Transcriptomic profiling suggests that Promysalin alters metabolic flux, motility, and iron regulation in *Pseudomonas putida* KT2440

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Supporting Information

Table S1. All enriched proteins from AfBPP experiment. (Yellow highlighted shown in text)

Locus	Gene	Protein	-Log t-test p-value	t-test Difference
PP_0133	*algB*	Alginate biosynthesis transcriptional regulatory protein AlgB	1.17	1.81
PP_0310	*dgcA*	Putative dimethylglycine dehydrogenase subunit (EC 1.5.8.-)	2.85	3.78
PP_0325	*soxA*	Sarcosine oxidase subunit alpha (EC 1.5.3.1)	3.86	3.04
PP_0328	*fdhA*	Formaldehyde dehydrogenase (EC 1.2.1.46)	2.08	4.67
PP_0395	*ycgB*	Putative type IV piliation protein	2.76	1.74
PP_0397	*yeaG*	Protein kinase (EC 2.7.11.1)	2.57	1.97
PP_0412	PP_0412	Polyamine ABC transporter, periplasmic polyamine-binding protein	2.49	1.63
PP_0614	PP_0614	Putative N-carbamoyl-beta-alanine amidohydrolase/allantoine amidohydrolase 1 (EC 3.5.1.6) (EC 3.5.3.9)	1.26	4.13
PP_0658	PP_0658	Homocysteine S-methyltransferase family protein	1.06	1.16
PP_0743	*yfcH*	Conserved protein with NAD(P)-binding Rossmann-fold domain	1.32	2.44
PP_0766	*PP_0766*	Uncharacterized protein	2.03	2.02
PP_0901	*colR*	DNA-binding response regulator	1.05	4.08
PP_0940	*tldD*	Putative protease involved in Microcin B17 maturation and in sensitivity to the DNA gyrase inhibitor LetD	2.47	1.05
PP_1141	*livK*	Branched-chain amino acids ABC transporter-periplasmic leucine binding subunit	3.08	1.65
PP_1291	PP_1291	PhoH family protein	2.46	2.27
PP_1478	PP_1478	Putative Xenobiotic reductase	5.55	3.09
PP_1502	PP_1502	OmpA family protein	1.83	2.09
PP_1743	*actP-I*	Acetate permease	2.95	3.78
PP_2112	*acnA-I*	Aconitate hydratase (Aconitase) (EC 4.2.1.3)	2.63	1.29
PP_2215	PP_2215	Acetyl-CoA acetyltransferase (EC 2.3.1.9)	1.41	1.57
PP_2335	*prpC*	Citrate synthase	2.07	3.50
PP_2364	PP_2364	Uncharacterized protein	2.06	1.16
PP_3668	*katG*	Catalase-peroxidase (CP) (EC 1.11.1.21) (Peroxidase/catalase)	2.89	1.22
PP_4011	*icd*	Isocitrate dehydrogenase [NADP] (EC 1.1.1.42)	1.66	2.32
PP_4034	*hyuC*	N-carbamoyl-beta-alanine amidohydrolase/allantoine amidohydrolase 2 (EC 3.5.1.6) (EC 3.5.3.9)	5.27	2.53
PP_4036	*pydB*	D-hydantoinase/dihydropyrimidinase (EC 3.5.2.2)	2.50	2.68
PP_4038	*pydA*	NADP-dependent dihydropyrimidine dehydrogenase subunit PreA (EC 1.3.1.2)	2.66	1.65
PP_4064	*ivd*	Isovaleryl-CoA dehydrogenase (EC 1.3.8.4)	1.62	1.85
PP_4065	*mccB*	Methylcrotonyl-CoA carboxylase biotin-containing subunit beta (EC 6.4.1.4)	1.31	1.65
PP_4067	*mccA*	Methylcrotonyl-CoA carboxylase biotin-containing subunit alpha (EC 6.4.1.4)	1.25	1.82
PP_4116	*aceA*	Isocitrate lyase (EC 4.1.3.1)	2.66	2.06
PP_4193	*sdhC*	Succinate dehydrogenase membrane b-556 subunit (EC 1.3.5.1)	1.60	1.45
PP_4279	*xdhB*	Xanthine dehydrogenase subunit XdhB (EC 1.17.1.4)	1.71	1.78

PP_4299	*glxR*	Tartronate semialdehyde reductase (EC 1.1.1.60)	2.10	2.24
PP_4402	*bkdAB*	Branched-chain alpha-keto acid dehydrogenase complex, beta subunit (EC 1.2.1.25) (EC 1.2.4.4)	4.04	1.85
PP_4487	*acsA1*	Acetyl-coenzyme A synthetase 1 (AcCoA synthetase 1) (Acs 1) (EC 6.2.1.1) (Acetate--CoA ligase 1) (Acyl-activating enzyme 1)	3.48	2.77
PP_4591	*rnd*	Ribonuclease D (RNase D) (EC 3.1.13.5)	1.20	1.13
PP_4620	*hmgB*	Fumarylacetoacetase (EC 3.7.1.2)	2.06	1.58
PP_4621	*hmgA*	Homogentisate 1,2-dioxygenase (HGDO) (EC 1.13.11.5) (Homogentisate oxygenase) (Homogentisic acid oxidase) (Homogentisicase)	1.39	1.80
PP_4666	*mmsB*	3-hydroxyisobutyrate dehydrogenase (HIBADH) (EC 1.1.1.31)	2.65	3.06
PP_4667	*mmsA-II*	Methylmalonate-semialdehyde dehydrogenase (EC 1.2.1.27)	1.83	2.66
PP_4702	*acsA2* *acsB*	Acetyl-coenzyme A synthetase 2 (AcCoA synthetase 2) (Acs 2) (EC 6.2.1.1) (Acetate--CoA ligase 2) (Acyl-activating enzyme 2)	1.78	1.82
PP_4945	*rlmJ*	Ribosomal RNA large subunit methyltransferase J (EC 2.1.1.266) (23S rRNA (adenine(2030)-N6)-methyltransferase) (23S rRNA m6A2030 methyltransferase)	1.52	1.15
PP_5165	*plpB*	NLPA lipoprotein	1.35	1.24
PP_5186	*argE*	Acetylornithine deacetylase (EC 3.-.-.-) (EC 3.5.1.-) (EC 3.5.1.16)	1.65	1.15

Figure S1: Workflow of AfBPP. Three experiments were run where (1) promysalin photoaffinity probe was incubated with the bacteria, (2) 5x excess promysalin was incubated with the bacteria **prior** to exposure to the probe, and (3) an inactive probe molecule was incubated with the bacteria. Experiments 2 and 3 will allow for the identification of non-specific targets to be taken into account when analyzing the data from experiment 1. In all cases bacteria and compound were incubated prior to irradiation. Upon covalent interaction the cells were clicked with biotin-azide, enriched, digested, and labeled for analysis via LC-MS/MS.

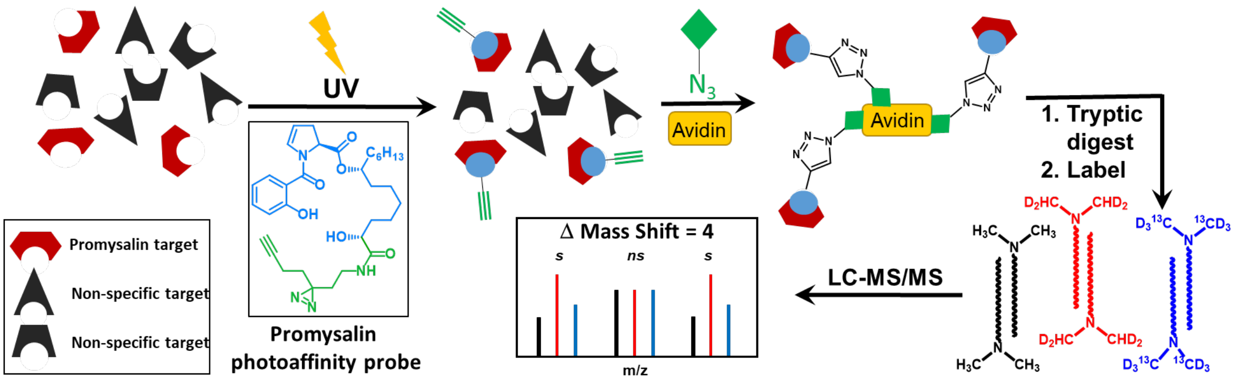

Table S2: RNA Sequencing reads mapping statistics of cDNA libraries.

[a] Reads were mapped to the *Pseudomonas putida* KT2440 genome. [b] quantity of reads when *Pseudomonas putida* KT2440 cells were treated with 50 uM promysalin. [c] quantity of reads when *Pseudomonas putida* KT2440 cells were treated with DMSO control.

Sequence read type[a]	Promysalin treated[b]	Control[c]
Total reads	109,540,045	151,278,351
Aligned 0 times	3,488,427	12,422,474
Aligned >1 time	2,522,000	3,054,800
Uniquely aligned	103,539,618	135,801,077

Figure S2: Swimming motility of P.putida KT2440 on swimming medium in the presence of DMSO (control) or promysalin. Swim plates were inoculated with bacteria from grown overnight on TSB plates containing 1.5% agar. Bacteria were resuspended in TSB and a sterile needle was used to inoculate the swim plates. Plates were kept at room temperature for 20-24 hrs and then photographed. Two independent assays were performed with two technical replicates for each independent assay.

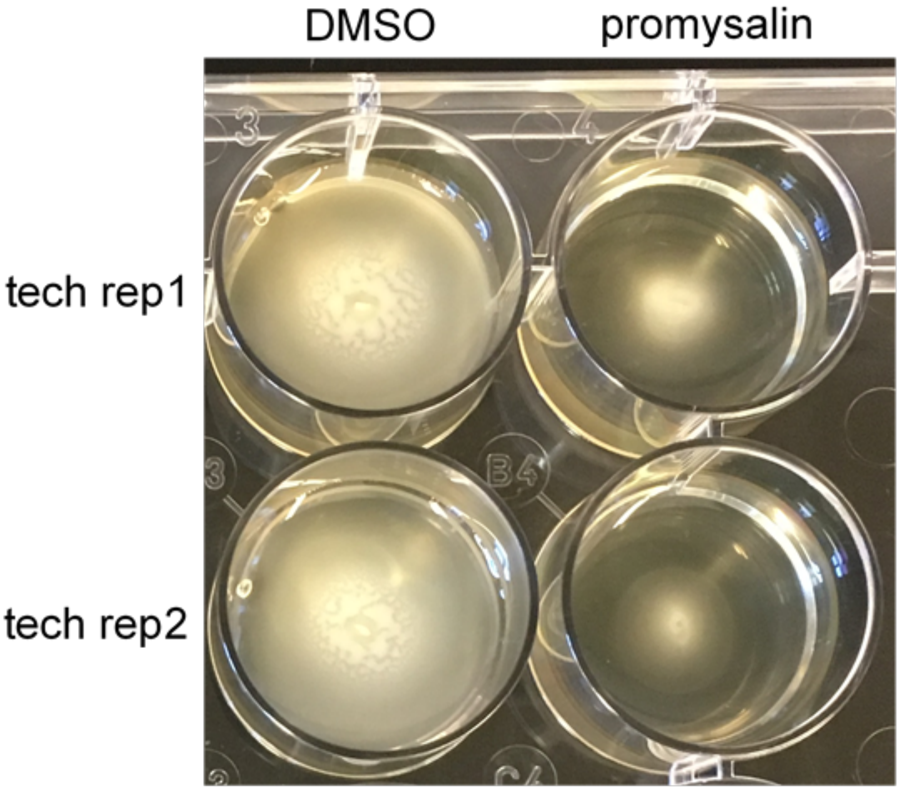

Figure S3: Swimming and swarming assays with promysalin treatment. Promysalin promotes swarming and inhibits swimming. Together this suggests flagella are not expressed or inhibited in proper function. *pictures taken after 20 hours

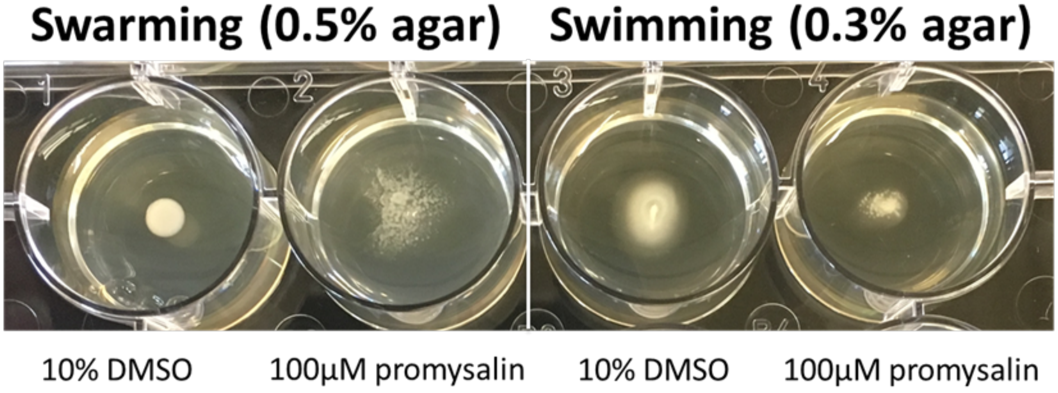

Figure S4: Western blot of *P. putida* KT2440 cells when exposed to DMSO (control) or promysalin.

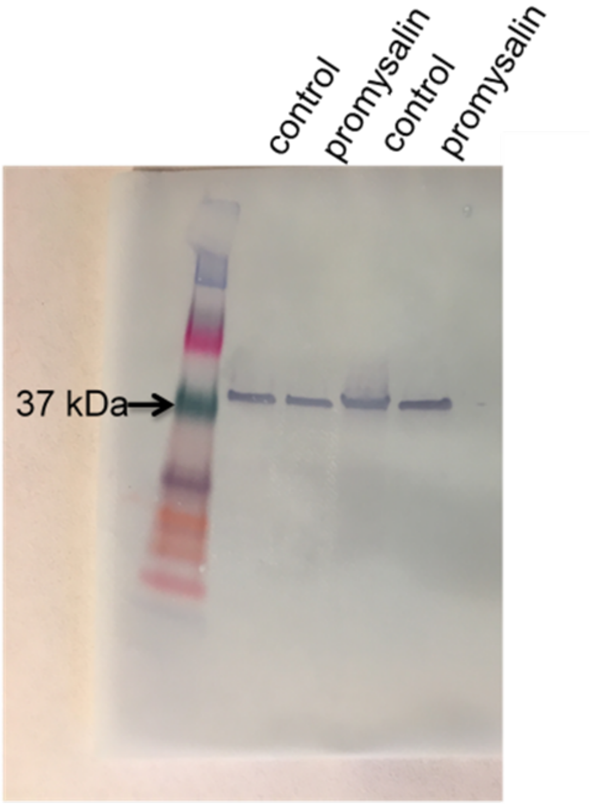

Figure S5. Volcano plots showing mean counts (y-axis) and fold change (log2; x-axis) of genes involved in iron-binding and/or homeostasis. Points colored orange indicate those genes predicted to encode iron binding proteins.

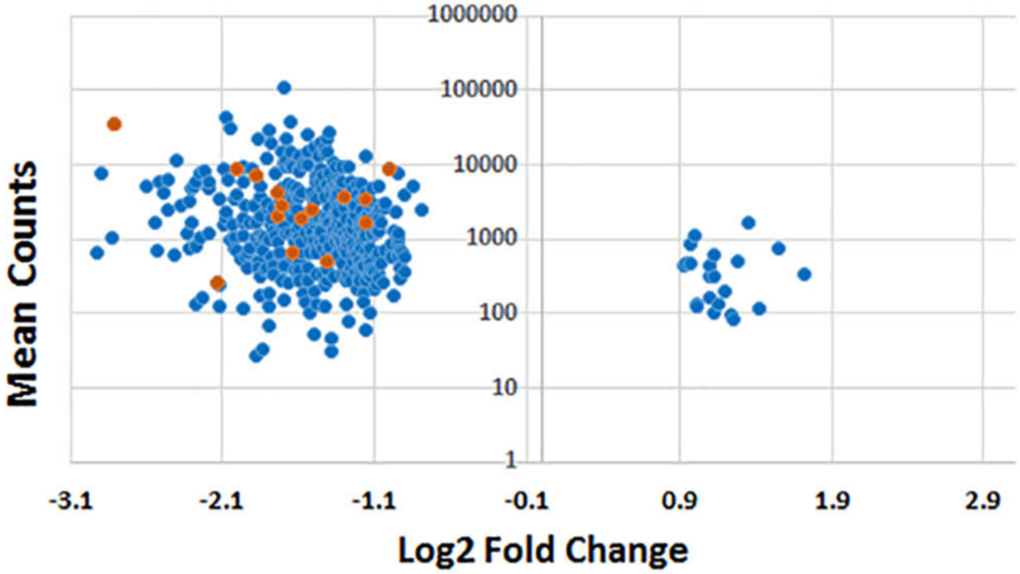

Table S3: Genes involved in iron homeostasis that were down-regulated in *P. putida* KT2440 with promysalin treatment.

Locus	Gene	Protein	Fold Change	P-value (adj)
PP_0160	PP_0160	Putative ferrioxamine receptor	-2.45	6.66E-03
PP_0481	*katA*	Catalase	-3.68	2.86E-05
PP_0482	*bfr-I*	Bacterioferritin	-4.03	6.64E-04
PP_0489	*fdoG*	Formate dehydrogenase-O major subunit	-2.00	1.28E-03
PP_1082	*bfr-II*	Bacterioferritin	-7.01	1.32E-04
PP_3332	PP_3332	Putative cytochrome c-type protein	-4.38	1.16E-02
PP_3823	PP_3823	Cytochrome c-type protein	-2.66	1.16E-03
PP_4251	*ccoO-I*	Cytochrome c oxidase subunit, cbb3-type	-3.34	2.21E-03
PP_4253	*ccoP-I*	Cbb3-type cytochrome c oxidase subunit	-2.23	1.82E-04
PP_4324	*ccmD*	Heme exporter protein D	-3.10	1.83E-03
PP_4325	*ccmC*	Protoheme IX reservoir complex subunit	-3.25	2.56E-02
PP_4839	PP_4839	Putative iron-regulated membrane protein	-2.99	8.46E-03
PP_4856	PP_4856	Ferritin, Dps family protein	-3.36	5.61E-04
PP_5212	PP_5212	Oxidoreductase, iron-sulfur-binding	-2.85	6.02E-03
PP_5267	PP_5267	Putative cytochrome c5	-2.23	5.20E-04

Figure S6: Relative expression levels measured by qRT-PCR. Quantitative real-time PCR was performed using cDNA prepared from the samples. The fold-induction of the genes in promysalin treated samples over that in control samples was calculated using the $2^{-\Delta\Delta Ct}$ method.