Computational methods for microbial cell factory engineering aided by evolution

Increasing global temperatures and limited fossil resources make it increasingly urgent to find alternative ways of producing fuels and chemicals. Metabolic engineering offers a promising solution to this problem by using microbes as cell factories for manufacturing a diverse set of products from renewable resources. However, cell factory development requires extensive knowledge of microbial biology as well as expensive and time-consuming strain engineering. Nonrational methods allow the strain development process to be accelerated by taking advantage of evolutionary processes. This thesis addresses the integration of adaptive laboratory evolution into cell factory development workflows through computational methods. By studying a large set of Escherichia coli strains evolved to tolerate 11 different chemicals of industrial relevance, it was shown that there is significant cross-tolerance between compounds of the same chemical class, and that pre-evolving strains to tolerate a product can improve production rates when the evolved strain is engineered with a production pathway. Metabolic profiling of the evolved strains using direct-injection mass spectrometry showed that strains evolved in the same conditions had converged to similar metabolic phenotypes, suggesting that metabolism is involved in chemical tolerance. It was shown that the effects of individual mutations could be predicted, both by directly comparing the metabolic profiles of evolved strains to previously measured metabolic profiles of knockout strains, as well as using deep neural networks to predict metabolite level changes directly from genetic perturbations.

Adaptive laboratory evolution can be used to optimize growth rates under various growth conditions, but through clever strain engineering it is possible to couple production to growth, thereby allowing optimization of production rate. This thesis also presents an algorithm based on genome-scale metabolic modelling that can predict genetic modifications that enable growth-coupling in combination with addition of specific supplements to the growth medium. The algorithm could predict known growth-coupled strain designs that are shown to work in vivo as well as novel promising strain designs, for production of itaconic acid, propionic acid and for product methylation.