Engineering Alternative Model Yeast Species for Biofuel Production

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Project Goals: The Great Lakes Bioenergy Research Center is developing the production of sustainable biofuels and bioproducts from dedicated energy crops grown on marginal lands. Our research is focused on engineering various species of yeast to convert sugars derived from lignocellulosic biomass into advanced biofuels such as isobutanol. By studying alternative model systems, we aim to identify and bypass engineering roadblocks, gain insight into the robustness and breadth of applicability of metabolic engineering strategies, and discover and develop new biological tools.

Saccharomyces cerevisiae has long been considered the model yeast species in many arenas, including the production of biofuels. Producing large quantities of isobutanol from sugars found in lignocellulosic hydrolysates will likely require the deletion of genes in competing pathways that normally direct most of pyruvate flux into ethanol fermentation: pyruvate decarboxylase (*PDC*). Deletion of all three *PDC* isoforms in *S. cerevisiae* renders the mutant unable to grow on glucose.[1] This presents a significant roadblock to further genetic engineering of this species to produce alternative end products. In contrast, the yeast *Kluyveromyces lactis* contains only a single isoform of *PDC*, and deletion of this gene does not prohibit growth on glucose, making further engineering of alternative metabolism on glucose feasible. Here we report initial characterization of the Kl*pdc1Δ* strain during growth on glucose, and its potential for use to bypass the growth deficiency of its *S. cerevisiae* counterpart.

After the removal of competing reactions that would otherwise divert carbon flux into non-target products, the next step of rationally designed engineering approaches is often to introduce new genes or upregulate existing genes for the desired pathway. For the production of isobutanol, these are the *ILV* genes that direct pyruvate through branched-chain amino acid (BCAA) biosynthesis, as well as a decarboxylase and alcohol dehydrogenase. These approaches to isobutanol production in *S. cerevisiae* have shown some success, and applying similar strategies to *K. lactis* will inform us of the robustness of these engineering approaches when applied to other yeast species.

Finally, we are exploring alternative approaches to rational design for engineering organisms to produce specific end products. *K. lactis*, as well as several other yeast species, produce a red, iron-binding molecule during growth called pulcherrimin. We recently identified and characterized a secondary metabolite gene cluster in this species responsible for the production and utilization of this iron-binding siderophore.[2] The evolution of this gene cluster suggested an

ecological role for this molecule, possibly in competition for iron in the environment. Because the carbon in pulcherrimin is derived from two leucine molecules, its production is a result of carbon flux through BCAA biosynthesis. We are exploring the utility of this molecule to link secondary metabolism with primary metabolism and develop alternative strategies to increasing BCAA flux in yeasts.

References

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