Fundamentals and Applications of Systems Metabolic Engineering

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Metabolic engineering has recently experiencing a huge leap towards systems-level engineering of microorganisms aided by the tools of systems and synthetic biology, which is referred to as 'systems metabolic engineering' [1, 2]. This is in sharp contrast with conventional random approaches, as this approach engineers only the specifically targeted parts of the organism without triggering unexpected changes that could undermine the organism's performance in a bioreactor. We have focused our efforts on constructing strains that overproduce value-added chemicals, including L-valine [3] and L-threonine [4], in this framework. For the production of L-valine as an example of the systems metabolic engineering approach, we rationally knocked out feedback inhibition and attenuation in *Escherichia coli* [3]. Feedback inhibition of acetohydroxy acid synthase isoenzyme III by L-valine was removed by site-directed mutagenesis, and the native promoter containing the transcriptional attenuator leader regions of the *ilvGMEDA* and *ilvBN* operon were replaced with the *tac* promoter. We, then, obtained a base strain of *E. coli* producing L-valine by knocking out competing metabolic pathways and amplifying several core metabolic pathway fluxes. After the development of rationally engineered base strain, stepwise improvements were made based on transcriptome analysis and *in silico* simulations using genome-scale metabolic network of *E. coli* [3, 5]. Comparative transcriptome profiling was performed during batch fermentation. Among the down-regulated genes, one global regulator and an L-valine exporter were selected as the amplification targets; when these two genes were amplified, L-valine production increased by 113%. Further improvement was achieved by using *in silico* gene knockout simulations, which predicted three additional gene knockout targets that are otherwise hard to be predicted with intuition [3, 5]. By knocking out the genes selected *in silico*, we were able to produce an impressively high yield of 0.378 g L-valine per g glucose. A similar strategy was also successfully applied for developing the L-threonine overproducing *E. coli* strain [4]. In addition to the rational genome engineering employed for the L-valine overproducing *E. coli* strain, *in silico* genome-scale flux response analysis was performed to examine the desired expression level of target genes for overexpression, which is suggested by transcriptome profiling and to reduce byproduct accumulation during the fed-batch culture. This is the first example of modulating the metabolic fluxes to a desired levels on the basis of the *in silico* analysis using the target genes identified by transcriptome profiling. The final engineered strain was able to produce a high yield of 0.393 g L-threonine per

g glucose, and 82.4 g/L L-threonine by fed-batch culture. These results suggest that an industrially competitive strain can be successfully developed by metabolic engineering based on combined rational modification, transcriptome profiling and systems-level *in silico* analysis. The above examples will be accompanied by the fundamental strategies of systems metabolic engineering for the development of industrial strains efficiently producing chemicals, fuels, and materials.

References

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