"MASSIVELY PARALLEL FITNESS PROFILING REVEALS MULTIPLE NOVEL ENZYMES IN *PSEUDOMONAS PUTIDA* LYSINE METABOLISM"

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Despite intensive study for 50 years, the biochemical and genetic links between lysine metabolism and central metabolism in *Pseudomonas putida* remain unresolved. To establish these biochemical links, we leveraged Random Barcode Transposon Sequencing (RB-TnSeq), a genome-wide assay measuring the fitness of thousands of genes in parallel, to identify multiple novel enzymes in both L- and D-lysine metabolism. We first describe three pathway enzymes that catabolize L-2-aminoadipate (L-2AA) to 2-ketoglutarate (2KG), connecting D-lysine to the TCA cycle. One of these enzymes, PP_5260, contains a DUF1338 domain, a family with no previously described biological function. Our work also identified the recently described CoA independent route of L-lysine degradation that metabolizes to succinate. We expanded on previous findings by demonstrating that glutarate hydroxylase CsiD is promiscuous in its 2-oxoacid selectivity. Proteomics of select pathway enzymes revealed that expression of catabolic genes is highly sensitive to particular pathway metabolites, implying intensive local and global regulation. This work demonstrates the utility of RB-TnSeq for discovering novel metabolic pathways in even well-studied bacteria, as well as a powerful tool for validating previous research.