Omics-driven identification and elimination of valerolactam catabolism in *Pseudomonas putida* KT2440 for increased product titer

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Supplemental Figures

Figure S1: Growth of *P. putida* on glucose with various nitrogen sources. Growth of *P. putida* in minimal medium supplemented with either 10 mM glucose, and 10 mM of either caprolactam, valerolactam, NH₄, or no nitrogen. Shaded area represents the 95% confidence interval (cI), n=3.



Figure S2: RB-TnSeq and Cellular Shotgun Proteomics Results. (A) Genes that show significant (t < -4) and large (fitness < -2) fitness defects specific to either L-lysine, 5AVA, or valerolactam, but not glucose. All non-valerolactam fitness experiments are from Thompson et. al 2019. (B) Fitness of the *oplA* and *oplB* genes on all carbon sources (C) Venn diagram showing the number of specific proteins found in the 100 most abundant proteins found within *P. putida* grown on either glucose, valerolactam, or 5-aminovalerate, based on emPAI. Below we can see the 5 most abundant proteins specific to growth on valerolactam. OplA and OplB are highlighted.

-	-3 -2	-1	0	1	2	3				
Λ			.og ₂ Fitnes	55						
PP_0157	-0.33		-0.45	-1.1	0.13	transcriptional activator of gcdH, LysR family	. Valer	olactam		5AVA
PP_0213	-2.5	-4.2			0.00084	succinate-semialdehyde dehydrogenase (NADP+)				
PP_0214	-2.7	-3.5	-2.5	-2.6	0.28	4-aminobutyrate aminotransferase		100	120	
PP_0356	-2.8				0.068	malate synthase G		1	1	
PP_0382	-3.2	-0.18				5-aminopentanamidase			i i	4
PP_0383	-2.5					Lysine 2-monooxygenase		5 /		
PP_0384	-2.5	0.15				Transcriptional regulator, AsnC family		1 1		6
PP_0787	-3.6	-2.3		-3.3	-0.46	quinolinate phosphoribosyltransferase (decarboxylating) monomer		1 100		
PP_0949	-2.9	0.89				putative ATP-binding protein UPF0042				i i i
PP_1673	-2.1					cobyrinate a,c-diamide synthase		i /i		4
PP_1941	-2					conserved protein of unknown function				
PP_2326	-2.7	-0.15		-0.48		Universal stress protein		i <i>i</i> i		13
PP_2868	-2.2					Transcriptional regulator, Cro/CI family				
PP_2905	-0.21	-3.4				carbon starvation induced protein				
PP_2910	-4.4	-3.8		-3.5	-0.056	L-2-hydroxyglutarate oxidase		1 3		
PP_3592	-4.4	0.057		0.46		Transcriptional regulator, RpiR family			\	/2 i/
PP_3593	-5	0.065				Amino acid ABC transporter, periplasmic binding protein			N	and the second
E PP_3594	-4.7	-0.34				Amino acid ABC transporter, membrane protein				and and
PP_3595	-3.5	0.025				Amino acid ABC transporter, membrane protein				/
PP_3596	-3	0.25	-0.49			D-lysine oxidase		ì		
PP_3597	-6.6	-0.6				Amino-acid ABC transporter, ATP-binding protein		× •	7	
PP_3603	-3.1	3.5	-0.24	2.4	-0.45	Transcriptional regulator, GntR family			1	
PP_3649	-4	0.16		-0.65		Transcriptional regulator, GntR family		•	·	ARAZ
PP_3790	0.13	-1.8		-2	-0.38	diaminopimelate epimerase				
PP_3821	0.83	-0.21				UTP-glucose-1-phosphate uridylyltransferase			Glue	9202
PP_3924	-3.7	-0.28		0.11	-0.15	conserved protein of unknown function			Olut	
PP_4116	-2.2	-1.3				isocitrate lyase				_
PP_4482	-3.4	-0.82				Transcriptional regulator, AraC family	Carbon_Source	Uniprot	emPAI	Desc
PP_4493	-2.7	-0.39				putative oxidoreductase	Valerolactam	Q88H50_PSEPK	1.7	Hydantoin utilization protein A
PP_4695	-3.4	0.43	-0.86		-0.49	Sensory box histidine kinase	Valerolactam	Q88H51_PSEPK	1.91	Hydantoin utilization protein B
PP_4829	-2.4	1				putative precorrin-3B synthase	Valerolactam	Q88QU0_PSEPK	1.19	Uncharacterized protein
PP_4937	-2.8	-0.28				toluene tolerance protein	Valerolactam	Q88NR4_PSEPK	2.09	Branched-chain amino acid ABC transporter
PP_5257	-4	0.1				L-pipecolate oxidase	Valerolactam	ILVC_PSEPK	1.01	Ketol-acid reductoisomerase
PP_5256	-4.5	-0.44		-0.55		L-piperidine-6-carboxylate dehydrogenase				
PP_5260	-6.1	-0.064				metalloprotein, putative enzyme				
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Figure S3: Distribution of OpIBA orthologs: Phylogenomics of selected OpIBA homologs across bacteria. The boxes represent the gene neighborhood for each homolog. The genes have been colored to represent their annotated functions.



- B12 biosynthesis
- Branched-chain amino acid aminotransferase (EC 2.6.1.42)
- LysR-family transcriptional regulator STM3020
- N-methylhydantoinase B (EC 3.5.2.14)
- N-methylhydantoinase A (EC 3.5.2.14)
- Transcriptional regulator, AraC family
- Putative exported protein
- possible membrane protein
- Alkanesulfonates-binding protein
- luciferase-like protein
- transcriptional regulator, LysR family

Figure S4: Complementation of *oplBA* mutants with plasmid expressed genes. *P. putida oplBA* mutants harboring either pBADT-RFP (left) or pBADT-*oplBA* (right) were grown in minimal media with 10 mM valerolactam and varying concentrations of arabinose (% w/v)to induce expression from the plasmid. Shaded area represents the 95% confidence interval (cI), n=3.



Figure S5: Biochemical characterization of OpIAB. When purified OpIA and OpIB were incubated in the presence of valerolactam with or without ATP for 4 hours, there was no significant decrease in lactam concentration compared to boiled enzyme control. Error bars represent 95% cI, n=3.



Table S1: Specific growth rates of *P. putida* and valerolactam catabolic mutants on various carbon sources.

Strain	Carbon Source	Maximal Growth Rate (1/hr)
WT	5AVA	0.304339
	Glucose	0.561722
	Valerolactam	0.492629
ΔdavT	5AVA	0
	Glucose	0.568278
	Valerolactam	0
ΔoplBA	5AVA	0.283833
	Glucose	0.567119
	Valerolactam	0