Engineering *Streptomyces* to Capture Value from Lignocellulosic Biofuel Conversion Residue

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Project Goals: To increase the economic viability of biofuels by generating fatty acid and isoprenoid bioproducts from the organics remaining after biofuel synthesis.

Current methods of switchgrass fermentation to bioethanol leave behind about 60% of the organic material in the hydrolysate after ethanol distillation. This material is referred to as conversion residue (CR). To increase the economic viability of lignocellulosic biofuels, we are engineering *Streptomyces* species to maximize the conversion of CR carbon into valuable isoprenoid and fatty acid bioproducts. From a library of 120 phylogenetically distinct *Streptomyces* isolates, more than half were capable of growth on undiluted, pH-adjusted CR. From these, we generated a collection of *Streptomyces* that produce the isoprenoid lycopene or fatty acid-derived melanin from CR as reporters to assess the production potential of these isolates. The genetic elements used in constructing these reporters are mobilizable between *Streptomyces* species and constructed using a combination of traditional cloning techniques and Golden Gate assembly to allow for rapid alterations in expression levels and the generated bioproduct.

Initial screens of the engineered *Streptomyces* reporter strains showed a wide range of native production levels of lycopene and melanin. We targeted four strains for further development using a design-test-learn approach to enhance bioproduct formation. These strains showed differences in carbon utilization on both CR and synthetic CR, a defined medium containing approximately one-third the organic compounds of CR at comparable concentrations. We will leverage insight into the mechanisms underlying these differences in carbon utilization from transcriptomic analyses and molecular modeling to increase CR catabolism and bioproduct formation. Other opportunities for further engineering include increasing isoprenoid precursor pools by introducing refactored MEP and mevalonate pathways and generating new bioproducts such as isoprene, limonene, pinene, and bisabolene.

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