Engineering Bacteria to produce Bio-Styrene and Other Aromatic Chemicals

David R. Nielsen, Rebekah McKenna, Shawn Pugh,
Matthew Sawtelle, Warinsinee Phusitkanchana

Chemical Engineering, Arizona State University
ECG 301, 501 E. Tyler Mall
Tempe, AZ, 85281, USA
David.R.Nielsen@asu.edu

Aromatic compounds represent a diverse class of fine and commodity chemicals with important commercial applications ranging from their use as molecular building blocks, flavor agents, and monomers. Today, conventional chemocatalytic synthesis routes for all aromatic compounds rely upon petroleum-derived BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds as feedstock. However, through *de novo* pathway engineering, our lab has been exploring the 'bottom up' development of microbial biocatalysts to produce a number of useful aromatics from renewable feedstocks.

Styrene, for example, is an important commodity chemical with versatile commercial applications, predominantly as a monomer building block for the production of many useful plastics. Today, all styrene is produced via chemocatalytic routes from petroleum-derived benzene or ethylbenzene according to an energy intensive process. Recently, our group engineered a non-natural enzyme pathway to instead produce styrene from renewable feedstocks. By over-expressing both *PAL2* from *Arabidopsis thaliana* and *FDC1* from *Saccharomyces cerevisiae* in a phenylalanine over-producing *Escherichia coli* host we have engineered the first styrene-producing microbe. Initial shake flask cultures have produced ~300 mg/L styrene, which approaches the determined toxicity threshold. Subsequent works have since sought to overcome several of critical limitations of this preliminary work. Here we present our progress in this regard on several fronts, including by developing strategies to increase pathway flux, overcome product toxicity, and by exploring alternative substrate feedstocks.

Building upon this theme, we have also begun applying the same pathway engineering strategies and principles to engineer bacteria to produce other, additional aromatic chemicals from renewable sugars. One such example is the chiral building block (*S*)-styrene oxide whose conventional applications include its use as a reactive plasticizer and as a chemical intermediate for cosmetics, surface coatings, and agricultural and biological chemicals. Our current titers here already approach the toxic threshold of (*S*)-styrene oxide, determined as ~1.6 g/L. In more recent applications we have also developed pathways for useful aromatic acids and alcohols. This presentation will provide an overview and summary of our past and current research related to this area.