In silico study of the metabolic impact of a gradual shift in the stoichiometry of the Ebdem-Meyerhof pathway in *Escherichia coli*

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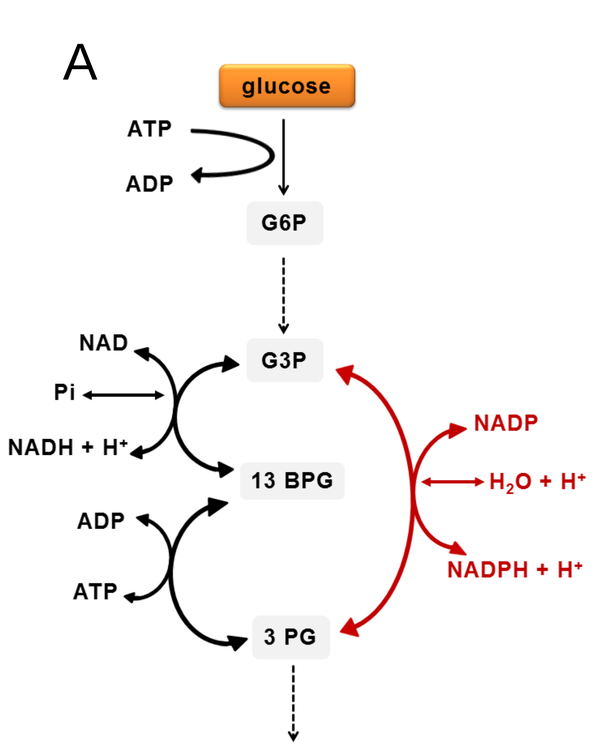
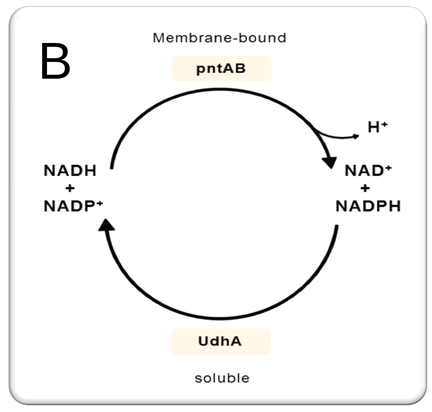
Abstract: Although the network topology of the metabolism is well known, the understanding of the principles that regulate the distribution of the fluxes through the metabolic pathways lags behind. In this project we show the results of different *in silico* experiments where the native NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (GAP) is gradually replaced by the NADP-dependent non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GANP). This way it is possible to analyze the effects of intermediate situations rather than a complete substitution of one enzyme by the other. The induced shift provoked changes in the distributions of the metabolic fluxes and the mechanisms to accomplish the NAD(P)(H) balances. We also analyzed the impact of the aforementioned shift in the generation of poly-hidroxy-butyrate (PHB) using a NADPH-consuming pathway. The results suggest that a total substitution of GAP by the NADPH-producing GANP is not the best strategy to enhance the production of PHB in this bacterium.

Methods:

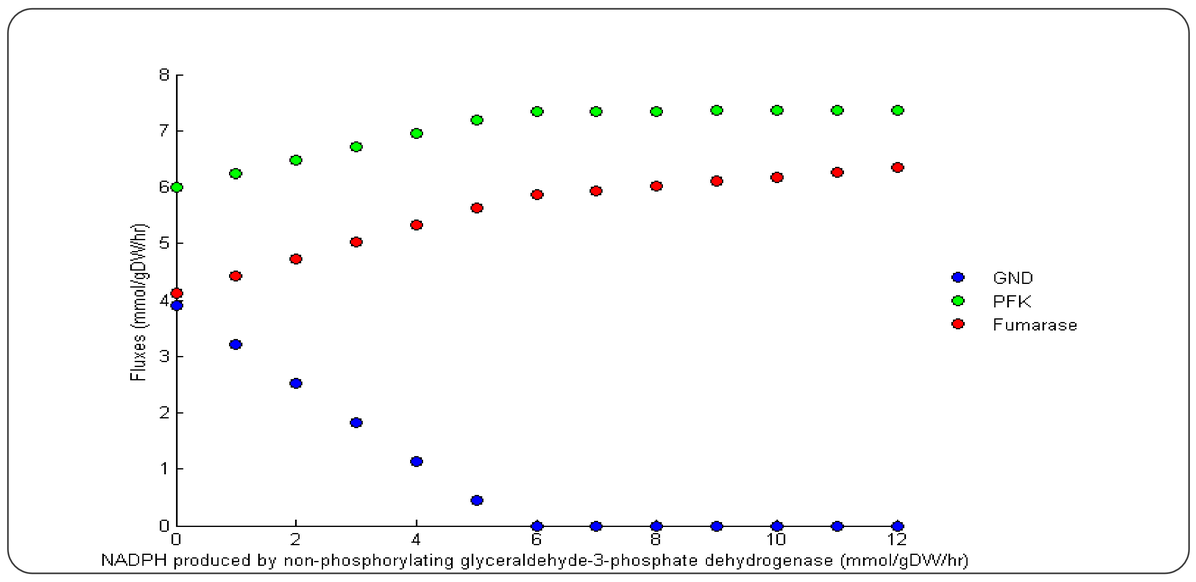
Flux Balance Analysis (**FBA**) (Orth, 2010) was performed with the COBRA toolbox v 2.0 (Schellemberger, 2011) in MATLAB. It was employed the *in-silico* model Ecolicore embracing 95 reactions and 72 metabolites. The Ecolicore model and COBRA are freely available from the web site of the Systems Biology Research Group, UCSD, USA.

Graphic representations were done in MATLAB and Microsoft Excel.

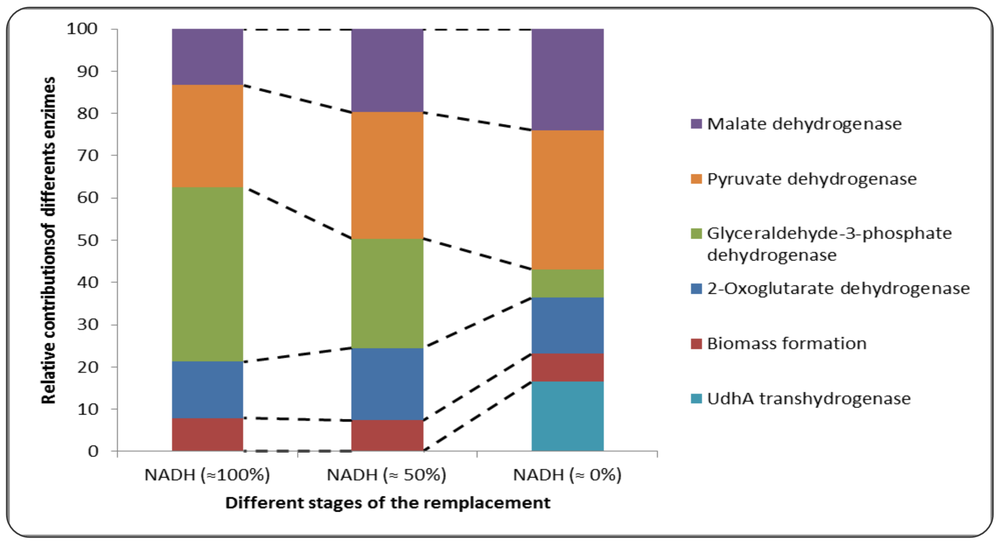




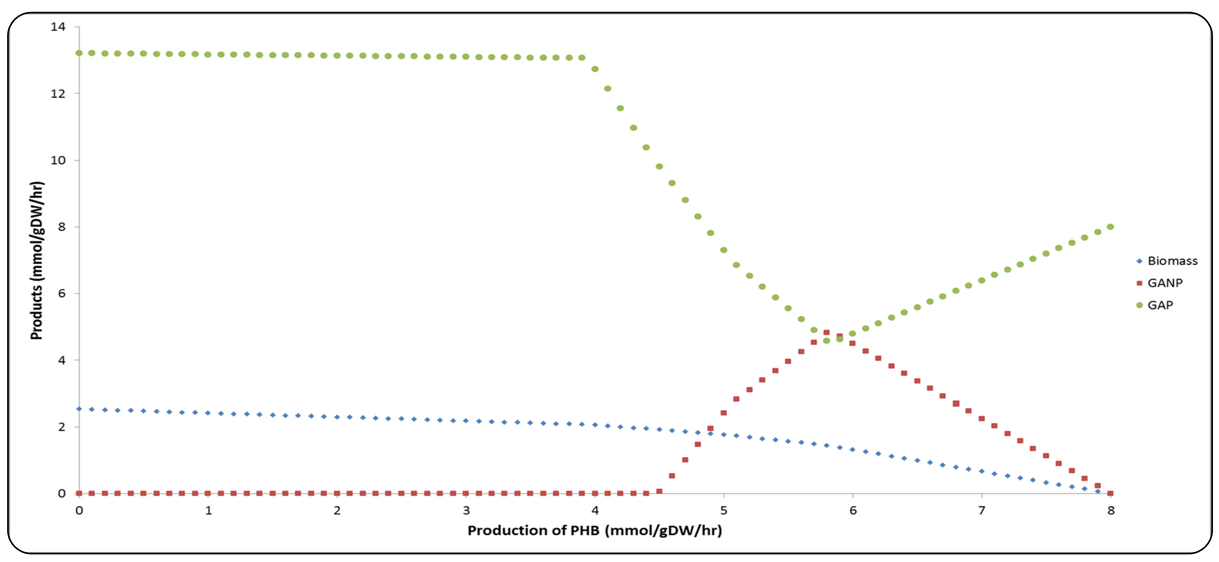
From the point of view of the cofactor balance, the replacement of GAP by GANP (A) is similar to an increase in the flux through the transhydrogenase pntAB (B). However, in the case of the shift from GAP to GANP, the generation of the cofactors NADH, NADPH and ATP is directly coupled to the carbon flux.



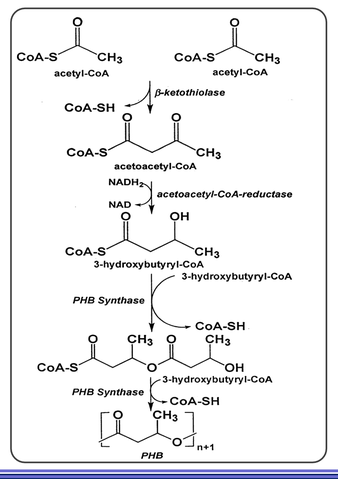
Changes in the metabolic fluxes through the oxidative branch of the pentose-phosphate pathway (GND), the upper Ebdem-Meyerhof pathway (PFK) and the lower Kreb's cycle (fumarase) coupled to the increase in the flux through GANP. The result points to a diminution in the flux through an exclusive NADPH producing pathway and a increase in the fluxes through NADH-producing pathways. Some reducing equivalents from NADPH must be transferred to NAD using the UdhA (see bars diagram).



Relative contributions of the different NADH and NADPH sources during the transition from GAP to GANP. The intermediate columns represent intermediate situations. In the most extreme case, GANP produced more than 50% of the total NADPH.



Impact on the fluxes through GAP, GANP and the biomass formation reaction of an increase in the flux through the pathway producing PHB. Although the biomass steadily decrease (as expected) with the increment in the generation of PHB, the changes in GAP and GANP are no so obvious: the flux through the NADPH-producing GANP has a peak and a further diminution.



Conclusions:

- During the shift from an exclusive use of GAP to a predominant use of GANP, the NADH sources are diversified while GANP becomes the predominant source of NADPH.
- **FBA** allowed us to identify a non obvious effects of the substitution of GAP by GANP: the excess of NADPH decreases the growth rate.
- Despite PHB production requires NADPH, the complete substitution of GAP by GANP will not necessarily improves the yield of this polymer.

Perspectives:

- Compare the effects of increasing the flux through the membrane-bound transhydrogenase pntAB with the effects of the gradual shift from GAP to GANP.
- Construction of a recombinant *E. coli* strain with the capacity to gradually shift from the use of GAP to the use of GANP.

References:

- **Orth, J.D.,** Thiele, I. & Palsson, B. What is flux balance analysis? *Nat Biotechnol* **28**, 245-248 (2010).
- **Schellenberger, J. et al.** Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0. *Nat Protoc* **6**, 1290-1307 (2011).