

Community-Level Bacterial Evolution for the Bioremediation of Biofuel n-Butanol

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Background/Objectives. n-Butanol is a strong biofuel candidate since it is more energy dense and less volatile than ethanol; additionally, butanol can be utilized in existing pipelines and infrastructure. The bottleneck for industrial production of n-butanol is its high toxicity. Most microbes cannot survive above 1.5% v/v, although some genetically engineered microbial strains have been improved to tolerate 2% v/v. Due to the high toxicity, a biofuel spill of n-butanol could be devastating. We propose to develop bioremediation capability along with the biofuel capacity to mitigate future spills of n-butanol.

Approach/Activities. To acclimatize and adapt bacteria to metabolize toxic n-butanol, we used high throughput tolerance assays, community-level ecological competition and evolutionary experiments with various selection pressures, and phenotypic and genomic characterizations. Communities were tested in a range of butanol concentrations in carbon and nutrient rich media. Bacterial communities that could grow in the 1-2% v/v concentrations of n-butanol were successively passaged to adapt to the stressful environment. The bacterial community composition was assessed over time and strains were isolated. The bacterial communities tolerant to n-butanol were then transitioned into a less carbon-rich media to look for breakdown of the butanol. Nuclear magnetic resonance (NMR) was used to quantify how much butanol remained in the cultures and identify breakdown products.

Results/Lessons Learned. The tolerable concentration of n-butanol was found to be lower than 2% v/v for most communities tested, with growth mainly at 1% v/v. In the ecological experiment, bacterial community dynamics were heavily influenced by n-butanol concentration. Two bacterial strains were found and further tested.