RESEARCH

Introduction to Focus Areas in Bioinformatics Project Week 9

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Abstract

Goal of the project: Use CobraPy to simulate the lycopene production with knockouts and overexpressions from Alper et. al. 2005 [1].

Main results of the project: The results could be accurately modeled without applying combinatorial knockouts.

Personal key learnings:
Sina & Christina: working with CobraPy
Gokul : Understanding gene knockouts, stochiometric and Flux Balance Analysis
Swetha : Understanding the biological and metabolic working of lycopene biosynthesis

Estimation of the time:
Sina: 7h
Christina: 7h
Swetha : 4h
Gokul: 4h

Project evaluation: 2

Number of words: 1239

1 Scientific Background

The introduction of genetic controls was to improve cellular phenotype, such as metabolite overproduction, and is the core goal of metabolic engineering. When evaluating the full bioreaction network, it was unclear how to systematically identify gene targets [2]. In the context of lycopene production in Escherichia coli, these difficulties were tackled computationally and experimentally.

The computational search makes use of a stoichiometrically balanced, genome-wide bioreaction network of E. coli metabolism, whose fluxes are estimated in the context of flux balance analysis (FBA) to maximize cell growth yield [3]. The challenges of gene target identification were addressed further in the context of heterologous lycopene production in E. coli using the non-mevalonate pathway. Lycopene is synthesized in E. coli utilizing glycolytic intermediates to create precursor monomers, which are then polymerized to make the 40 carbon biopolymer [4]. To build a rich library of carotenoids, the isoprenoid pathway and downstream reactions were used.

Several possible single- and multiple-gene knockouts with increased lycopene production were discovered through a computational search [5]. Scan of the E. coli genome for single gene knockout targets. FBA was used to mimic the phenotype of

each potential single gene deletion, with MOMA as an extra constraint. The regulation of multiple genes is required to optimize a secondary-metabolite phenotype, such as lycopene synthesis.

Multiple gene knockouts must be examined in the same way. The problem is that a thorough examination of all potential gene knockout combinations quickly leads to a combinatorial explosion: 965C2 combinations of all possible double mutants, and so on [6]. As a result, single gene knockouts are frequently explored in the genetic background of deletion mutants selected for their enhanced phenotype from prior iterations, using sequential and iterative optimization methodologies [1]. Double mutants were found by examining the effect of further gene knockouts in the genetic background of the single gene knockout, and so on for higher mutants, after identifying a gene whose deletion produced maximum lycopene enhancement [1]. An approach for rational strain creation that uses a global stoichiometric analysis to identify single and multiple gene knockout targets. The pre-engineered strain employed in the study had dxs, idi, and ispFD chromosomal over expressions.

2 Modeling

Identification of genes that affect the product accumulation phenotype of recombinant strains is an important problem in industrial strain construction and a central tenet of metabolic engineering [7]. The concurrent changes of metabolic intermediates and retraining of regulatory pathways are typically required for metabolic phenotype optimization.

2.1 Gene Knockouts

Gene knockout is a gene deletion approach in an organism and is usually implemented to investigate the gene function with the effect of a targeted gene loss. Here we experimented to understand the specific role of the target gene by comparing the knockout system to a wild type with the same scientific background. Gene knockouts are crucial tools in the development of drugs, understanding metabolic pathways and genetic deficiencies with help of a target organism.

2.2 Stoichiometric Modeling

Stoichiometric models describe cellular biochemistry with systems of linear equations. [8] The systems that are dependent on the steady-state hypothesis are quite simple to create and could be adapted to networks as large as the genome. Stoichiometric models are often used to assess the metabolic flux allocation in a cell under specific conditions at a specific time (metabolic flux analysis), as well as to estimate it based on an optimality premise (flux balance analysis).

2.3 Flux Balance Analysis

Apart from traditional methods for constructing a metabolic model, Flux Balance Analysis (FBA) requires a minimal intense input which leads in a precise estimation of the effect of gene knockouts in a metabolic model. Flux Balance Analysis (FBA) plays a key biotechnological role in bioprocessing engineering, to make modifications in microscopic biological systems to increase the production yield. In our work, it is lycopene (a bright red carotenoid pigment) produced by significant strains of E.coli.

3 Results

First, the model for the E. coli wild type was loaded. To simulate the growth in an aerobic and glucose minimal area, the lower bounds for oxygen and glucose were set. Afterward, the missing metabolites and reactions were added.

3.1 What is the theoretical maximum yield of lycopene (mol lycopene/mol glucose)?

To simulate the theoretical maximum yield of lycopene, we adjusted the objective for the lycopene biosynthesis. This resulted in a maximum yield of 0.1102 mol/mol glucose.

3.2 How much lycopene is produced by the wild-type strain that has been extended with the lycopene pathway?

Additionally, the lycopene produced by the wild-type strain was calculated. The model showed a yield of 0.0 mol/mol glucose. There is no lycopene production because the biosynthesis is inactive in the wild-type strain. It can be activated through overexpression. This is also reflected in Figure 2, where the yield for all wild-type simulations is zero.

3.3 How much lycopene is produced in mutant strains with gene knockouts?

Then, we analyzed how knockouts influence lycopene production in the wild type. Alper et. al. [1] mentions four stoichiometrically and three combinatorially found knockout genes. These genes were compared to the model. However, the three combinatorial genes are not part of the model. They correspond to hypothetical proteins, which are not distinct in the model documentation. Therefore, only combinations of the four stochiometric genes, gdhA, aceE, yjtC, and fdhF, could be tested. These targets are also validated by Alper et. al. in other papers [4].

Figure 1 shows, how the growth rate was influenced by different knockouts. In the wild type (orange bars), deactivating gdhA or aceE seems to have the greatest influence. On the contrary, yjtC and fdhF do not change the growth rate. Since the wild type does not produce lycopene, no changes can be seen in the lycopene production (Figure 2). However, we used the model with a maximized theoretical yield to test the knockouts (green bars). Here, the lycopene yield was not influenced by the knockouts.

3.4 How much lycopene is produced in mutant strains with genes overexpressed?

Next, we applied overexpressions used by Alper et. al. [1] to produce lycopene in in-vitro cells. These overexpressed genes are dxs, idi, ispF, and ispD. First, an FBA was conducted, that analyzed every reaction the genes take part in. This was used to maximize the lower bound of each reaction. The resulting lycopene yield is 0.049974 and the growth rate is 0.542634. These cells are the only growing cells that produce lycopene and with that the only cells viable for in-vitro experiments.

3.5 How much lycopene is produced in mutant strains with overexpression and knockouts?

Finally, we combined the overexpression with the different knockouts. That way, we could see the influence of the different knockouts on realistic cells. It is notable,

that neither the growth rate nor the lycopene yield is severely influenced by the knockouts. Only in the lycopene production yield differences are visible. The values vary between 0.499743 and 0.499753.

These results are much less clear than those of the in vitro experiments in the paper [1]. A reason for that could be the missing combinatorial knockouts. The results seem similar to the production without combinatorial knockouts.

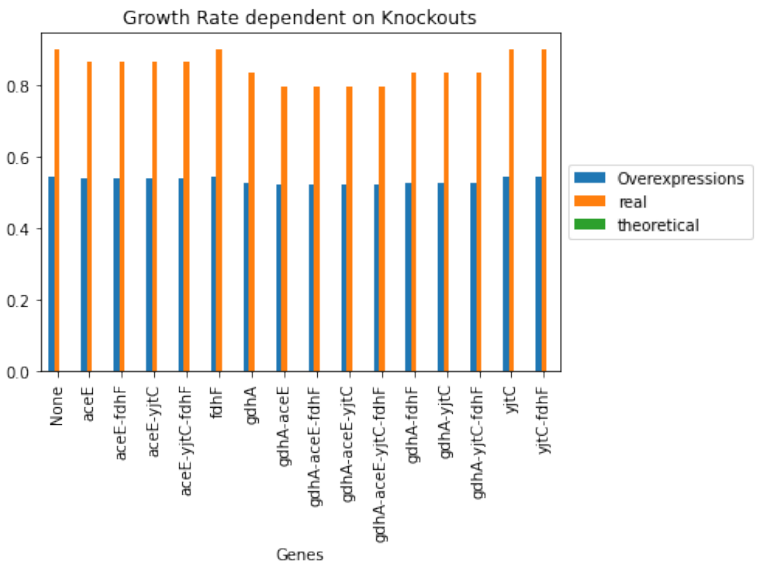

Figure 1: **Growth Rate** This plot shows, how the different knockouts influence the growth rate. The bars are colored by the settings of each model.

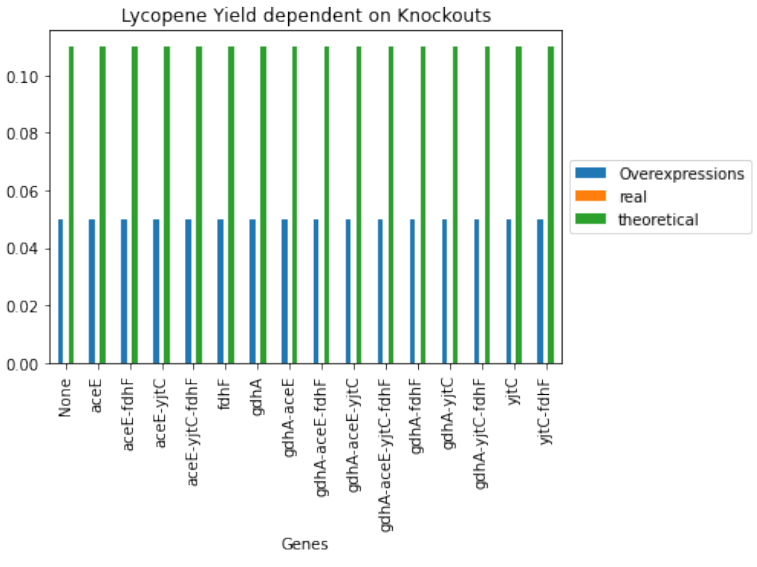

Figure 2: **Lycopene Yield** This plot shows, how the different knockouts influence the lycopene yield.

Competing interests
The authors declare that they have no competing interests.

Author's contributions
Gokul Thothathri and Swetha Rose Maliyakal Sebastian wrote about the Scientific Background and the Modeling. Sina Glöckner and Christina Kirschbaum implemented the model and wrote the Results.

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