

436n Identification of Organic Acid Tolerance Genes in E. Coli for Biorefinery Applications

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Synthesis of building block chemicals at high titers can be toxic to host microorganisms. We have developed Mixed Library Parallel Gene Trait Mapping (ML-PGTM) as a technique that identifies the effect of specific genes on the expression of a specific bacterial phenotype. We have utilized this method to select for organic acid tolerance. Selections using 3-hydroxypropionic acid (3-HP) were carried out in both minimal media and buffered minimal media systems. Genes identified conferred both acid tolerance and resistance to 3-HP and 3-hydroxypropionaldehyde (3-HPA), a toxic intermediate formed during 3-HP production. We have identified one loci with putative functions involving oxidative stress for which increased copy confers increased 3-HP tolerance. Confirmations of increased tolerance were carried out by determining minimum inhibitory concentrations and constructing growth curves of selected transformants in the presence of 3-HP and other organic acids. A 50% increase in tolerance was observed for 3-HP when compared with wild type E. coli.