153e Engineering Escherichia Coli Solvent Tolerance Phenotypes to Improve Chemical Production

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This project is focused on improving the conversion of naphthalene into 1-naphthol, a conversion which is traditionally performed chemically, as a model system for examining how to engineer stress tolerance phenotypes. It has been observed that higher 1-naphthol yields are difficult to obtain because of the toxicity of 1-naphthol. We hypothesize that some mechanisms for increasing 1-naphthol tolerance should result in increased 1-naphthol production. Toluene ortho-monooxygenase (TOM) from *Burkholderia cepacia* G4 catalyzes naphthalene oxidation, as well as oxidation of chlorinated ethenes and toluene, but TOM is not naturally expressed by *Escherichia coli*. Using a library screening approach, we have identified genes for which increased copy number confers increased 1-naphthol tolerance in *E. coli*. One corresponds to the marRAB operon, which has been shown to control resistance to multiple antibiotics. We have also identified additional loci. We next plan to test the extent to which overexpression of such loci in *E. coli* cells, containing the TOM operon, improves 1-naphthol production and then to apply modern genome engineering tools to further improve production. For example, genome shuffling is a powerful tool, enabling directed evolution of superior strains through the genetic recombination of a genetically diverse collection of mutants. However, application of this technique to *E. coli* has been difficult. We have used genome shuffling with two *E. coli* strains with different resistance markers to investigate the important process variables, such as lysozyme concentration, osmoprotectant (sucrose) concentration and quality, fusant (PEG) concentration, and recovery temperature. Both PEG concentration and sucrose quality strongly affect the survival of shuffled cells. We will present these results, as well as any new significant findings resulting from the completion of this study.