Metabolic engineering of *Escherichia coli* for biotechnological chondroitin production

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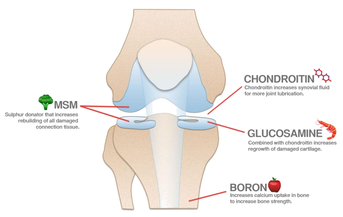
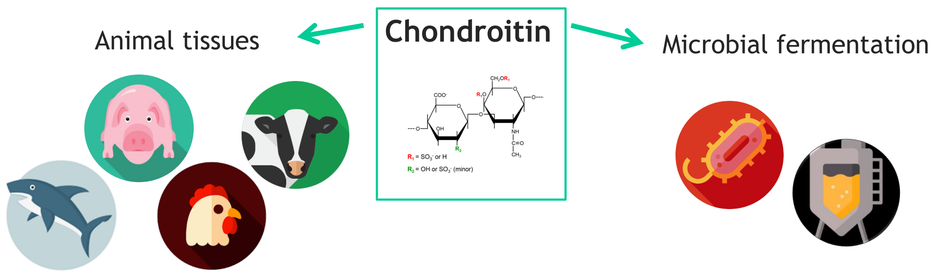
Introduction

Chondroitin is a **glycosaminoglycan** which main application is as anti-inflammatory supplement for **osteoarthritis.**



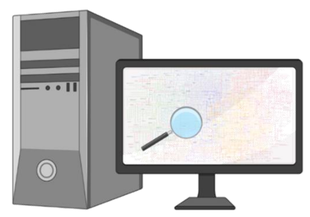
Most of the **commercial chondroitin** results from chemical extraction from **animal** cartilage. There is a need to design efficient **microbial cell factories to replace animal sources.**



× High heterogeneity
× Risk of pathogen contamination
× Environmentally hazardous extraction

✓ Vegan/ vegetarian alternative
✓ Safer
✓ Easy control of process and product quality

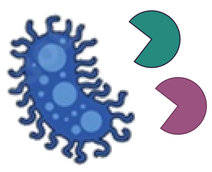
The construction of **genome-scale stoichiometric models** for engineered microorganisms harbouring the chondroitin biosynthetic pathway and further *in silico* analysis are useful tools to **predict novel and more efficient strains.**



Methods

Construction of the metabolic model

Introduction of missing genes, metabolites and reactions in the metabolic model of a **non-pathogenic *Escherichia coli*** (iB21_1397), for chondroitin production



Heterologous enzymes:
KfoA/ UAE: UDP-N-acetylglucosamine 4-epimerase

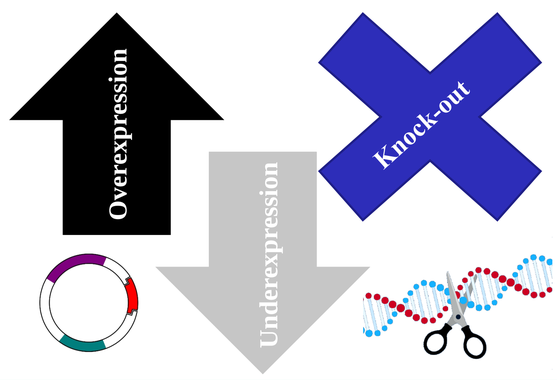
KfoC/ CHSY: chondroitin synthase

Evolutionary optimization

Prediction of modifications in expression of genes (knock-outs, underexpressions and overexpressions) that would enhance chondroitin yields.
Algorithm combining 2 objectives: Biomass-coupled yield (BPCY) and Weighted Yield (WYield)

Optflux — Phenotype simulation

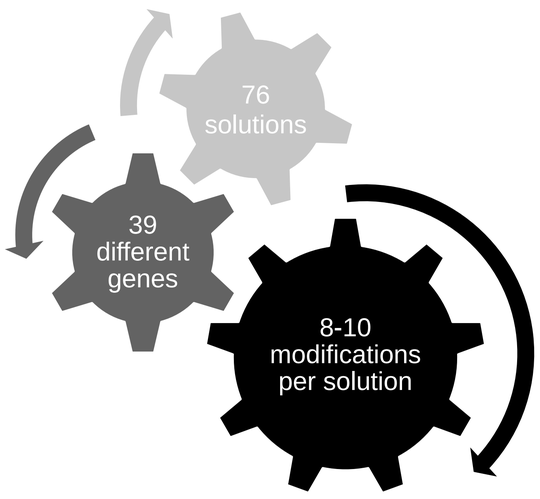
Metabolic engineering



Results

Evolutionary optimization

Novel predictions of modifications to improve chondroitin production!



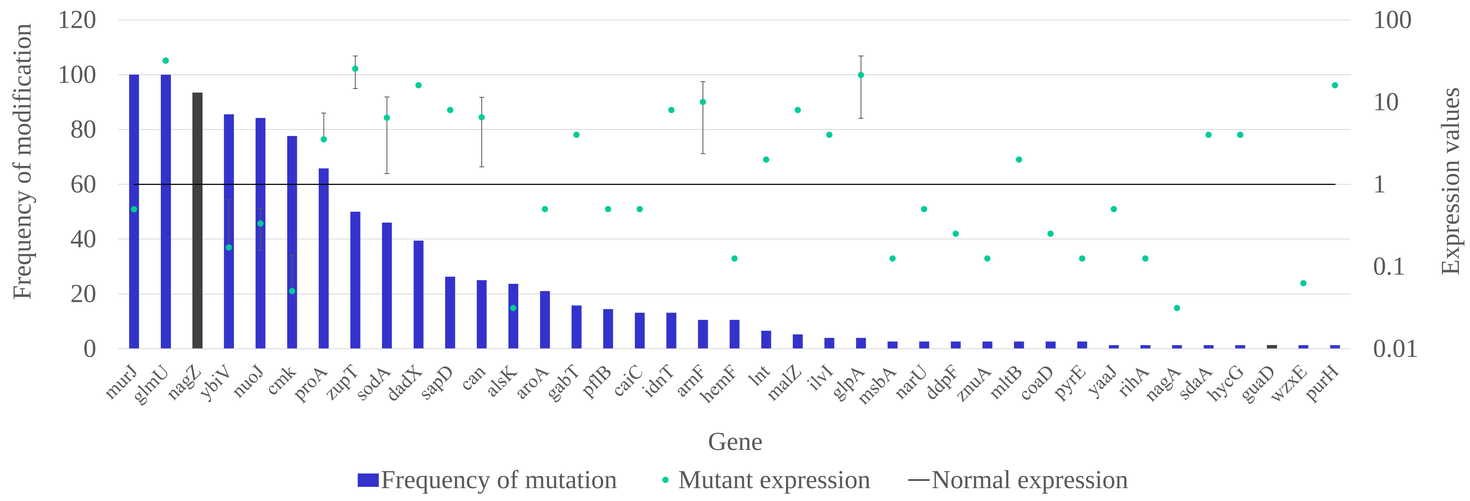


Figure 1. Frequency of gene modifications in the obtained 76 solutions and value of gene expression for each gene. Grey bars of genes *nagZ* and *guaD* correspond to knock-outs, where expression values are 0.

Table 1. Solutions from evolutionary optimization of *Escherichia coli* BL21 model with highest Biomass-Product Coupled Yield (BPCY) with the corresponding Weighted Yield (WYield), genes modified according to the type of expression modification, and the estimated biomass and chondroitin, as calculated in Optflux.

BPCY	WYield	Knock-Out	Underexpression	Overexpression	Biomass ($mol\ g_{cells}^{-1}\ h^{-1}$)	Chondroitin ($mol\ g_{cells}^{-1}\ h^{-1}$)
1.41649	2.91027	*nagZ*	*ybiV, alsK, aroA, pflB, murJ, narU*	*sodA, glmU, mltB*	0.30401	2.90794
1.41649	2.91027	*nagZ*	*ybiV, aroA, pflB, murJ, znuA*	*sodA, lnt, glmU, purH*	*	*

*Biomass and chondroitin yields too low to be handled by Optflux

Metabolic engineering

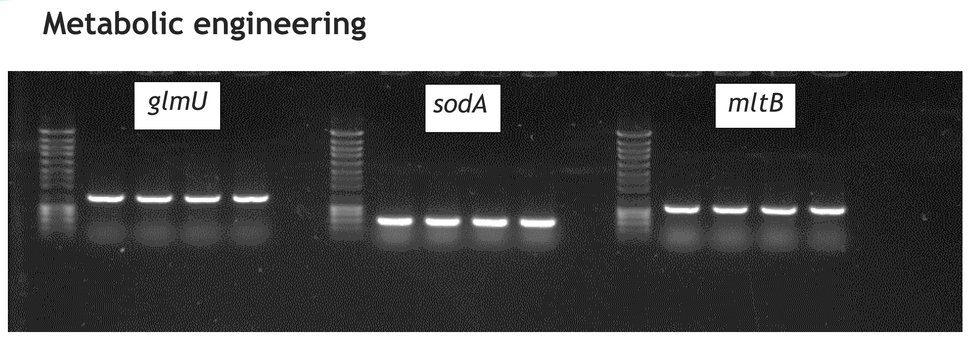


Figure 2. Gene amplification for cloning and expression in *Escherichia coli* strains engineered with chondroitin biosynthetic pathway.

Conclusions

The *in silico* prediction of *Escherichia coli* mutants for chondroitin production can provide novel and more efficient strains to replace the use of animal tissues as chondroitin source. The predicted mutations will be further validated *in vivo*.

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