THESIS ABSTRACT

In silico analysis, modelling and engineering of Escherichia coli metabolic network for succinic acid production

Introduction: the main purpose of metabolic engineering is to design and redesign cellular metabolism to redirect carbon flux through central metabolism and to obtain metabolites with high economic impact (succinic acid) from renewable resources - using by-products (waste) resulted from different industries. *Escherichia coli* can be used successfully, but succinic acid production in minimal medium is very low and to improve the production metabolic pathways should be modified- (metabolic engineering, genetic engineering). The metabolism of microorganisms is very complex, hence metabolic manipulations are very difficult. For the rational design *in silico* analysis like systems biology has proven to be successful in metabolic engineering. Using this method we can analyze the phenomena and processes in biology taking into account systems approaches.

The first step is to obtain mathematical model based on physiological, biochemical processes. Flux Balance Analysis is the most widely used method for process optimization in biology.

The main aim of research was to *in silico* optimize the metabolic pathways for succinic acid production from glucose and glycerol and characterization of metabolic pathways before and after metabolic engineering.

Methods: the used *iJO1366 E. coli* K12 MG1655 model published in 2011 contains more than 2,000 reactions. Different platforms such as, COBRA Toolbox (MATLAB), OptFlux and GEMSiRV were used for simulations. Conditions: carbon sources glucose and glycerol, minimal medium (M9), under aerobic, microaerobic and anaerobic conditions.

Results: the metabolic model was updated by changing 11 important reactions. The first step was to identify by-products pathways; under anaerobic conditions four pathways have been eliminated: formic acid, lactic acid and ethanol, phosphotransferase system for glucose, respectively. With these changes under anaerobic conditions the yield of succinic acid increased from 0.008 to 1.25 mol mol^{-1} glucose. In case of glycerol, metabolic pathways cannot be eliminated (zero growth rate) and the use of different co-substrates is crucial. Amino acids and glucose were tested and with glucose as co-substrate the yield was about 0.6 mol mol^{-1} glycerol. The importance of genes was determined under different conditions before and after metabolic engineering. Changing redox metabolism does not change the number of essential genes, but the number of genes with a negative impact on the growth rate increased in mutant strains. A similar tendency was observed after changing the carbon source to glycerol. Lethal genes were not significantly modified by environmental conditions, but genes with negativ impact were influenced by the presence of substrates and oxygen or even by changes in redox pathways. Using dynamic simulations under aerobic and microaerobic conditions we have a phenomenon called diauxic (diauxic growth) - previously produced metabolites can be metabolized. The robustness, the impact of important parameters on the growth rates were analyzed using phenotypic phase plane analysis. Qualitatively distinct phenotypes were obtained, determining the isoclines between phases and the line of optimality (which is the optimal relationship between the two metabolic fluxes). For alternative solutions were tested bi-level optimization algorithms like OptKnock and GDLS. The results confirmed the anaerobic conditions for glucose and microaerobic for glycerol without co-substrates (0.6 mol mol^{-1} glycerol). Genes have been eliminated from the system using λ-Red recombineering methods and the metabolic profile was determined using gas chromatography coupled with mass spectrometry.

Original contributions: up-dated metabolic model; role of redox metabolic pathways under different conditions using minimal medium; genetically engineered strains have been designed for the over-production of succinic acid; the effects of co-substrate on biomass and on succinic acid production have been identified for glycerol; genetic changes and environmental conditions effect on genes of the system were identified; diauxic growth identified; several distinct phenotypes were identified for each condition separately; the optimal conditions for succinic acid production.