Modifying terpenes for the development of biofuels and bioproducts

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Project Goals: We aim to develop and demonstrate a platform for on-demand prioritization and validation of routes to biofuels and bioproducts at JBEI. Modifying terpenes show the possibility to expand the portfolio of isoprenoid biosynthesis. Combining chemical and biological catalysis shows advantages to achieve both productivity and diversity during product development. In this study, chemical and biological routes were investigated for modifying terpene molecules toward the production of biofuels and bioproducts. We also develop an enzyme fusion strategy by linking terpene synthase and P450 together to improve the productivity of terpene oxidation.

The functionalization of terpenes is a versatile route to the development of useful derivatives that can be further converted to value-added products. Combining chemical and biological catalysis shows advantages to achieve both productivity and diversity during product development. In this study, the chemical conversion of monoterpene (limonene and 1,8-cineole) to *p*-cymene was investigated. While limonene bioproduction is constrained by its toxicity, 1,8-cineole, a less toxic precursor to limonene, can be also converted to p-cymene with similar efficiency using a bifunctional metal/acid chemical catalyst. This suggests that 1,8-cineole could be a preferred hand off point from biological to chemical conversion. In a biological conversion, cytochrome P450 enzymes were investigated for the enzymatic oxidation of monoterpene. Biosynthetic pathways were further explored to develop possible derivatives, such as carvolactone, a monomer of thermoplastic polyester. Particularly, we developed an enzyme fusion strategy by linking terpene synthase and P450 together to overcome the low availability of hydrophobic and volatile terpene molecules for the P450 reaction [1]. The engineered fusion proteins of 1,8-cineole synthase and $P450_{cin}$ showed an increase of the hydroxylation of 1,8-cineole up to 5.4-fold. Structural analysis of the fusion proteins indicated a dimer formation with preferred orientations of the active sites of two domains. We also applied the enzyme fusion strategy to the oxidation of a sesquiterpene epi-

isozizaene, in which a 90-fold increase was observed in albaflavenol production. This study demonstrated a platform for the development of biofuels and bioproducts via terpene modification.

References

1. Wang, X., Pereira J. H., Tsutakawa, S., Fang, X., Adams, P. D., Mukhopadhyay, A., Lee, T. S. Efficient production of oxidized terpenoids via engineering P450 fusion proteins. *Metabolic Engineering*, 2021, 64:41–51.

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