Pinching Metabolism for the Production of Citramalic Acid using Metabolically Engineered Escherichia coli

Metabolic Engineering

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Abstract

Citramalic acid (citramalate) is a five carbon hydroxy-dicarboxylic acid and potential resource for the production of methacrylic acid (MAA). The most common approach for MAA synthesis currently involves the hydrolysis of methacrylamide sulfate obtained from acetone cyanohydrin by a hazardous and environmentally process. We have recently examined citramalate production in Escherichia coli by overexpressing citramalate synthase which catalyzes the specific condensation of pyruvate and acetyl-CoA. Several enzymes compete for these two central metabolites, and therefore our research has compared the benefits of numerous gene knockouts on citramalate production. Not surprising, the gltA coding citrate synthase which controls entry of acetyl CoA into the TCA cycle is particularly important for elevated citramalate yield and productivity. Unfortunately, a gltA gene deletion causes cells to require that the medium be supplemented with glutamate. Glutamate addition allows cells to produce citramalate with a yield of 0.77 g/g from glucose, but significant glutamate is also consumed. To overcome the additional growth requirement, we examined introducing point mutations into citrate synthase to pinch but not eliminate its activity. Escherichia coli with decreased citrate synthase activity was able to accumulate over 60 g/L citramalate in 130 hours at a yield of 0.55 g/g using glucose as the sole carbon source.
Protein engineering for production of the high-value biochemical paraxanthine

Metabolic Engineering

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Abstract

The main metabolite of caffeine (1,3,7-trimethylxanthine) in humans is paraxanthine (1,7-dimethylxanthine), which has lower toxicity than caffeine and has been suggested as a therapeutic for patients with hepatic fibrogenesis and chronic liver diseases. Paraxanthine may also serve as a neuroprotective agent against Parkinson’s disease. Unfortunately, the high cost of paraxanthine ($1,200 per gram versus $0.04 per gram for caffeine) has limited its ability to be tested on a larger scale. Although caffeine is toxic to most bacteria, some have evolved metabolic pathways to use caffeine for energy and nutrients. The soil bacterium Pseudomonas putida CBB5 metabolizes caffeine to xanthine using three positional-specific N-demethylase enzymes. First, the N1-demethylase NdmA converts caffeine to theobromine (3,7-dimethylxanthine). Theobromine is further N3-demethylated to 7-methylxanthine by NdmB, followed by N7-demethylation to xanthine by NdmC. Although NdmB is active toward the N3-methyl group, its activity is blocked by the N1-methyl group present on the caffeine molecule. However, NdmA does exhibit a slight promiscuity toward the N3-methyl group, resulting in small amounts of paraxanthine produced from caffeine.

Recent elucidation of the NdmA and NdmB crystal structures has revealed nine amino acids involved in substrate binding. Surprisingly, only two of these nine amino acids differ between NdmA and NdmB. By mutating two amino acids at the NdmA active site to mimic the NdmB active site, we have produced a mutant enzyme with a paraxanthine:theobromine ratio of at least 3:1, over 100-fold improvement from the wild-type ratio (1:39). However, the activity is also reduced, with only 20% of the caffeine consumed over 2 hours by E. coli expressing the mutant enzyme. Additional targets for mutagenesis to improve activity have been identified on loops near the active site by further analyzing the crystal structures of NdmA and NdmB, and the effects of these mutations are currently being studied. These rationally engineered enzymes will provide an inexpensive, environmentally friendly method for synthesis of the high-value biochemical paraxanthine, and will enable further studies regarding its potential as a pharmaceutical ingredient.
Establishing a platform Escherichia coli strain to generate xylose-derived products

Metabolic Engineering

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Abstract

Xylose is the most abundant C5 sugar in lignocellulosic biomass and also represents a source of carbon from non-edible feedstocks. Here, we report the construction of a platform Escherichia coli strain for the production of high value compounds via the nonphosphorylative xylose metabolism. Firstly, a 3,4-dihydroxybutanal over-producing pathway was constructed by efficient enzymes screening and host strain engineering. Then two 3,4-dihydroxybutanal dehydrogenases were identified to efficiently convert 3,4-dihydroxybutanal into 3,4-dihydroxybutyric acid (3,4-DHBA), which is the hydrolyzed form of 3-Hydroxy-?-butyrolactone (3HBL). 3HBL is one of the top value-added building block for synthesis of various drugs and nutraceuticals. This novel 3,4-DHBA biosynthetic pathway produced 1.27 g/L of 3,4-DHBA in shake flasks, which is the highest titer reported so far. The application of this platform was further demonstrated by building an artificial pathway for biosynthesis of 1,4-butanediol (1,4-BDO). The 3,4-dihydroxybutanal over-producing strain was transformed into a 1,2,4-butanetriol (1,2,4-BTO) over-producing strain by over-expression of alcohol dehydrogenase, which enabled 1.5 g/L 1,2,4-BTO produced. Then the Klebsiella oxytoca diol dehydratase was engineered to achieve non-native catalysis of 1,2,4-BTO into 1,4-BDO by a series of rational protein engineering strategies. Those efforts enabled 209 mg/L 1,4-BDO produced via a novel metabolic route from xylose. This work demonstrates great potential for large-scale production of 3,4-DHBA and sets an example to build novel biosynthetic pathways via rational protein engineering.