Chemical Engineering Doctoral Defense

Metabolic Engineering for the Biosynthesis of Styrene and its Derivatives

School for Engineering of Matter, Transport and Energy

Rebekah McKenna

Advisor: David Nielsen

abstract

A demand for commodity chemicals by renewable means rather than fossil fuels has been increasing in recent years. Utilizing the tools of metabolic engineering, researchers have successfully catalyzed a wide array of biochemical reactions producing important biofuels, pharmaceuticals, and other fine chemicals. Styrene is a versatile, widely-used commodity chemical for which 60% of its global annual consumption supports the production of numerous, industrially-important polymers and co-polymers. Today, all commercially-available styrene is derived from the world's dwindling petroleum resources. Conventional styrene synthesis is achieved through the chemocatalytic dehydrogenation of petroleum-derived ethylbenzene which requires exorbitant amounts of energy in the form of steam. In this work, a pathway for the biosynthesis of styrene has been engineered in Escherichia coli from glucose by utilizing the pathway for the naturally occurring amino acid phenylalanine. Styrene production was accomplished via over-expression of PAL2 from Arabidopsis thaliana and FDC1 from Saccharomyces cerevisiae in the E. coli phenylalanine overproducer, E. coli NST74. The styrene pathway was then extended by just one enzyme to either (S)-styrene oxide (StyAB from Pseudomonas putida S12) or (R)-1,2-phenylethanediol (NahAaAbAcAd from Pseudomonas sp. NCIB 9816-4) which are both used in pharmaceutical production. Overall, these pathways suffered from limitations due to product toxicity as well as limited precursor availability. In an effort to overcome the toxicity threshold, the styrene pathway was transferred to a yeast host (Saccharomyces cerevisiae BY4741) with a higher toxicity limit. For styrene biosynthesis in yeast to be realized, S. cerevisiae BY4741 was first engineered, via random mutagenesis and high-throughput selection, to overproduce the pathway precursor phenylalanine. Subsequently, by overexpression of PAL2 and relying on the native expression of FDC1, styrene was achieved in yeast, for the first time, from renewable resources. These works have successfully demonstrated the use of microorganisms as cellular factories for the production of styrene, (S)-styrene oxide, and (R)-1,2-phenylethanediol.

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