Massively parallel fitness profiling reveals multiple novel enzymes in Pseudomonas putida lysine metabolism - DTU Orbit (19/08/2019)

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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, P. putida 5260 (PP_5260), contains a DUF1338 domain, representing a family with no previously described biological function. Our work also identified the recently described coenzyme A (CoA)-independent route of L-lysine degradation that results in metabolism to succinate. We expanded on previous findings by demonstrating that glutarate hydroxylase CsiD is promiscuous in its 2-oxoacid selectivity. Proteomics of selected pathway enzymes revealed that expression of catabolic genes is highly sensitive to the presence of particular pathway metabolites, implying intensive local and global regulation. This work demonstrated the utility of RB-TnSeq for discovering novel metabolic pathways in even well-studied bacteria, as well as its utility a powerful tool for validating previous research. IMPORTANCE P. putida lysine metabolism can produce multiple commodity chemicals, conferring great biotechnological value. Despite much research, the connection of lysine catabolism to central metabolism in P. putida remained undefined. Here, we used random barcode transposon sequencing to fill the gaps of lysine metabolism in P. putida. We describe a route of 2-oxoadipate (2OA) catabolism, which utilizes DUF1338-containing protein P. putida 5260 (PP_5260) in bacteria. Despite its prevalence in many domains of life, DUF1338-containing proteins have had no known biochemical function. We demonstrate that PP_5260 is a metalloenzyme which catalyzes an unusual route of decarboxylation of 2OA to D-2-hydroxyglutarate (D-2HG). Our screen also identified a recently described novel glutarate metabolic pathway. We validate previous results and expand the understanding of glutarate hydroxylase CsiD by showing that it can use either 2OA or 2KG as a cosubstrate. Our work demonstrated that biological novelty can be rapidly identified using unbiased experimental genetics and that RB-TnSeq can be used to rapidly validate previous results.