149c Benefits and Costs of Improving Stress Tolerance

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Metabolic engineering involves the manipulation of an organism's genome in order to redirect metabolic functions in a specific manner. There are many tools available for strain improvement including the following genetic techniques: 1) random mutagenesis, 2) plasmid based gene over expression, and 3) gene disruption. Such strategies are commonly employed in metabolic engineering efforts. A problem associated with any genetic engineering approach is the possibility that mutations that benefit the expression of a particular phenotype may unintentionally impose a cost to biological fitness. Our objective here was to improve understanding of this phenomenon, which entailed a “systems-biology” view of a “synthetic-biology” problem. In this study, we have engineered tolerance to three closely related amino acid analogs: L-aspartic acidβ hydroxamate, L-glutamic acidγ hydroxamate, and L-tryptophan hydroxamate. Using this system we have attempted to determine if the benefit to cost ratios associated with different mutants was a function of either the genetic engineering technique applied or the particular amino acid hydroxamate employed. We have determined that increased copy strategies appear to work more efficiently at identifying the best performing mutants and the genes underlying such improvements. In addition, we have determined that the particular hydroxamate employed, and thus the anti-metabolite target, plays a critical role in the evolution of stress tolerant phenotypes. Finally, we have identified several new amino acid hydroxamate tolerance genes, which yield insights into novel strategies for improving amino acid production in bacteria.