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, NH_4, or no nitrogen. Shaded area represents the 95% confidence interval (cI), n=3.

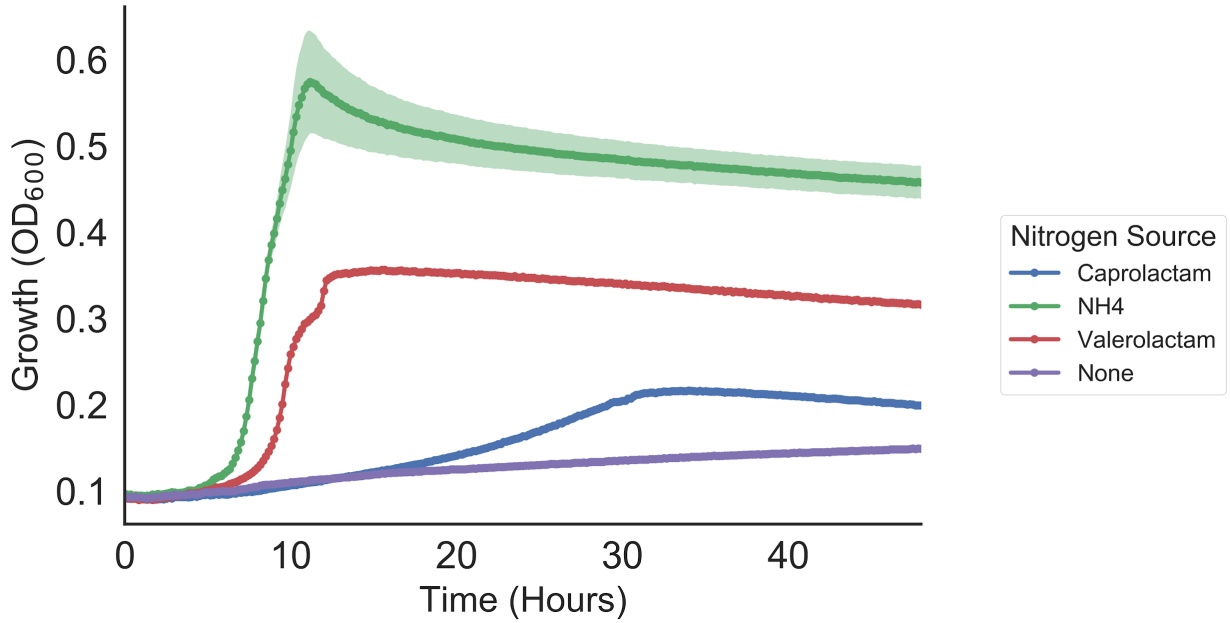
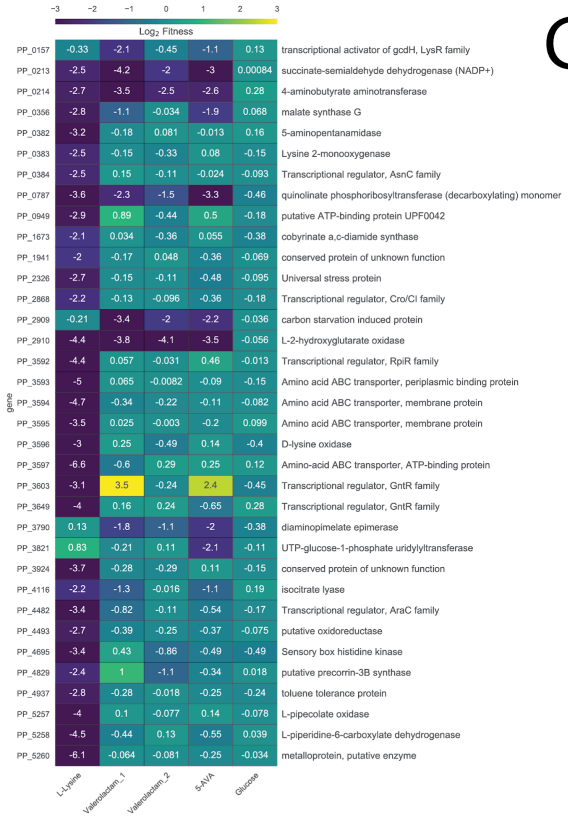


Figure S2: RB-TnSeq and Cellular Shotgun Proteomics Results. (A) Genes that show significant (t < -4) and large (fitness < -2) fitness defects specific to either L-lysine, 5AVA, or valerolactam, but not glucose. All non-valerolactam fitness experiments are from Thompson et. al 2019. (B) Fitness of the *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.

A



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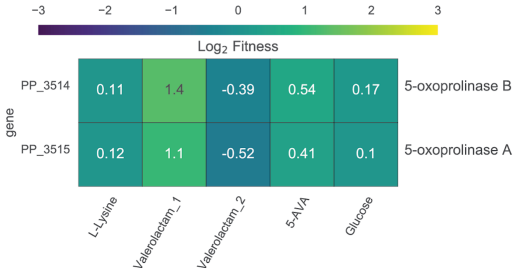
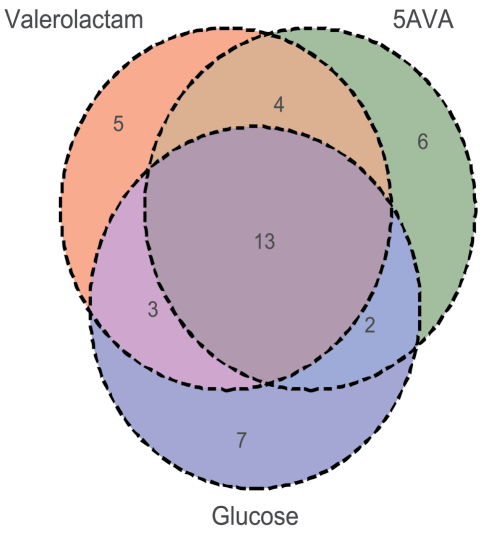


C



Carbon_Source	Uniprot	emPAI	Desc
Valerolactam	Q88H50_PSEPK	1.7	Hydantoin utilization protein A
Valerolactam	Q88H51_PSEPK	1.91	Hydantoin utilization protein B
Valerolactam	Q88QU0_PSEPK	1.19	Uncharacterized protein
Valerolactam	Q88NR4_PSEPK	2.09	Branched-chain amino acid ABC transporter
Valerolactam	ILVC_PSEPK	1.01	Ketol-acid reductoisomerase

Figure S3: Distribution of OplBA orthologs: Phylogenomics of selected OplBA homologs across bacteria. The boxes represent the gene neighborhood for each homolog. The genes have been colored to represent their annotated functions.

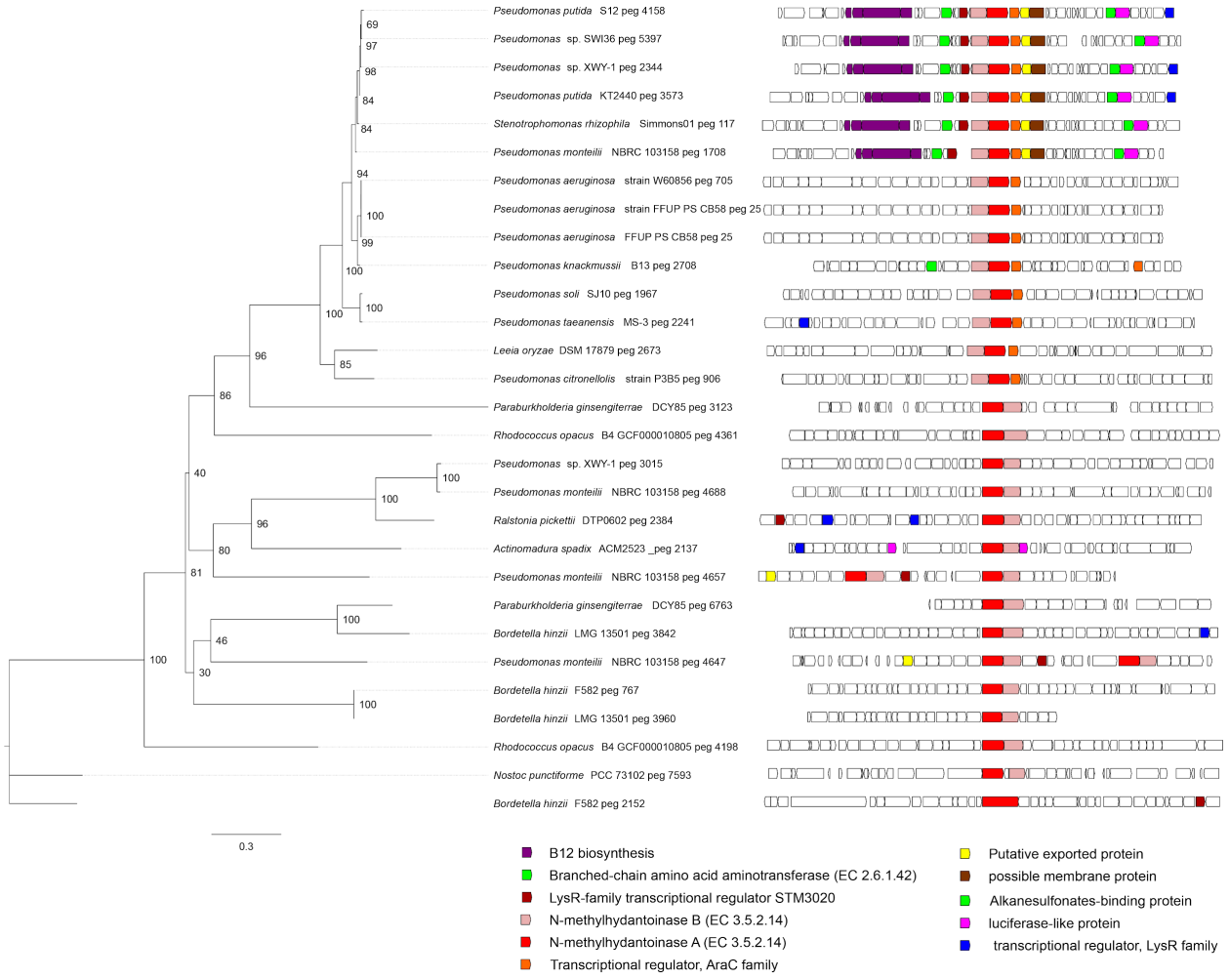


Figure S4: Complementation of *oplBA* mutants with plasmid expressed genes. *P. putida* *oplBA* mutants harboring either pBADT-RFP (left) or pBADT-*oplBA* (right) were grown in minimal media with 10 mM valerolactam and varying concentrations of arabinose (% w/v)to induce expression from the plasmid.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

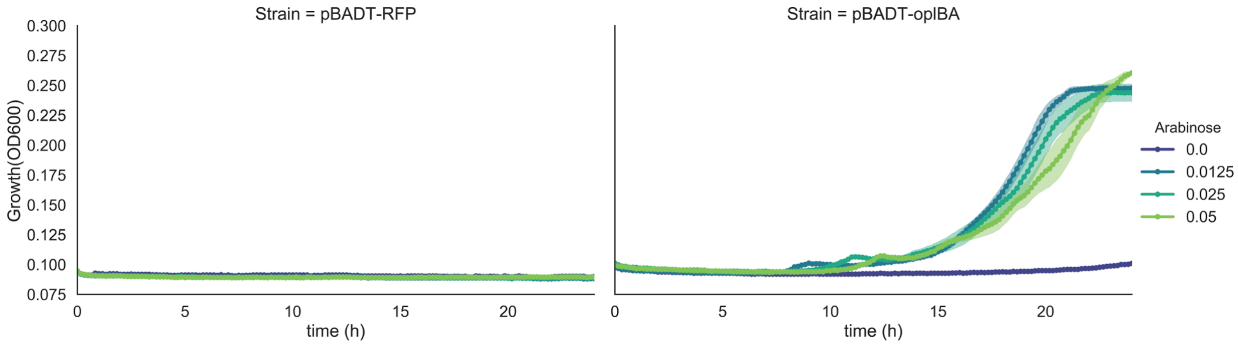


Figure S5: Biochemical characterization of OplAB. When purified OplA and OplB were incubated in the presence of valerolactam with or without ATP for 4 hours, there was no significant decrease in lactam concentration compared to boiled enzyme control. Error bars represent 95% cI, n=3.

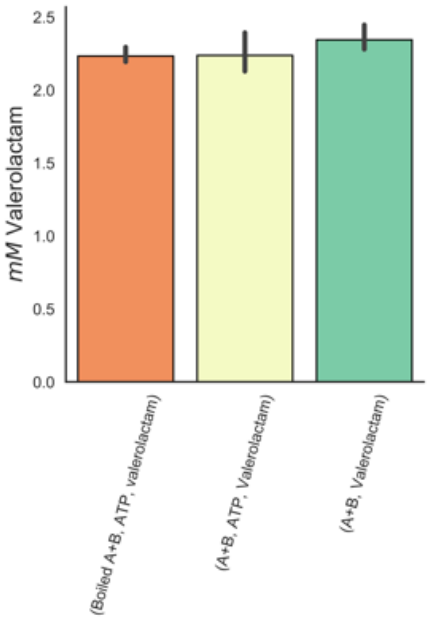


Table S1: Specific growth rates of *P. putida* and valerolactam catabolic mutants on various carbon sources.

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