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Microbial synthesis of phloroglucinol and xylitol

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Catalyzed by recent developments in bioenergy, industrial biotechnology, the use of enzymes and microorganisms to produce chemicals, biomaterials, and biofuels from renewable feedstocks, is on the verge of exponential growth. My group has been focused on the development and application of new protein engineering and metabolic engineering tools for industrial biotechnology. In this talk, I will present two specific examples. The first example concerns the development of a new bioprocess for synthesis of phloroglucinol, a precursor that is used to produce a variety of high value bioactive compounds and energetic compounds and is currently produced at 140 tons/year using chemical methods. Such a bioprocess may offer significant advantages over the current chemical manufacture of phloroglucinol, including environmental friendliness and reduction in the cost of phloroglucinol. Specifically, we discovered a novel type III polyketide synthase, PhlD, from *Pseudomonas fluorescens* that enables the direct biosynthesis of phloroglucinol from D-glucose (Achkar et al., 2005; Zha et al., 2006). Heterologous expression of PhlD in *Escherichia coli* led to the production of phloroglucinol *in vivo*, with an estimated amount of 0.7 g/L under the shaking flask condition and 10 g/L by using a continuous fermentation (Xie et al., 2006). To further improve the phloroglucinol yield, directed evolution was applied to enhance the activity of PhlD, which is facilitated by a high through-put screening to assay the improvement in phloroglucinol production (Zha et al., 2008). In addition, metabolic pathway engineering was carried out to redirect the carbon flux inside *E. coli* to pathways responsible for the synthesis of phloroglucinol. The second example concerns the development of a new bioprocess for synthesis of xylitol, a five-carbon sugar alcohol with many industrial applications and one of Department of Energy (DOE)'s top 12 platform chemicals for biorefinery. The current processes for xylitol manufacture, based on either chemical synthesis or fermentation, all rely on the use of pure D-xylose as a feedstock, resulting in relatively high cost of production. To address this limitation, we used protein engineering techniques to create a xylose reductase (XR) mutant with decreased specificity toward L-arabinose, while maintaining its high activity toward D-xylose. The *Neurospora crassa* XR was chosen for protein engineering work due to several favorable properties over other XRs, in addition to its innate >2-fold catalytic efficiency toward D-xylose than L-arabinose (Woodyer et al., 2005). A directed evolution strategy was developed that consists of a combined structure–function-based semi-rational design involving active site residue mutagenesis followed by random mutagenesis and selection for desired substrate specificity. After the first round of evolution, a mutant was identified with 14-fold preference for D-xylose over L-arabinose (Nair and Zhao, in press). Such engineered xylose-specific XR mutants will enable the direct use of inexpensive hemicellulose hydrolysates (mainly D-xylose and L-arabinose) as substrates in large-scale fermentation.

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Synthesis of size-tunable upconversion nanophosphors with a consistent shape for photodynamic therapy (PDT)

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Lanthanide, Yb/Ln (Ln = Er, Tm and Ho), doped $NaYF_4$ upconversion nanophosphors (UCNPs) were synthesized in one step via thermal decomposition of lanthanide trifluoroacetate precursors in the low volatile organic solvents, such as octadecene (ODE), oleic acid (OA), trioctylphosphone (TOP), and trioctylphosphone oxide (TOPO). The UCNPs as-synthesized have a consistent hexagonal prism shape and the sizes can be tuned in the range from 5 to 200 nm by applying different ligands and monotonically adjusting the precursor ratios. Surface modifications by coating SiO_2 and polymer layers were conducted. For photodynamic therapy (PDT) in cancer treatment, these UCNPs were encapsulated with porphyrins and further conjugated with antibody. Specific drug delivery targeting at tumor cells was also performed.

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Production of poly(3-hydroxybutyrate) from molasses and peach pulp

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Nowadays, plastic materials are taking an important place in our every day life. Their physical properties make them very convenient in utilization. But these nondegradable plastics are accumulating in the environment at the rate of 25 million tonnes per year (Jacquel et al., 2008; Choi and Lee, 1997). Poly 3-hydroxybutyrate (PHB), the best known member of the polyhydroxyalkanoates (PHA), is an energy and/or carbon storage material synthesized and accumulated as intracellular by numerous microorganisms (Ustian,