Session 1
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17:00 h

Stoichiometric genome-scale models for the chondroitin production in *Escherichia coli*

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Chondroitin is a natural-occurring glycosaminoglycan with applications as a nutraceutical and pharmaceutical ingredient. It can be extracted from animal tissues, though chondroitin-like polysaccharides using microorganisms emerged as a safer and more sustainable alternative source. However, chondroitin yields using either natural or recombinant microorganisms are still far from meeting the increasing demand. In this work, stoichiometric models containing the heterologous pathway necessary for producing chondroitin in *E. coli* were constructed and investigated for mutant predictions that would potentially improve chondroitin yields. Four models of *E. coli* BL21 (BIGG ID: iECBD_1354, iECD_1391, iEC1356_Bl21DE3, iB21_1397) and one of *E. coli* K12 (BIGG ID: iJO1366), from which the other models were derived, were used to insert the heterologous pathway composed by two enzymatic steps catalyzed by UDP-*N*-acetylglucosamine 4-epimerase (UAE) and chondroitin synthase/polymerase (CHSY). The models were imported in Optflux, and the evolutionary optimization was then performed for gene deletion predictions using Strength Pareto Evolutionary Algorithm 2 (SPEA2) and the parsimonious Flux Balance Analysis (pFBA) as the simulation method. Chondroitin production was not predicted to improve by combining gene deletions, probably because the competing pathways that use the intermediates are critical for cell growth. However, gene over and underexpression search allowed to identify several targets. Most of the resulting solutions were composed by the overexpression of one of the genes responsible for the production of the heterologous pathway precursor (either *glmU* or *glmM* encoding glucosamine-1-phosphate *N*-acetyltransferase/UDP-*N*-acetylglucosamine diphosphorylase and phosphoglucosamine mutase, respectively) combined with the underexpression of one of the genes associated with cell wall recycling pathways (such as membrane-bound lytic transglycosylases *mltA*, *mltB* and *mltC*, or the anhydromuropeptide permease *ampG*), which contain reactions known to consume such precursors. The solutions herein obtained will be further validated *in vivo* by constructing the *E. coli* mutants predicted to improve chondroitin production.

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