Dissecting the genetic and metabolic mechanisms of adaptation to the knockout of a major metabolic enzyme in Escherichia coli

Unraveling the mechanisms of microbial adaptive evolution following genetic or environmental challenges is of fundamental interest in biological science and engineering. When the challenge is the loss of a metabolic enzyme, adaptive responses can also shed significant insight into metabolic robustness, regulation, and areas of kinetic limitation.

In this study, whole-genome sequencing and high-resolution C-13-metabolic flux analysis were performed on 10 adaptively evolved pgi knockout strains of Escherichia coli. Pgi catalyzes the first reaction in glycolysis, and its loss results in major physiological and carbon catabolism pathway changes, including an 80% reduction in growth rate. Following adaptive laboratory evolution (ALE), the knockouts increase their growth rate by up to 3.6-fold. Through combined genomic-fluxomic analysis, we characterized the mutations and resulting metabolic fluxes that enabled this fitness recovery. Large increases in pyridine cofactor transhydrogenase flux, correcting imbalanced production of NADPH and NADH, were enabled by direct mutations to the transhydrogenase genes sthA and pntAB. The phosphotransferase system component crr was also found to be frequently mutated, which corresponded to elevated flux from pyruvate to phosphoenolpyruvate.

The overall energy metabolism was found to be strikingly robust, and what have been previously described as latently activated Entner-Doudoroff and glyoxylate shunt pathways are shown here to represent no real increases in absolute flux relative to the wild type. These results indicate that the dominant mechanism of adaptation was to relieve the rate-limiting steps in cofactor metabolism and substrate uptake and to modulate global transcriptional regulation from stress response to catabolism.