Adaptive Laboratory Evolution Provides a Route to Improve Biofuel Production Under Ionic Liquid Stress

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Project Goals: To use adaptive laboratory evolution (ALE) to identify strains with the capacity produce high titers of biofuels in the presence of ionic liquids.

The production of biofuels in microbes needs to consider many upstream and downstream bottlenecks. Upstream bottlenecks include the toxicity inherent from crude biomass or pretreatment inhibitors, both which reduce the ability to consolidate fermentation processes. Downstream bottlenecks such as the toxicity of the target molecule also limit production titers. We hypothesized that lab adaptation could yield strains that are better able to reach high metabolic rates in biomass hydrolysates and potentially provide an improved background to express biosynthetic pathways and final products. Our adaptation studies led to the discovery of strains with the desired improved strain phenotype but tracked to mutations that functioned independently of any existing background mutants. Here, we report a hereditable mutation in an essential gene that bestows high IL tolerance. We confirmed that the mutant background not only provides improved IL tolerance but also results in increased production a final biojetfuel product, d-Limonene. The production of limonene production at 200 mg/L is the highest reported biogasoline production in the presence of typical levels of residual ILs in the culture medium. Our study suggests that the mutant strain mounts a distinct global physiological response which alleviates the cytotoxicity of [EMIM]OAc allowing for greater metabolic flux into the target gene pathway.

This work was part of the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the U. S. Department of Energy, Office of Science, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.