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Application of a genome-scale metabolic model to the inference of nutritional requirements and metabolic bottlenecks during recombinant protein production in *Escherichia coli*

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Background

Escherichia coli has been the organism of choice for the production of many recombinant proteins with high therapeutic value. However, while the research on molecular biology has allowed the development of very strong promoters, there are still some phenomena associated with this process that hamper the full use of those technologies like the so-called stringent response, caused by an imbalance in intracellular amino acid uptake and the endogenous amino acid synthesis rates. It occurs due to high demands of certain amino acids for recombinant protein production [1], causing several cellular responses, from the inhibition of the synthesis of rRNA to the induction of proteases. The main consequence of this response is a decrease in process productivity due to the lower specific growth and production rates observed.

An important research topic is the identification of the metabolic fluxes that suffer major changes during protein production, giving information that can be used for the formulation of new strategies for improving the process performance either by genetic or operational manipulations.

In this work, a recombinant protein production process was analysed using genome-scale models and Flux Balance Analysis (FBA) [2] in order to identify potential sources of metabolic bottlenecks that can trigger the stringent response.

Results

The existing genome-scale metabolic model of *E. coli* [3] was modified by including an equation for protein production (the eYFP – enhanced Yellow Fluorescent Protein), based on its amino acids content. Additionally, equations that represent the metabolic burden caused by the presence of plasmids were added, based on knowledge about the precursor balances and energetic requirements for plasmid replication and marker protein expression [4]. This modified metabolic model was used on simulations using the FBA approach [2], optimizing for biomass and for recombinant protein synthesis.

When comparing the distribution of internal fluxes for both cases, it was observed that most fluxes over amino acid biosynthetic reactions suffered an increase in the recombinant protein production experiment (flux variation (a) in Table 1).

Differences in amino acid composition between average proteins in *E. coli* and eYFP. The variation of fluxes across major amino acid biosynthetic routes is also shown when only recombinant protein is produced (a) and when both biomass and recombinant proteins are being formed (b)

Interestingly, there is not a clear correspondence between those differences and the ones found in the relative amino acid composition of biomass and eYFP. This demonstrates that it is very difficult to predict the consequences of the production of recombinant proteins in amino acid biosynthetic pathways.

Table 1

Amino acids	Biomass	eYFP	Flux variation (a)	Flux variation (b)
L-ala	9,6	3,8	-38%	1%
L-arg	5,5	2,5	-19%	-11%
L-asn	4,5	5,5	122%	69%
L-asp	4,5	7,6	23%	13%
L-cys	1,7	0,8	31%	17%
L-gln	4,9	3,4	-59%	-33%
L-glu	4,9	6,7	38%	22%
Gly	11,0	9,2	21%	12%
L-his	1,8	4,2	351%	199%
L-ile	5,4	5,0	75%	42%
L-leu	8,4	8,4	84%	48%
L-lys	6,4	8,4	142%	80%
L-met	2,9	2,5	57%	32%
L-phe	3,5	5,5	189%	107%
L-pro	4,1	4,6	106%	60%
L-ser	4,0	3,8	20%	12%
L-thr	4,7	5,9	101%	57%
L-trp	1,1	0,4	-6%	-3%
L-tyr	2,6	5,0	268%	152%
L-val	7,9	6,7	58%	33%

Table 2: Differences in carbon dioxide secretion rate and in requirements regarding ammonium, sulphates and oxygen when the metabolism is optimized for recombinant protein production as opposed to biomass production.

	Flux Variation (a)	Flux Variation (b)
NH_4 requirements	16%	9%
O_2 requirements	-27%	-15%
SO_4 requirements	31%	17%
CO_2 secretion rate	-23%	-13%

In a second experiment (Flux variation (b) in Table 1), the solution space of the genome-scale metabolic model was constrained by imposing limits on the protein production flux according to what was observed experimentally – 0.06 g protein.g^{-1} biomass. h^{-1} - while optimizing for biomass, forcing the model to represent both growth and protein production simultaneously. Taking again the optimization of biomass growth with no restrictions as reference, a trend similar as before was observed, although the variations are lower.

Finally, cellular requirements represented by input fluxes to the cell of relevant nutrients were compared. It can be seen that recombinant protein production imposes additional demands regarding ammonium and sulphates, while both oxygen requirements and carbon dioxide excretions are lower (see Table 2).

Conclusion

The application of Flux Balance Analysis to a modified genome-scale model of *E. coli* allowed the identification of the metabolic fluxes for which there is a predicted increase when recombinant proteins are being expressed, elucidating the shift in metabolism that is necessary to occur upon induction. This insight can help to identify the main sources for the observed stringent response and to design strategies to avoid the occurrence of that phenomenon.

The need of high amino acid content for recombinant protein production also affected the ammonium, sulphates and oxygen requirements, giving useful indications for medium design.

However, for the clarification of these phenomena associated with recombinant protein production it is now important to proceed with the integration of the information obtained from genome-scale models with experimental data obtained from transcriptomics, proteomics and metabolomics.

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