Discovery of a Novel Bacterial Enzyme Enabling First-Time Biochemical Production of Toluene

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Project Goals: The Joint BioEnergy Institute (JBEI) aims to produce a chemically diverse suite of biofuels from lignocellulosic biomass. Our objective in this project was enzyme discovery to enable first-time biochemical production of toluene, an important octane booster and petrochemical with a global annual market of 29 million tons, from cellulosic sugars.

Although anaerobic bacterial biosynthesis of toluene from phenylacetic acid was reported more than two decades ago, the biochemistry underlying this novel metabolism has never been elucidated. Here we report the discovery of a toluene synthase (phenylacetate decarboxylase) from an anaerobic, sewage-derived enrichment culture that quantitatively produces toluene from phenylacetate. The discovery process (Zargar et al. 2016) included metagenome sequencing of the culture (which included more than 340,000 protein-coding genes), anaerobic FPLC (Fast Protein Liquid Chromatography) of cell-free extracts of the culture, and differential metaproteomic analyses to identify proteins present in active (toluene-producing) FPLC fractions but absent in adjacent inactive FPLC fractions (i.e., toluene synthase candidates). Toluene synthase candidates included a novel glycyl radical enzyme (GRE) and its cognate activating enzyme [AE; a radical SAM (*S*-adenosyl-L-methionine) enzyme]. Recombinant, N-terminally tagged, codon-optimized versions of the GRE and AE genes were expressed in *E. coli* and purified under anaerobic conditions. After *in vitro* reconstitution of the AE to restore its [4Fe-4S] cluster, its activity was confirmed *in vitro* by measuring conversion of SAM to methionine. *In vitro* assays with the purified GRE, AE, and SAM were shown to successfully convert ^{13}C-labeled phenylacetate to ^{13}C-labeled toluene, whereas no toluene was produced in control assays lacking SAM. Thus, using an omics-enabled approach, we have discovered a novel glycyl radical enzyme (only 6 are currently known) that catalyzes decarboxylation of phenylacetate to form toluene. Heterologous expression of the GRE and AE in a phenylacetate-overproducing microbial host should enable toluene production from lignocellulosic biomass.

Publications

1. Zargar, K., R. Saville, R. Phelan, S. G. Tringe, C. J. Petzold, J. D. Keasling, and H. R. Beller. 2016. *In vitro* characterization of phenylacetate decarboxylase, a novel enzyme catalyzing toluene biosynthesis in an anaerobic microbial community. *Scientific Reports* (*Nature*) **6**, 31362 doi: 10.1038/srep31362.

This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.