EVALUATING ORNITHINE EFFECTS ON CLAVUNIC ACID BIOSYNTHESIS WITH THE AID OF METABOLIC FLUX ANALYSIS

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Introduction. The aim of metabolic flux analysis (MFA) is to illustrate how flux is distributed through a metabolic pathway. It provides a holistic treatment of cellular metabolism and is the most commonly modeling tool currently used. MFA involves the calculation or estimation of in vivo fluxes from substrate and product experimental data, using a system of linear equations built just from the knowledge of reaction stoichiometry [1].

In this work, we used MFA to study the *Streptomyces clavuligerus (Sc)* metabolism for clavulanic acid (CA) production. We were interested in discerning which of the measured metabolic fluxes did significantly impact the cellular system, and, how CA biosynthesis was affected by the dilution rate (D). A quantitative description for the individual flux variation effect of CA accumulation was also studied.

Methods. The proposed metabolic pathway relied on literature information [2]; the metabolic model consisted of 60 biochemical reactions and 47 metabolites, including internal balanced metabolites, internal energy cofactors and extracellular metabolites. In order to solve a determined system, a set of 13 measurements were provided based on experimental work at two different D [3]. These did include, glycerol (GLC), asparagine, NH4 and oxygen uptake; CA, aminoacid biosynthesis and biomass production were taken from Sánchez et al., (2012) [4]. MFA was performed using the software Cell Net Analyzer, to provide an admissible flux distribution that satisfied the steady state balance. Metabolic concentrations values were normalized based on the initial GLC concentration, at the highest D.

Results A closer look at the Fig.1, allows us to observe how ornithine (ORN) concentration changes as a result of D variations, showing, not only the same flux of CA, but a reduction (to a half) at higher D; aspartate (ASP) related flux distribution showed lower values at the lower values of the D. Conversely, high D values caused decreasing of the cofactor NADPH. Flux through the pentose phosphate pathway (PP) was favored at high D, as it was expected. In turn, lower D also did favor TCA precursor consumption. Putting together all these observations, did let us to infer, as a first result, that a feasible strategy for increasing CA biosynthesis would be to amend the urea cycle, by intervening on ORN flux more than on the one of arginine (ARG).

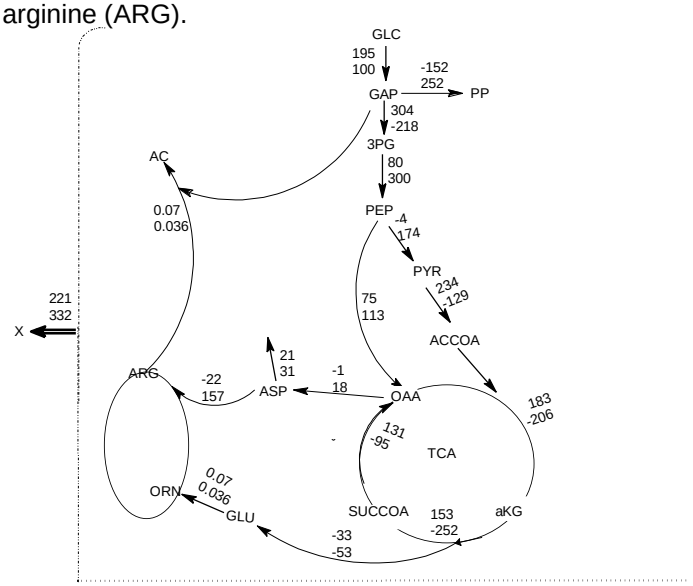

Fig. 1. . Distribution of metabolic fluxes of CA production from *Sc*. The upper values correspond to the $0.02h^{-1}$ D; the lower ones correspond to the $0.03h^{-1}$ D. abbreviations: glyceraldehyde phosphate (GAP), three phospoglycerate (3PG), phosphoenolpyruvate (PEP), acetylCoA (ACCOA), oxaloacetate (OAA), alphacetoglutarate (aKG), glutamate (GLU), pyruvate (PYR).

Conclusions. By observing the complete metabolic flux distribution, one can state that the CA precursor ORN, augmented at lower dilution rate, thus improving the CA.

High Dilution rates did also favor ASP and TCA precursor consumption, but not CA production. Therefore, a feasible strategy for increasing CA biosynthesis might be the ORN supplementation. It is also strategic to explore more about the urea cycle, for the purpose of understanding the role of ORN and ARG precursors and its influence on CA biosynthesis.

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