

## Using Fungal Diversity to Improve Biofuel Conversion

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**Project Goals: Our major goal is to improve the conversion of sugars from lignocellulosic hydrolysates into specialty biofuels such as isobutanol. We are engineering non-traditional model species of budding yeast to develop an improved understanding of how flux can be directed from the typical ethanol fermentation into new pathways such as isobutanol production. We also aim to use budding yeast diversity to better understand adaptations to growth and production of biofuels under anaerobic conditions.**

The production of specialty biofuels from dedicated bioenergy crop hydrolysates will require genetically engineered microbes capable of rapidly converting sugars into biofuels in an oxygen-free environment. The budding yeasts are a diverse group of microorganisms containing many species that have these key traits. We are currently using budding yeast diversity to address several key challenges to producing the specialty biofuel isobutanol in industrial conditions. First, the primary model yeast species, *Saccharomyces cerevisiae* is unable to grow on glucose when lacking genes encoding pyruvate decarboxylase, which is a barrier to completely transitioning this organism from ethanol production to isobutanol production. In contrast, the yeast *Kluyveromyces lactis* is capable of growth in the absence of any ethanol production machinery. We will present progress in engineering *K. lactis* for isobutanol production in the absence of ethanol production. One drawback to *K. lactis* as a biofuel organism is its inability to grow under strict anaerobic conditions. We are currently screening over 1,000 species of budding yeasts for anaerobic growth. These experiments will improve our understanding of how anaerobic growth evolved within the budding yeast subphylum Saccharomycotina, as well as shed light on how we might engineer obligate aerobic yeast species to grow anaerobically. By leveraging genetic diversity among yeasts, as well as performing genetic engineering in non-traditional model yeast species, we are employing novel strategies to overcoming barriers to specialty biofuel production.

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