In silico optimization of the production of amino-acids in Escherichia coli

Rui Pereira¹, Paulo Vilaça^{1,2}, Miguel Rocha², Isabel Rocha¹

¹IBB-Institute for Biotechnology and Bioengineering / Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

² Department of Informatics / CCTC, University of Minho, Campus de Gualtar, 4710-057 Braga – PORTUGAL

Abstract

The increasing need to replace chemical synthesis of compounds of interest by more environmentally friendly biological processes is driving the research for microbial cell factories. The industrial production of amino and organic acids includes several examples of success stories using microorganisms to convert inexpensive substrates into added value products. Traditionally, the design of such microbes relied on cycles of random mutagenesis followed by phenotypic selection [1], but a deeper knowledge of the microbial physiology allowed a more rational approach to this optimization problem [2,3]. However, this task is not straightforward, since the cell metabolism has proved to be highly complex and hard to predict.

One of the approaches to tackle this problem is to use Systems Biology simulation tools to predict the microorganism behavior when subjected to genetic modifications. Using genome scale stoichiometric models, such as the latest iAF1260 for *Escherichia coli* [4] one can simulate a great diversity of possible metabolic phenotypes under steady state conditions by imposing flux-balance constrains. The use of flux balanced analysis (FBA) allows the determination of flux values through all the reactions in the network under a set of environmental conditions and genetic manipulations, by using an objective function, such as the maximization of growth [5]. In this work, we used genetic algorithms, such as OptGene [6] to search for sets of gene knockouts that result in the overproduction *in silico* of amino-acids in *Escherichia coli*.

From all the proteinogenic amino-acids, glycine yielded the best results in the optimizations. A careful analysis of the *in silico* flux distribution in some of the mutants revealed an interesting and non-intuitive mechanism behind glycine accumulation. Furthermore, in these mutants the growth is coupled to the production of glycine, which makes them excellent candidates for *in vivo* implementation.

We are reaching a point where bioinformatics tools are advanced enough to aid in complex tasks, such as the optimization of microbial cell factories. Here we described an effort to optimize *in silico* the production of amino-acids in *Escherichia coli*, which resulted in the discovery of a potential set of knock-outs that leads to glycine overproduction. This serves to show the increasing importance of *in silico* optimizations to aid in the metabolic engineering projects, especially to search for non-intuitive beneficial genome modifications.

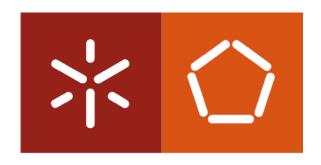
References

- 1. K. Okamoto et al. *Bioscience, Biotechnology, and Biochemistry* **61** (1997) 1877-1882.
- 2. A. Ozaki et al. Agricultural and Biological Chemistry 49 (1985) 2925–2930.
- 3. M. Ikeda Applied Microbiology and Biotechnology 69 (2005) 615-626.
- 4. A.M. Feist et al. *Metabolic Engineering* **12** (2010) 173-186.
- 5. K.J. Kauffman et al. Current Opinion in Biotechnology 14 (2003) 491-496.
- 6. K. Patil et al. BMC Bioinformatics 6 (2005) 308.



Detailed Program

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