2590	Atu2590, bp 35-36	putative glycosyltransferase belonging to the MurG superfamily
Atu2591	Atu2591, bp 42-43	putative glycosyltransferase belonging to the RfaB superfamily
Atu2592	Atu2592, bp 878-879	putative glycosyltransferase belonging to the MurG superfamily
Atu2577-1	Atu2577, bp 2288-2289	ABC transporter, nucleotide binding/ATPase protein
Atu2577-2	Atu2577, bp 1010-1011	ABC transporter, nucleotide binding/ATPase protein

Table S1: PYO-sensitive transposon insertion mutants identified in this study.

<u>Table S2:</u> Top 15 genes (by fold change) upregulated by PYO in WT. The fold change is for PYOtreated cultures relative to cultures treated with the solvent control. Base Mean = mean of normalized transcript counts across all replicates and conditions. Bolded genes were previously computationally predicted to be regulated by SoxR on the basis of a SoxR box-containing promoter. Genes that were confirmed to be regulated by SoxR are highlighted in green.

Gene	Base Mean	Log ₂ Fold Change	Adjusted p-value	Product
Atu4582	8108	11.30	2.22E-170	ferredoxin
Atu4581	3644	10.51	1.89E-114	putative flavin reductase
Atu4583 (sodBII)	106513	10.35	0	superoxide dismutase
Atu5305	1548	8.08	9.83E-149	hypothetical protein
Atu2361	10590	7.74	0	MFS permease
Atu5152	932	7.41	1.60E-117	hypothetical protein
Atu4742	4465	7.38	0	hypothetical protein
Atu3915 (soxR)	8621	7.27	0	MerR family transcriptional regulator
Atu0942	29490	7.19	0	MFS permease
Atu4316	2029	4.98	0	ABC transporter permease
Atu2482 (<i>mexE</i>)	34410	4.42	0	AcrB/AcrD/AcrF family protein
Atu2483 (<i>mexF</i>)	19007	4.39	0	HlyD family secretion protein
Atu1475	1784	4.08	0	hypothetical protein
Atu4741	6479	4.06	0	putative transcriptional regulator
Atu4366	343	3.86	3.24E-82	short chain dehydrogenase

<u>**Table S3:**</u> Genes that were expressed at least 2-fold more highly in $\triangle actR$ than in WT only upon **PYO treatment.** The fold change is for $\triangle actR$ relative to WT. Base Mean = mean of normalized transcript counts across all replicates and conditions.

Gene	Base Mean	Log2 Fold Change	Adjusted p-value	Product
Atu3298	542	2.95	9.59E-89	C4-dicarboxylate transporter DctA
Atu4080	1096	2.31	2.21E-104	glutamine amidotransferase
Atu2511	1697	2.17	5.82E-133	D-alanine aminotransferase
Atu4311	90	1.85	6.14E-10	sarcosine oxidase delta subunit
Atu4642	24522	1.60	3.41E-201	catalase
Atu2510	1005	1.54	5.97E-55	aminopeptidase
Atu3180	179	1.51	1.61E-12	ABC transporter permease
Atu2733	132	1.50	1.79E-09	hypothetical protein
Atu3329	2939	1.35	2.48E-79	beta alanine-pyruvate transaminase
Atu4732	324	1.25	2.32E-15	fimbrial chaperone
Atu8166	341	1.21	4.54E-12	hypothetical protein
Atu4073	1211	1.18	6.68E-38	glycogen debranching protein
Atu4709	36	1.16	4.10E-03	NAD-dependent formate dehydrogenase subunit delta
Atu2248	985	1.16	2.90E-30	hypothetical protein
Atu3790	151	1.13	3.70E-07	potassium-transporting ATPase subunit A
Atu3340	250	1.12	2.74E-10	trehalose/maltose ABC transporter permease
Atu3472	87	1.10	1.04E-04	2-oxoisovalerate dehydrogenase subunit beta
Atu0727	2186	1.09	3.92E-45	ferredoxin I
Atu3038	169	1.08	2.87E-07	dipeptide ABC transporter ATPase
Atu0236	373	1.08	8.07E-13	hypothetical protein
Atu0496	363	1.06	6.67E-12	putative universal stress protein
Atu4077	4410	1.05	8.76E-59	glycogen branching protein
Atu4076	1716	1.04	6.31E-38	glucose-1-phosphate adenylyltransferase
Atu4074	4597	1.02	3.87E-56	phosphoglucomutase
Atu3039	358	1.02	1.32E-11	hydantoinase beta subunit-like protein
Atu3037	136	1.01	1.68E-05	dipeptide ABC transporter permease
Atu2477	1029	1.00	1.01E-23	DNA oxidation protective protein

<u>**Table S4:**</u> Genes that were statistically significantly upregulated in $\triangle actR$ vs. WT regardless of PYO treatment. Values listed in the table are for PYO-treated $\triangle actR$ relative to PYO-treated WT. Base Mean = mean of normalized transcript counts across all replicates and conditions. Genes are ordered by decreasing magnitude of fold change between $\triangle actR$ and WT, and are highlighted with colors according to their function. Pink = genes related to the respiratory ETC; green = genes related to amino acid metabolism, including proteases; blue = genes related to sugar transport/metabolism; purple = genes related to fatty acid metabolism; orange = genes related to carbon oxidation, glycolysis, and the TCA cycle.

Gene	Base Mean	Log2 Fold Change	Adjusted p-value	Product
Atu4090	2240	4.63	0.00E+00	ABC transporter permease
Atu4089	1925	3.75	0.00E+00	ABC transporter permease
Atu8036	554	2.92	1.07E-91	protein YBGT-like protein
Atu4092	2595	2.89	5.99E-246	cytochrome d oxidase subunit II
Atu4081	2514	2.07	1.36E-120	aspartate racemase
Atu4314	552	2.05	7.30E-55	serine hydroxymethyltransferase
Atu4091	3678	2.02	1.44E-124	cytochrome d oxidase
Atu4088	837	1.93	4.88E-70	transcriptional regulator
Atu0143	1520	1.92	9.53E-100	MFS permease
Atu4310	380	1.90	4.97E-36	sarcosine oxidase beta subunit
Atu4313	180	1.83	1.29E-17	sarcosine oxidase gamma subunit
Atu4447	306	1.73	5.54E-19	sorbitol ABC transporter substrate-binding protein
Atu4312	1016	1.67	5.02E-62	sarcosine oxidase alpha subunit
Atu2249	567	1.62	8.95E-37	hypothetical protein
Atu3342	695	1.59	2.95E-41	trehalose utilization-like protein
Atu4315	114	1.47	1.95E-08	formyltetrahydrofolate deformylase
Atu3343	1005	1.35	3.64E-39	trehalose utilization-like protein
Atu3341	325	1.34	1.06E-16	trehalose/maltose ABC transporter ATPase
Atu0649	1691	1.33	8.93E-59	cyclopropane-fatty-acyl-phospholipid synthase
Atu4660	582	1.28	1.07E-25	alpha-galactosidase
Atu0543	7394	1.27	1.19E-104	flaB
Atu3338	1077	1.21	2.33E-37	trehalose/maltose ABC transporter substrate-binding protein
Atu0726	4368	1.15	2.40E-75	ring hydroxylating dioxygenase, alpha-subunit
Atu3471	350	1.15	3.85E-13	branched-chain alpha-keto acid dehydrogenase subunit E2
Atu3339	338	1.13	5.35E-13	trehalose/maltose ABC transporter permease
Atu1338	2909	1.10	1.28E-09	3-oxoacyl-(acyl carrier protein) reductase
Atu4078	7728	1.01	4.18E-68	glycogen phosphorylase
Atu4661	277	0.99	4.95E-09	alpha-galactoside ABC transporter substrate-binding protein
Atu2200	11449	0.98	6.88E-63	cold shock protein
Atu8170	9043	0.97	2.35E-60	hypothetical protein

Atu1567	1372	0.95	7.00E-25	glutathione-independent formaldehyde dehydrogenase
Atu1865	3965	0.93	1.17E-28	hypothetical protein
Atu5489	783	0.92	2.16E-13	hypothetical protein
Atu2631	2010	0.91	1.34E-30	hypothetical protein
Atu1632	9825	0.90	1.39E-52	dimethylglycine dehydrogenase
Atu3894	180	0.87	3.03E-05	sugar ABC transporter permease
Atu3506	2431	0.86	1.60E-27	hypothetical protein
Atu4442	496	0.84	9.55E-11	hypothetical protein
Atu0063	2389	0.83	6.24E-25	ABC transporter, substrate binding protein (sugar)
Atu0542	682	0.82	1.81E-12	flagellin
Atu3474	279	0.82	9.90E-07	acyl-CoA dehydrogenase
Atu1174	1835	0.81	6.36E-24	H+ translocating pyrophosphate synthase
Atu1805	4548	0.81	3.79E-36	hypothetical protein
Atu4708	534	0.78	5.77E-10	formate dehydrogenase alpha subunit
Atu4727	756	0.75	2.47E-11	hypothetical protein
Atu5450	5849	0.73	5.59E-34	hypothetical protein
Atu3893	106	0.73	3.34E-03	sugarl ABC transporter permease
Atu5270	77	0.72	9.36E-03	permease component of C4 dicarboxylate transporter
Atu3336	1843	0.72	1.05E-19	hypothetical protein
Atu1864	4280	0.71	1.63E-18	putative homoserine/homoserine lactone efflux protein
Atu1777	6119	0.71	6.67E-30	hypothetical protein
Atu4728	8613	0.71	1.63E-05	ABC transporter permease
Atu5449	13865	0.71	7.79E-39	heat-shock protein
Atu0373	471	0.69	2.46E-07	methyl-accepting chemotaxis protein
Atu3891	286	0.69	2.57E-05	hypothetical protein
Atu0738	1062	0.67	1.41E-10	chemotaxis methyl-accepting protein
Atu4014	16149	0.67	2.87E-32	transcriptional regulator
Atu0591	12637	0.64	1.76E-29	ABC transporter, substrate binding protein (alpha- glucoside)
Atu4061	732	0.63	1.99E-08	exopolysaccharide production repressor protein
Atu3895	170	0.62	2.91E-03	sugar ABC transporter ATPase
Atu1832	926	0.62	2.40E-09	membrane protein associated metalloendopeptidase
Atu0545	4409	0.61	1.79E-21	flagella associated protein
Atu0108	4839	0.60	1.98E-21	putative metalloprotease M20 family
Atu0590	1735	0.60	6.22E-14	transcriptional regulator repressor
Atu0064	549	0.60	1.70E-06	ABC transporter, membrane spanning protein (sugar)
Atu4062	736	0.60	8.80E-08	hypothetical protein
Atu0476	1401	0.59	1.48E-11	aquaporin
Atu0517	1013	0.58	3.63E-09	Chemotaxis protein histidine kinase

Atu2768	1085	0.58	7.83E-09	hypothetical protein
Atu0871	5443	0.57	1.06E-20	branched-chain-amino-acid aminotransferase
Atu0984	1007	0.57	3.09E-08	aminopeptidase N
Atu5052	22384	0.55	9.03E-20	small heat shock protein
Atu1661	1362	0.54	4.06E-09	soluble pyridine nucleotide transhydrogenase
Atu5053	3354	0.53	6.22E-14	hypothetical protein
Atu3185	795	0.51	2.31E-06	glycerol-3-phosphate ABC transporter substrate-binding protein
Atu1392	15199	0.47	6.00E-19	citrate synthase
Atu2617	1342	0.46	1.85E-07	chemotaxis protein
Atu3896	234	0.46	8.13E-03	sugar ABC transporter ATPase
Atu2644	2277	0.46	3.55E-09	succinate dehydrogenase hydrophobic membrane anchor
Atu8094	1653	0.44	8.99E-08	hypothetical protein
Atu2827	819	0.43	1.50E-04	hypothetical protein
Atu1405	617	0.43	3.02E-04	transcriptional regulator, GntR family
Atu0526	2120	0.42	1.58E-07	methyl-accepting chemotaxis protein
Atu1210	1939	0.42	3.05E-07	putative 2,3-cyclic nucleotide 2-phosphodiesterase/3- nucleotidase
Atu3898	763	0.41	3.72E-04	DeoR family transcriptional regulator
Atu0573	1619	0.40	5.35E-05	transcriptional regulator
Atu3596	5621	0.39	3.82E-10	electron transfer flavoprotein alpha subunit
Atu2645	2303	0.36	3.61E-06	succinate dehydrogenase cytochrome B-556 subunit
Atu2643	6452	0.36	1.25E-08	succinate dehydrogenase flavoprotein subunit
Atu3595	3695	0.36	2.03E-07	electron transfer flavoprotein subunit beta
Atu4319	2386	0.35	1.06E-05	AraC family transcriptional regulator
Atu2196	5340	0.35	1.41E-08	aspartate aminotransferase A
Atu3695	4263	0.34	1.35E-07	hypothetical protein
Atu1616	9523	0.34	2.67E-09	fumarate hydratase
Atu0767	4159	0.33	1.20E-06	cytochrome c oxidase subunit II
Atu2239	1761	0.32	3.00E-04	ubiquinol-cytochrome C reductase iron-sulfur subunit
Atu2223	802	0.31	5.77E-03	methyl-accepting chemotaxis protein
Atu2642	3397	0.31	1.61E-05	succinate dehydrogenase iron-sulfur
Atu1717	869	0.29	5.89E-03	long-chain fatty acid transport protein
Atu0594	3253	0.29	2.91E-05	alpha-glucosidase
Atu1870	14872	0.28	2.98E-07	isocitrate dehydrogenase
Atu0768	5078	0.27	1.81E-04	cytochrome-c oxidase chain I
Atu2675	4519	0.26	5.78E-04	hypothetical protein
Atu3706	2778	0.26	1.00E-03	D-3-phosphoglycerate dehydrogenase
Atu2685	14712	0.25	1.77E-05	aconitate hydratase
Atu1426	7399	0.24	1.01E-04	enolase

Atu2238	2861	0.22	3.24E-03	ubiquinol-cytochrome c reductase cytochrome b subunit
Atu1307	1977	0.22	7.56E-03	dihydroorotase
Atu0592	2054	0.21	8.63E-03	ABC transporter, membrane spanning protein
Atu2348	6953	0.21	8.02E-04	sugar binding protein
Atu0977	51857	0.20	1.05E-03	serine protease DO-like protease
Atu2682	17487	0.19	8.74E-04	hypothetical protein

<u>Table S5:</u> Genes that were statistically significantly downregulated in $\triangle actR$ vs. WT regardless of **PYO treatment.** Values listed in the table are for PYO-treated $\triangle actR$ relative to PYO-treated WT. Base Mean = mean of normalized transcript counts across all replicates and conditions. Genes are ordered by decreasing magnitude of fold change between $\triangle actR$ and WT. Genes mentioned in the main text because of their relevance to the respiratory ETC are highlighted in pink.

Gene	Base Mean	Log2 Fold Change	Adjusted p-value	Product
Atu0142	2962	-2.88	1.43E-295	cytochrome o ubiquinol oxidase subunit II
Atu0141	3309	-2.53	8.03E-253	cytochrome O ubiquinol oxidase subunit I
Atu0140	874	-2.46	5.49E-97	cytochrome o ubiquinol oxidase subunit III
Atu0138	89	-2.36	6.03E-13	surfeit 1
Atu0139	645	-2.27	5.64E-71	cytochrome o ubiquinol oxidase subunit IV
Atu4171	1181	-1.70	4.77E-63	cold-shock dead-box protein A
Atu4096	142	-1.43	1.03E-08	nicotinate-nucleotide pyrophosphorylase
Atu1528	731	-1.38	2.85E-27	nitrogen fixation protein FixI
Atu2333	2854	-1.37	9.58E-78	ATP-dependent RNA helicase
Atu1529	238	-1.36	3.00E-13	nitrogen fixation protein FixH
Atu1155	400	-1.32	1.98E-19	hypothetical protein
Atu3122	576	-1.30	3.51E-21	cold shock protein
Atu4097	474	-1.29	1.58E-21	L-aspartate oxidase
Atu1798	155	-1.27	1.99E-08	ankyrin repeat protein
Atu3124	434	-1.25	6.16E-17	hypothetical protein
Atu1221	984	-1.23	3.30E-29	hypothetical protein
Atu1536	268	-1.21	1.36E-11	cytochrome C oxidase, FixO chain
Atu2112	168	-1.20	3.81E-08	soluble lytic transglycosylase
Atu1530	1171	-1.17	2.85E-29	nitrogen fixation protein FixG
Atu5384	640	-1.15	4.16E-21	hypothetical protein
Atu4372	247	-1.08	9.70E-09	ribose ABC transporter permease
Atu1537	1510	-1.04	8.66E-07	cytochrome-c oxidase, FixN chain
Atu5126	740	-1.03	1.10E-19	ABC transporter nucleotide binding/ATPase(putrescine)
Atu3998	220	-1.00	1.62E-07	8-amino-7-oxononanoate synthase
Atu1535	58	-0.99	3.28E-03	cytochrome c oxidase, FixQ chain
Atu4695	595	-0.99	4.94E-14	oligopeptide ABC transporter substrate-binding protein

Atu3771	431	-0.97	1.35E-11	ferrochelatase
Atu0895	7152	-0.95	9.15E-58	ABC transporter, substrate binding protein
Atu1956	22144	-0.94	1.01E-68	DNA-directed RNA polymerase beta chain
Atu2273	651	-0.93	1.92E-15	hypothetical protein
Atu3817	403	-0.92	1.78E-10	dehydratase
Atu2833	4918	-0.92	1.04E-45	transcription termination factor Rho
Atu1601	1118	-0.91	1.03E-05	oxygen-independent coproporphyrinogen III oxidase
Atu5108	287	-0.91	2.25E-07	conjugal transfer coupling protein TraG
Atu2806	489	-0.91	1.09E-11	hypothetical protein
Atu2804	2183	-0.90	3.68E-31	cobaltochelatase subunit
Atu2805	1465	-0.90	9.38E-26	cobalamin synthesis protein
Atu2283	816	-0.88	1.60E-04	pseudoazurin
Atu0885	176	-0.88	1.96E-05	hypothetical protein
Atu1923	14379	-0.87	3.51E-54	DNA-directed RNA polymerase alpha subunit
Atu2803	698	-0.84	2.56E-13	Cobalamin biosynthesis associated protein
Atu2673	2180	-0.83	1.25E-25	hypothetical protein
Atu4609	3074	-0.82	1.15E-31	glycosyltransferase
Atu1771	286	-0.81	1.27E-06	hypothetical protein
Atu5516	1337	-0.81	6.38E-18	hypothetical protein
Atu3191	768	-0.81	2.30E-10	outer membrane protein
Atu5162	590	-0.80	1.34E-08	type IV secretion protein AvhB1
Atu1955	33189	-0.79	1.03E-43	DNA-directed RNA polymerase beta' chain
Atu0663	385	-0.78	2.00E-07	hypothetical protein
Atu3121	8101	-0.77	1.01E-39	cold shock protein
Atu1654	117	-0.77	1.47E-03	nitroreductase
Atu2183	2132	-0.76	9.49E-23	lipopolysaccharide core biosynthesis mannosyltransferase
Atu2801	780	-0.76	9.76E-12	precorrin-2 C20 methyltransferase
Atu2835	1143	-0.76	1.37E-14	uroporphyrinogen decarboxylase
Atu2834	285	-0.76	4.89E-06	hypothetical protein
Atu0107	369	-0.75	1.61E-06	hypothetical protein
Atu1534	460	-0.75	3.76E-07	cytochrome-c oxidase, FixP chain
A tu 2085	5204	0.72	7 95E 22	UDP-3-0-(3-hydroxymyristoyl) N-
Atu2005	3294	-0.75	7.83E-32	ABC transporter membrane spanning protein
Atu5127	414	-0.70	7.90E-07	(mannopine)
Atu0033	2568	-0.69	3.74E-22	two component sensor kinase
Atu2824	935	-0.69	9.89E-12	hypothetical protein
Atu0070	1714	-0.69	4.02E-17	ribonucleoside-diphosphate reductase 2 alpha chain
Atu2394	3017	-0.68	2.43E-03	Regulator of Biofilm formation, Fnr Family
Atu2792	184	-0.68	8.38E-04	hypothetical protein

Atu5110	109	-0.68	5.43E-03	conjugal transfer protein
Atu0983	225	-0.68	2.84E-04	hypothetical protein
Atu3230	375	-0.67	8.05E-06	hypothetical protein
Atu0769	848	-0.67	2.03E-10	protoheme IX farnesyltransferase
Atu5161	800	-0.66	1.14E-07	hypothetical protein
Atu2073	1393	-0.66	1.39E-13	membrane protein
Atu0365	744	-0.66	3.21E-09	hypothetical protein
Atu2710	1129	-0.65	7.82E-11	hypothetical protein
Atu4667	534	-0.64	4.78E-07	ABC transporter permease
Atu0250	344	-0.64	2.61E-05	hypothetical protein
Atu4608	4774	-0.64	3.10E-24	hypothetical protein
Atu4098	778	-0.64	1.90E-08	quinolinate synthetase
Atu2026	1377	-0.64	1.39E-12	exodeoxyribonuclease V
Atu8135	2663	-0.64	5.43E-16	hypothetical protein
Atu2800	556	-0.63	4.80E-07	precorrin-3B C17-methyltransferase
Atu2471	136	-0.61	6.53E-03	hypothetical protein
Atu0106	11129	-0.60	2.10E-23	cold shock protein
Atu0345	1829	-0.60	8.82E-14	DNA mismatch repair protein, MutS family
Atu0034	4246	-0.60	5.26E-21	two component response regulator
Atu0071	839	-0.59	2.42E-08	ribonucleoside-diphosphate reductase 2 beta chain
Atu2287	144	-0.59	6.48E-03	outer membrane heme receptor
Atu3203	2501	-0.58	4.53E-15	RND multidrug efflux membrane permease
Atu5130	355	-0.58	9.65E-05	attachment protein
Atu0012	7125	-0.58	1.20E-21	DNA gyrase subunit B
Atu0032	266	-0.58	6.08E-04	hypothetical protein
Atu2730	7718	-0.56	3.48E-21	beta (1>2) glucan biosynthesis protein
Atu5129	1722	-0.56	5.08E-12	ABC transporter substrate binding protein (mannopine)
Atu5001	1437	-0.56	1.90E-10	replication protein B
Atu4666	677	-0.56	2.11E-05	HlyD family secretion protein
Atu5128	444	-0.55	8.22E-05	ABC transporter membrane spanning protein (mannopine)
Atu1447	3047	-0.55	7.51E-15	two component sensor kinase
Atu3229	259	-0.54	1.86E-03	hypothetical protein
Atu1658	1096	-0.53	5.86E-08	conserved protein involved in phosphoglycerol modification of cyclic glucan
Atu0973	1235	-0.53	6.64E-09	cycH protein
Atu2303	694	-0.53	3.47E-06	hypothetical protein
Atu2494	443	-0.52	1.39E-04	NAD(P)+ transhydrogenase beta chain
Atu1445	863	-0.51	1.25E-06	two component sensor kinase
Atu2129	380	-0.51	4.90E-04	hypothetical protein

Atu3704	2031	-0.51	1 03E-10	hypothetical protein
Atu0721	2154	-0.50	1.05E-10	hypothetical protein
Atu3202	3935	-0.49	5.04E-14	RND multidrug efflux transporter
Atu4491	1679	-0.49	3 73E-09	hypothetical protein
Atu2380	583	-0.49	6 13E-05	hypothetical protein
Atu4619	1063	-0.49	3.67E-07	ATP-dependent DNA helicase
Atu2320	487	-0.49	2.30E-04	transcriptional regulator. TetR family
Atu5469	562	-0.48	1.03E-04	DNA polymerase IV
Atu3325	756	-0.48	1.69E-05	exopolysaccharide production protein
Atu2057	2428	-0.48	9.28E-11	DNA helicase II
Atu5000	1652	-0.48	6.13E-08	replication protein A
Atu1262	16332	-0.47	7.08E-14	histone-like protein
Atu8200	3772	-0.47	1.07E-10	hypothetical protein
Atu1074	606	-0.47	1.33E-04	NAD/NADP dependent oxidoreductase
Atu5170	283	-0.47	7.31E-03	type IV secretion protein AvhB9
Atu0900	620	-0.46	1.05E-04	hypothetical protein
Atu5165	1769	-0.46	5.78E-06	type IV secretion protein AvhB4
Atu1131	43210	-0.46	3.23E-15	outer membrane protein
Atu1683	1453	-0.45	2.26E-07	hypothetical protein
Atu0933	729	-0.45	9.93E-05	beta-lactamase class D
Atu1877	4170	-0.45	4.18E-10	OmpA family protein
Atu1242	1157	-0.44	6.58E-06	cytochrome oxidase assembly factor
Atu2692	2234	-0.44	1.19E-07	intracellular septation protein
Atu2728	4808	-0.43	1.78E-09	beta (1>2) glucan export ATP-binding protein
Atu1602	891	-0.43	9.86E-05	transcriptional activator, Crp family
Atu3396	4110	-0.43	1.31E-09	ABC transporter substrate-binding protein
Atu1825	1610	-0.43	5.53E-06	cysteine desulfurase
Atu1467	436	-0.42	2.00E-03	hypothetical protein
Atu8142	743	-0.42	4.95E-04	hypothetical protein
Atu4026	14058	-0.42	5.49E-13	hypothetical protein
Atu5167	485	-0.42	4.41E-03	type IV secretion protein AvhB6
Atu2163	6391	-0.41	7.59E-12	hypothetical protein
Atu2521	1143	-0.41	2.85E-05	Protein regulated by acid pH
Atu5122	806	-0.41	1.92E-04	3-ketoacyl-ACP reductase
Atu2463	740	-0.41	2.50E-04	hypothetical protein
Atu2181	1052	-0.41	2.85E-05	hypothetical protein
Atu1077	1260	-0.39	3.73E-05	DNA repair protein
Atu3326	1373	-0.39	5.38E-05	exopolysaccharide production protein
Atu0898	602	-0.39	1.72E-03	hypothetical protein

Atu3295	803	-0.39	5.08E-04	hypothetical protein
Atu2653	450	-0.39	5.00E-03	Uroporphyrinogen III synthase HEM4
Atu1874	11481	-0.38	5.09E-09	RecA protein
Atu3507	2784	-0.38	3.23E-07	hypothetical protein
Atu2779	1792	-0.36	8.78E-06	gamma-glutamyl phosphate reductase
Atu2652	1401	-0.36	9.33E-05	porphobilinogen deaminase
Atu5124	1920	-0.35	1.17E-05	glutamate-1-semialdehyde aminotransferase
Atu0720	2876	-0.35	5.42E-06	ribonuclease HII
Atu2168	2744	-0.35	1.69E-05	DNA primase
Atu2522	696	-0.34	3.11E-03	agrobacterium chromosomal virulence protein B
Atu2780	1666	-0.34	7.80E-05	gamma-glutamyl kinase
Atu4499	683	-0.33	3.99E-03	hypothetical protein
Atu2001	7332	-0.33	3.74E-07	excinuclease ABC subunit B
Atu0974	639	-0.32	7.84E-03	cytochrome-c biosynthesis heme-carrier protein cycJ
Atu1780	1051	-0.31	1.66E-03	ATP-dependent DNA helicase
Atu1032	905	-0.31	5.48E-03	hypothetical protein
Atu0975	1765	-0.30	4.18E-04	cytochrome c-type synthesis protein
Atu1395	2869	-0.30	6.47E-05	LexA repressor
Atu0998	1891	-0.30	5.05E-04	undecaprenyl pyrophosphate phosphatase, possible bacitracin resistance protein
Atu4498	1078	-0.29	3.11E-03	hypothetical protein
Atu4178	2418	-0.29	2.60E-04	hypothetical protein
Atu5123	2497	-0.28	2.42E-04	acetolactate synthase catalytic subunit
Atu1433	4200	-0.28	7.83E-05	arylesterase
Atu1300	1132	-0.26	7.31E-03	MFS permease
Atu1446	1385	-0.26	3.78E-03	two component response regulator
Atu1028	6244	-0.24	1.13E-04	hypothetical protein
Atu1715	2517	-0.20	9.33E-03	exopolysaccharide production negative regulator

<u>Table S6:</u> Top six most highly expressed non-ribosomal genes in *A. tumefaciens* NT1 WT upon treatment with 100 μ M PYO. Expression values were calculated in a manner similar to RPKM (reads per kilobase per million mapped reads) values except that they were normalized to the upper quartile of gene expression instead of total mapped reads (Tjaden, 2015).

Gene	Product	Expression
rnpB	RNase P RNA	53179
sodBII	superoxide dismutase	25726
hspL	small heat shock protein	15130
Atu1020	outer membrane protein	14239
groEL	GroEL chaperonin	13833
groES	co-chaperonin GroES	12884

Fig. S1: Supplemental data from growth-based PYO sensitivity assays. A) Growth of WT and PYOsensitive transposon mutants after 24 hrs without PYO treatment. **B)** Growth of $\Delta actS$ and $\Delta actR$ after 24 hrs in the presence of different concentrations of PYO. **C)** Growth of WT pLacZ (vector control for overexpression constructs), $\Delta actR$ pLacZ, $\Delta actR$ pActR (overexpression vector for ActR), $\Delta soxR$ pLacZ, and $\Delta soxR$ pSoxR (overexpression vector for SoxR) after 24 hrs in the presence of different concentration of the $\Delta actR$ and $\Delta soxR$ clean deletion mutants. Expression from the vectors was induced by adding 1 mM IPTG at the start of the experiment. **D)** Growth of WT, $\Delta sodBII$, Δcyo , and $\Delta cyo/\Delta sodBII$ after 24 hrs in the presence of different concentrations of PYO, showing that Δcyo is slightly more dependent on SodBII than WT (i.e. it does not gain as large of a growth advantage upon loss of SodBII). Error bars in all panels represent standard deviations of biological replicates (n = 3).



Fig. S2: Structures and standard reduction potentials (E_0) of the redox-active molecules tested in this study. Reduction potentials are versus that of the normal hydrogen electrode (NHE).



<u>Fig. S3:</u> Supplemental gradient plate assays. Growth of WT, $\Delta actR$, and $\Delta soxR$ on agar plates containing either plain LB or a concentration gradient (low-high, left to right) of EDTA (up to 1 mM), myo-inositol (up to 1 M), or NaCl (up to 0.4 M). Images are representative of eight biological replicates.



Fig. S4: Supplemental qRT-PCR data for SoxR regulon candidates and *cydA*. A) Relative induction by 100 μ M PYO of Atu1475, Atu4316, and Atu4895 in WT and $\Delta soxR$, showing that these three genes are not regulated by SoxR. B) Relative expression of *cydA* in WT, $\Delta actR$, and Δcyo without PYO treatment. Expression was normalized to the housekeeping gene *rpoD*.



Fig. S5: Loss of ActR does not significantly alter the NADH/NAD+ ratio or PYO-mediated cyanideinsensitive oxygen consumption rate. A) NADH and NAD+ were extracted and quantified from exponential phase cultures of WT and $\Delta actR$. The slight increase in the mean NADH/NAD+ ratio in $\Delta actR$ is not statistically significant (p > 0.05, Welch's t-test, n = 4). B) Exponential phase cultures of WT or $\Delta actR$ in LB were treated with 5 mM potassium cyanide for 5 min to inhibit terminal oxidase activity; this concentration was sufficient to fully inhibit respiration. After 5 min, 10 μ M PYO was added, and after another 5 min, an additional 90 μ M PYO was added. The concentration of oxygen over time is plotted such that t = 0 is approximately the time of the second PYO addition. The concentration of oxygen. Each sample was fitted with a linear regression. The positive slope of the blank reference represents the rate of oxygen leakage into the system. The slope for the culture samples is a function of the leak rate minus the consumption rate. Hence, PYO-mediated oxygen consumption resulted in shallower slopes for the culture samples compared to the blank sample, although their rates of oxygen consumption were slower than the rate of oxygen leakage back into the system (resulting in a net positive slope). Two biological replicates are plotted for each strain.



Fig. S6: Inhibition of ATP synthesis does not necessarily correlate with PYO sensitivity. Data in all panels is from the same experiment (n = 3). A) Growth of WT and $\Delta actR$ after 5 hrs of treatment with +/-100 μ M DCCD combined with +/-10 μ M PYO. The difference between the OD₅₀₀ at 5 hrs and initial OD₅₀₀ was taken as a proxy for growth. B) ATP levels after 5 hrs of treatment. C) Percent growth of each strain in 10 μ M PYO relative to its growth in 0 μ M PYO, with or without 100 μ M DCCD, after 5 hrs. * p < 0.05, ** p < 0.01, *** p < 0.001 (in B, Welch's *t*-test followed by the Benjamini-Hochberg procedure to control the false discovery rate; in C, linear regression with custom contrast matrix to specify comparisons between 0 μ M and 100 μ M DCCD conditions). Legend on the right applies to all panels.



Fig. S7: EPR spectra and DHE oxidation in the presence of PYO. A) EPR spectra of 1) a cell-free sample containing 100 μ M PYO + 0.5% glucose (light gray); 2) WT + 100 μ M PYO + 0.5% glucose + exogenous superoxide dismutase (SOD) (100 units) (dark gray); 3) $\Delta actR$ + 100 μ M PYO + 0.5% glucose (pink); 4) WT + 100 μ M PYO + 0.5% glucose (black). G = Gauss. EPR signal intensity is directly proportional to the quantity of radicals in a sample (Eaton *et al.*, 2010). All spectra were collected using 25 mM BMPO as the spin trap for superoxide and hydroxyl radical. The cell-free and SOD control spectra are single scans while the other two spectra are averages of six consecutive scans and are representative of at least three biological replicates. The complete loss of signal upon addition of cell-impermeable SOD indicates that the signal from PYO-treated cell suspensions represents only extracellular superoxide. **B)** Fluorescence (510ex/580em) of 15 μ M dihydroethidium (DHE) after 1 hr of incubation with 0 μ M or 10 μ M PYO in cell-free phosphate buffer. Increased fluorescence in the PYO-treated sample indicates abiotic DHE oxidation, suggesting that DHE oxidation would not be a reliable reporter for intracellular superoxide. RFU = relative fluorescence units.



<u>**Table S7:**</u> **Primers used in this study.** Primers with "us" or "ds" were used to amplify the upstream or downstream flanking regions of the given gene, respectively, which were subsequently joined by overlap extension PCR and cloned into pNPTS138.

Primer	Sequence	Cut site
SS9arb1	GACCACGAGACGCCACACTNNNNNNNNNNCATGC	
Mar4	TAGGGTTGAGTGTTGTTCCAGTT	
arb3	GACCACGAGACGCCACACT	
Mar4-2	TCACCGTCATGGTCTTTGTAGTC	
soxR-us-F	GTAAAAATT-GAATTC-CACCGAAGAGCTGACCATCAAG	EcoRI
soxR-us-R	GAATAAGGGGCGGCTCAGCCGC-TCTGGCTCCTGCTTCCG	
soxR-ds-F	CGGAAGCAGGAGCCAGA-GCGGCTGAGCCGCCCCTTATTC	
soxR-ds-R	GTAAAAATT-ACTAGT-CCGACCGAAGACGGCATATTCCG	SpeI
actS-us-F	CTTGGTGCCATCATTCTCGAC	EcoRI (downstream)
actS-us-R	CGCTTCAACGTGGATGGTAAAG-CGTCTTCTCCTTTACCCTTTTC	
actS-ds-F	GAAAAGGGTAAAGGAGAAGACG-CTTTACCATCCACGTTGAAGCG	
actS-ds-R	GTAAAAATT-ACTAGT-TGCGCCGATGCATGTTG	SpeI
actR-us-F	GTAAAAATT- <u>GGATCC</u> -GCGTCGTCGCCAAGGAAATG	BamHI
actR-usR	CGCACTTTTACTGGAAATGCT-GTTTCCGGCCTTCCGGCAAC	
actR-ds-F	GTTGCCGGAAGGCCGGAAAC-AGCATTTCCAGTAAAAGTGCG	
actR-ds-R	GTAAAAATT-ACTAGT-CGCCAAATTGTGCGAAGGAG	SpeI
sodBI-us-F	GTAAAAATT- <u>GGATCC</u> -AATGGCTGAATACGGTGC	BamHI
sodBI-us-R	GAAAAATTCCGTTCGCCAAGCC-GGTTTTTACTCCTTTTTGCAGC	
sodBI-ds-F	GGCTTGGCGAACGGAATTTTTC	
sodBI-ds-R	GTAAAAATT-ACTAGT-GAATTGCTTGTCACTGAGTG	SpeI
sodBII-us-F	GTAAAAATT- <u>GGATCC</u> -TCGAAACCGTGCTTCTGCGC	BamHI
sodBII-us-R	GGGTCGGTGGGGCTCAAA-GGGGTATTCCTTTGTCAACTGTT	
sodBII-ds-F	AACAGTTGACAAAGGAATACCCC-TTTGAGCCCCACCGACCC	
sodBII-ds-R	GTAAAAATT-ACTAGT-ACGCGGCGAGATCGTCGG	SpeI
Atu0942-us-F	GTAAAAATT-GAATTC-CAAGGACGAACATCGCTGTG	EcoRI
Atu0942-us-R	GGTTCACTTTTTCTGGAAATGCT-GCGGGCTTTTTTATCCGATG	
Atu0942-ds-F	AGCATTTCCAGAAAAAGTGAACC	
Atu0942-ds-R	GTAAAAATT-ACTAGT-GATCGAACTTGCCTCGTCAG	SpeI
Atu2361-us-F	GTAAAAATT- <u>GGATCC</u> -CTACGAGCCGCAGAATTTCG	BamHI
Atu2361-us-R	CCCACCCCACGCTGCGCC-GGAACGTCCTTCGATAGTGT	
Atu2361-ds-F	ACACTATCGAAGGACGTTCC-GGCGCAGCGTGGGGTGGG	
Atu2361-ds-R	GTAAAAATT-ACTAGT-GGAATAGAGTGCATCGGATTCC	SpeI
mexF-us-F	GTAAAAATT-GAATTC-TCAACCCGATCTATGCAAGC	EcoRI
mexF-us-R	GCGGTGGATGGGGTGCGGCG-ATCCAGTCCCTCTCCGGG	

mexF-ds-F	CGCCGCACCCCATCCACCGC	
mexF-ds-R	GTAAAAATT- <u>ACTAGT</u> -GTTTTCCGTCCAACCTGCC	SpeI
Atu5152-us-F	GTAAAAATT- <u>GGATCC</u> -AGCCATTGGGATTGATGTCG	BamHI
Atu5152-us-R	CCACGTCAAAGGAGGGCGTC-ATTCCCGACCGCACTTGGTG	
Atu5152-ds-F	CACCAAGTGCGGTCGGGAAT-GACGCCCTCCTTTGACGTGG	
Atu5152-ds-R	GTAAAAATT- <u>ACTAGT</u> -CGTAGTGGTCATTGAGCTTGG	SpeI
Atu5305-us-F	TGCAGACCAATGTC <u>GAATTC</u>	EcoRI
Atu5305-us-R	GCATCCAACGGATCCACAT-GAGCCGCGACCGTCTTGC	
Atu5305-ds-F	ATGTGGATCCGTTGGATGC	
Atu5305-ds-R	GTAAAAATT- <u>ACTAGT</u> -GTAACGGTTGCGGCACATAG	SpeI
Atu4581-us-F	GTAAAAATT- <u>GGATCC</u> -CACGGAAAACGGCGTCAAC	BamHI
Atu4581-us-R	GGGAGAGAAAATCCCTAGTTGAG-ACCGCCATCGATCAGCCTG	
Atu4581-ds-F	CTCAACTAGGGATTTTCTCTCCC	
Atu4581-ds-R	GTAAAAATT- <u>ACTAGT</u> -CCCGCGCCACAATATTTTC	SpeI
Atu4582-us-F	GTAAAAATT- <u>GGATCC</u> -TGTCCGAGGAGACGCTGAAG	BamHI
Atu4582-us-R	ATGAGACATGCAGTGCCGGG-GGGGATGCTCAAATGGAGTTG	
Atu4582-ds-F	CCCGGCACTGCATGTCTCAT	
Atu4582-ds-R	GTAAAAATT- <u>ACTAGT</u> -GCCGCCTTTCTAAATGTCCG	SpeI
Atu4741-us-F	GTAAAAATT- <u>GGATCC</u> -GAACTGCTGGAAGAAAACCG	BamHI
Atu4741-us-R	CTGTGGCATATGCCTCACCT-AAACCACCGGCTCTAGACAG	
Atu4741-ds-F	AGGTGAGGCATATGCCACAG	
Atu4741-ds-R	GTAAAAATT- <u>ACTAGT</u> -CACAGGCATAAACGGCAACG	SpeI
Atu4742-us-F	GTAAAAATT- <u>GAATTC</u> -ATTTCCAACAACAGGCACGG	EcoRI
Atu4742-us-R	CGACTGAACTTCCGCTGCCA-CGTTATTCCGTTGCAATGG	
Atu4742-ds-F	TGGCAGCGGAAGTTCAGTCG	
Atu4742-ds-R	GTAAAAATT- <u>ACTAGT</u> -CATGTTCTGGCTCGTCTTCAG	SpeI
CyoABCD-us-F	GTAAAAATT- <u>GGATCC</u> -CGATCTGGAGGAAGATCAGC	BamHI
CyoABCD-us-R	GCCGCTGATTTATCGCTGC-GACGACCAGATTGCAGCCGC	
CyoABCD-ds-F	GCAGCGATAAATCAGCGGC	
CyoABCD-ds-R	GTAAAAATT- <u>ACTAGT</u> -GGTTCTAAGGAAAAGCCCACC	SpeI
pSRK-actR-F	GTAAAAATT- <u>CAT-ATG</u> AAGATTGAAGACCAGACCC	NdeI
pSRK-actR-R	GTAAAAATT- <u>GCTAGC</u> -TCACTTCGGAGCGCGTTTC	NheI
pSRK-soxR-F	GTAAAAATT- <u>CAT-ATG</u> GAAAATACCATCTTCAAACAC	NdeI
pSRK-soxR-R	GTAAAAATT- <u>GCTAGC</u> -TTATTCAGCGGAGACGAGG	NheI
Cyo-pSRK-Gib-F	GAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCATATGAACCCTT CGGGCGAC	Used with NdeI/NheI- cut pSRKKm
surf1-pSRK-Gib-R	CCTGAACCGACGACCGGGTCGAATTTGCTTTCGAATTGTCAATCAGCATCC CGTTTCG	Used with NdeI/NheI- cut pSRKKm
pSRK-Cyd-F	GTAAAAATT- <u>CAT-ATG</u> CTGCCGGCGCTGCTCTG	NdeI

pSRK-Atu8036-R	GTAAAAATT- <u>GCTAGC</u> -TCATTTTTCCGGCTTCGCTTCG	NheI
soxR-qpcr-F	CTGTCGGTGGGGGATGTTG	
soxR-qpcr-R	GATGATAACGACGCTGGTTGC	
sodBII-qpcr-F	CTTCTGGGAGATCATGGGGC	
sodBII-qpcr-R	CGAGGGCGAAATTCTGCTTG	
Atu0942-qpcr-F	GCGGGTTTTTATAGCTGGGC	
Atu0942-qpcr-R	AGATGAGGCAGCACAGAAGC	
Atu2361-qpcr-F	TTTTACACCATCCCCTCGCA	
Atu2361-qpcr-R	CTGCCAGATTGAAGATGCCG	
mexE-qpcr-F	CGCTGGTGAAGGAAGGTGAC	
mexE-qpcr-R	CACGGTCGAGTTCCGTCTTT	
mexF-qpcr-F	ACCGACGCCTATGACATCAC	
mexF-qpcr-R	TAATCACCTGCGCCGAAGAC	
Atu5152-qpcr-F	GGTTGGAGCCGTATTGTTGC	
Atu5152-qpcr-R	CCACAGAATGAAACCCAGCG	
Atu5305-qpcr-F	CCATAGCGAGCCCAAATCCA	
Atu5305-qpcr-R	AGTCACCTCAGTCTCGTTGC	
Atu4741-qpcr-F	GAAGCCACGGAAAATCGTCG	
Atu4741-qpcr-R	CATCTCACCGTCCCCTGAAC	
Atu4742-qpcr-F	CCATCAGGCAGAATCCCGAC	
Atu4742-qpcr-R	CACTGCCACGCCTTCTATCC	
CyoA-qpcr-F	GAGATGCCGTCGTAGACACC	
CyoA-qpcr-R	AACTCCTTCTTCGTTCCCGC	
CydA-qpcr-F	CGAAGCAATGTGGGAAACCG	
CydA-qpcr-R	TTTGCGTATCAAGCGAACGG	
Atu4316-qpcr-F	TGGCATAAACGCCGATGACT	
Atu4316-qpcr-R	CCAGATCATCACGCTCTCCC	
Atu1475-qpcr-F	AACGCCTGAACCCGATTTTG	
Atu1475-qpcr-R	GTCCGTATGGAACGTCGTGA	
Atu4895-qpcr-F	GGGCTCACTGCAAAACCAAC	
Atu4895-qpcr-R	TCCCAAATGCCAAGAGCGAC	

the NT1 parent strain. Strain/Plasmid Notes **Reference/Source** E. coli DH10b Cloning strain Invitrogen (Dehio and Meyer, ∆*dapA*::*erm* (Erm^r) *pir*::RP4 [::*kan* (Km^r) from SM10] β2155 1997) A. tumefaciens non-pathogenic derivative of strain C58; carries pDCI41E33 (Shaw et al., 1997; NT1 (plasmid with *traG*::*lacZ* fusion) Watson et al., 1975) soxR mariner transposon insertion in soxR This study This study actS mariner transposon insertion in actS rpoH *mariner* transposon insertion in *rpoH* This study Atu1426 mariner transposon insertion in Atu1426 This study Atu3738 mariner transposon insertion in Atu3738 This study *pykA* mariner transposon insertion in pykA This study

mariner transposon insertion in Atu2584

mariner transposon insertion in Atu2590

mariner transposon insertion in Atu2591

mariner transposon insertion in Atu2592

mariner transposon insertion in Atu2577

mariner transposon insertion in Atu2577

clean deletion of *soxR* coding sequence

clean deletion of *actS* coding sequence

clean deletion of *actR* coding sequence

clean deletion of *sodBI* coding sequence

clean deletion of sodBII coding sequence

clean deletion of Atu0942 coding sequence

clean deletion of Atu2361 coding sequence

clean deletion of Atu5152 coding sequence

clean deletion of Atu5305 coding sequence

clean deletion of Atu4581 coding sequence

clean deletion of Atu4582 coding sequence

clean deletion of Atu4741 coding sequence

clean deletion of Atu4742 coding sequence

clean deletion of cytochrome o oxidase biosynthesis operon

clean deletion of *mexF* coding sequence

clean deletion of *actR* and *soxR* coding sequences

clean deletion of sodBI and sodBII coding sequences

clean deletion of *soxR* and *sodBI* coding sequences

Atu2584

Atu2590

Atu2591

Atu2592

Atu2577-1

Atu2577-2

 $\Delta soxR$

 $\Delta actS$

 $\Delta actR$

 $\Delta sodBI$

 $\Delta sodBII$

 $\Delta actR/\Delta soxR$

 $\Delta sodBI / \Delta sodBII$

 $\Lambda sox R / \Lambda sod BI$

 $\Delta Atu0942$

ΔAtu2361

 $\Delta Atu5152$

ΔAtu5305

 Δ Atu4581

 Δ Atu4582

∆Atu4741

 $\Delta Atu4742$

 Δcvo

 $\Delta mexF$

Table S8: Strains and plasmids used in this study. All A. tumefaciens mutants were constructed from

Plasmid				
pSC189	R6K suicide plasmid carrying <i>mariner</i> transposon with hyperactive transposase; Ap ^R Km ^R	(Chiang and Rubin, 2002)		
pNPTS138	ColE1 suicide plasmid; <i>sacB</i> ; Km ^R	Constructed by M.R.K. Alley; gift of C. Fuqua		
pNPTS138::soxRdel	pNPTS138 carrying flanking regions of <i>soxR</i> for clean deletion	This study		
pNPTS138::actSdel	pNPTS138 carrying flanking regions of <i>actS</i> for clean deletion	This study		
pNPTS138::actRdel	pNPTS138 carrying flanking regions of <i>actR</i> for clean deletion	This study		
pNPTS138::sodBIdel	pNPTS138 carrying flanking regions of <i>sodBI</i> for clean deletion	This study		
pNPTS138::sodBIIdel	pNPTS138 carrying flanking regions of <i>sodBII</i> for clean deletion	This study		
pNPTS138::Atu0942del	pNPTS138 carrying flanking regions of Atu0942 for clean deletion	This study		
pNPTS138::Atu2361del	pNPTS138 carrying flanking regions of Atu2361 for clean deletion	This study		
pNPTS138::mexFdel	pNPTS138 carrying flanking regions of <i>mexF</i> for clean deletion	This study		
pNPTS138::Atu5152del	pNPTS138 carrying flanking regions of Atu5152 for clean deletion	This study		
pNPTS138::Atu5305del	pNPTS138 carrying flanking regions of Atu5305 for clean deletion	This study		
pNPTS138::Atu4581del	pNPTS138 carrying flanking regions of Atu4581 for clean deletion	This study		
pNPTS138::Atu4582del	pNPTS138 carrying flanking regions of Atu4582 for clean deletion	This study		
pNPTS138::Atu4741del	pNPTS138 carrying flanking regions of Atu4741 for clean deletion	This study		
pNPTS138::Atu4742del	pNPTS138 carrying flanking regions of Atu4742 for clean deletion	This study		
pNPTS138::cyoABCDdel	pNPTS138 carrying flanking regions of cyoABCD for clean deletion	This study		
pLacZ	alias for pSRKKm; broad host range P _{lac} vector; lacI ^Q ; Km ^R	(Khan et al., 2008)		
pActR	pSRKKm carrying P _{lac} -actR	This study		
pSoxR	pSRKKm carrying P _{lac} -soxR	This study		
рСуо	pSRKKm carrying P _{lac} -cyoABCD-surf1 (surf1 is co-transcribed with the other cyo genes)	This study		
pCyd	pSRKKm carrying P_{lac} -cydDCAB-Atu8036 (Atu8036 is co- transcribed with the other cyd genes)	This study		

Supplemental References

- Chiang, S. L., and Rubin, E. J. (2002). Construction of a *mariner*-based transposon for epitope-tagging and genomic targeting. *Gene*, 296, 179–185. https://doi.org/10.1016/S0378-1119(02)00856-9
- Dehio, C., and Meyer, M. (1997). Maintenance of broad-host-range incompatibility group P and group Q plasmids and transposition of Tn5 in *Bartonella henselae* following conjugal plasmid transfer from *Escherichia coli*. Journal of Bacteriology, 179(2), 538–540.
- Eaton, G. R., Eaton, S. S., Barr, D. P., and Weber, R. T. (2010). Quantitative EPR. New York: SpringerWienNewYork.
- Khan, S. R., Gaines, J., Roop, R. M., and Farrand, S. K. (2008). Broad-host-range expression vectors with tightly regulated promoters and their use to examine the influence of TraR and TraM expression on Ti plasmid quorum sensing. *Applied and Environmental Microbiology*, 74(16), 5053–5062. https://doi.org/10.1128/AEM.01098-08
- Shaw, P. D., Ping, G., Daly, S. L., Cha, C., Cronan Jr., J. E., Rinehart, K. L., and Farrand, S. K. (1997). Detecting and characterizing *N*-acyl-homoserine lactone signal molecules by thin-layer chromatography. *Proceedings of the National Academy of Sciences*, 94, 6036–6041. https://doi.org/10.1073/PNAS.94.12.6036
- Tjaden, B. (2015). De novo assembly of bacterial transcriptomes from RNA-seq data. *Genome Biology*, 16. https://doi.org/10.1186/s13059-014-0572-2
- Watson, B., Currier, T. C., Gordon, M. P., Chilton, M.-D., and Nester, E. W. (1975). Plasmid required for virulence of *Agrobacterium tumefaciens. Journal of Bacteriology*, 123(1), 255–264.