INTRODUCTION TO FOCUS AREAS IN BIOINFORMATICS - WS19/20

Project 8: Lycopene production in E.coli

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Abstract

Goal of the project: This week's project was centered on using flux-balance analysis to calculate the amount of lycopene in E.coli after inserting DNA for lycopene production.

Main result(s) of the project: We can optimize the theoretical maximum yield of lycopene by overexpressing and knocking out certain genes.

Personal key learnings: E.coli does not produce lycopene by itself since it doesn't need lycopene to grow. We have to modify gene expression to have E.coli produce lycopene.

Estimation of the time: 16 hours

Project evaluation on a scale of 1-5: 2

Word count: 501

Keywords: E.coli; lycopene; flux-balance analysis; Python

Creation of the model

Description of model analysis

Flux balance analysis (FBA) is a mathematical method for simulating metabolism in genome-scale reconstructions of metabolic networks. Assuming there is no change in the concentrations of the metabolite we can calculate steady-state metabolic fluxes. Because we want to accumulate lycopene to E.coli, we have to add lycopene-coding DNA to its wildtype strain. We can create such a model in the a Google Colab and calculate the flux.

Metabolites and reactions

Using the iJE660 E.coli model of the COBRApy framework, we now add the lycopene pathway by including three new metabolites. As mentioned in the lecture's supplementory paper by Alper et al. these are geranylgeranyl pyrophosphate (ggpp_c), phytoene (phyto_c) and of course yycopene (lyco_c) itself. For these we then had to introduce new reactions as well to have them make an impact on the model.

Methods to manipulate lycopene production

To see changes in the flux an therefore the production of lycopene we need to modify the pathway of E.coli in such a way that it produces more lycopene. This can be done by overexpressing genes or knocking out genes that hinder lycopene from being produced. We can use both methods as well as a combination of those to optimize the production of lycopene.

Results

Theoretical maximum yield

We initially set the objective to lycopene, so that the pathway proceeds with the lycopene production only. This approach lets us optimise the lycopene yield. Hence we can obtain the theoretical maximum yield which was 0.11 mol/mol glucose as seen in figure 1.

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Growth Rate (1/h): 0.0
Lycopene Production Rate (mmol/gdcw/h): 1.101916572717023
Lycopene Yield (mol/mol glucose): 0.1101916572717023
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Figure 1 Theoretical maximum yield of lycopene

Yield in a extended wildtype strain

Wild type strain only produces the low quantity of lycopene and the biomass as the reactions are slow in the pathway. Hence the lycopene will be less or minimum. The lycopene obtained was 0.00 mol/mol glucose

Yield with gene knockouts

Here we knocked out certain number of genes in order to know the significance of these genes. We knocked out gdhA, aceE, ytjC(gpmB), fdhF according to the reference from the article. The lycopene yield obtained was 0.00 mol/mol glucose indicates the genes not involved in the lycopene production pathway.

Yield with overexpression

Here, genes such as dxs, idi, ispFD when overexpressed changes the whole ecosystem of the metabolic pathway. Hence we get change in the lycopene yield with 0.09 mol/mol glucose.

Yield with gene knockouts and overexpression

In the mutant strains, when genes were both knocked out and overexpressed, the overexpression and the knock out both affects the quantity with the lycopene yield of 0.024 mol/mol glucose.

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Growth Rate (1/h): 0.7085755330435601
Lycopene Production Rate (mmol/gdcw/h): 0.2496644894851038
Lycopene Yield (mol/mol glucose): 0.02496644894851038
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Figure 2 Lycopene yield with gene knockouts and gene overexpression