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METABOLIC FLUX ANALYSIS TO STUDY THE PRODUCTION OF CDA, BY STREPTOMYCES COELICOLOR

Authors:
Raul Munoz-Hernandez (*The University of Manchester*)
Ana Katerine de Carvalho Lima Lobato (*The University of Manchester*)
Ferda Mavituna (*The University of Manchester*)

Short Abstract: Streptomyces coelicolor, produces many secondary metabolites. Of these, CDA is of great interest; especially for its potential applications in novel Drug Development. In this work, a metabolic model for it was constructed using the Metabolic Flux Balancing method. This model is being applied to develop strategies to increase CDA productivity.

Long Abstract:

Gram positive bacteria such as Staphylococcus aureus are the main cause of hospital-acquired infections and have increasingly acquired resistance to most common antibiotics who share structure and mechanism of action. So new drugs, with different structure and way of action are needed.

In September 2003, daptomycin, a new class of antibiotic, was approved by the US FDA for the treatment of infections caused by Gram positive bacteria. It has been proved that, in vitro, daptomycin has bactericidal activity against vancomycyn-resistant Staphylococcus aureus (VRSA) and vancomycyn-resistant Enterococcus faecium (VREF). As daptomycin, the Calcium Dependent Antibiotic (CDA) from Streptomyces coelicolor shares a similar structure and possibly mode of action.

Performing Metabolic Flux Analysis, previously Kim et al., (2004) found that CDA was concomitant with growth. In addition, they found that CDA produced in a batch culture was affected by nitrogen assimilation, pentose phosphate pathway, shikimate biosynthesis, and oxoglutarate fluxes. In this work, a novel, simplified Metabolic and Mathematical Model for S. coelicolor was solved in GAMS. The model was used for CDA optimization and obtains the internal fluxes. This information and model are being applied to develop strategies such as media formulation, precursor addition and identification of Genetic Engineering targets to increase CDA productivity.

To simulate S. coelicolor growth in fermentation conditions, a metabolic model was reconstructed using databases and specialised articles. The model includes around 250 reactions from primary and secondary metabolism including carbon metabolism, nitrogen metabolism, and nucleotide biosynthesis and other important reactions; as well as macromolecular components, biomass and CDA biosynthesis. To complete the biochemical model, some assumptions were made. Finally, CDA biosynthesis reactions were included in the model.

The mathematical (stoichiometric) model was based on metabolic flux balancing and consisted of linear algebraic equations obtained by applying a pseudo steady state mass balance (Stephanopoulos et al., 1988). Some metabolites were allowed to consume (reactants) or accumulate (products). A Matrix, containing the stoichiometric coefficients of

the set of equations was created in GAMS and solved there optimizing the objective function (biomass or CDA production). The solution gave not only the value of the maximized CDA, but also the value of all the unknown metabolic fluxes. The experimental values for specific growth and glucose uptake at the 75th h of the batch culture (exponential phase) were used as constraints in the model. For the maximisation of the specific growth rate only the experimental specific glucose uptake rate was used; and for the maximisation of the CDA rate, the experimental specific glucose uptake rate and the experimental specific growth rate were used.

Intracellular fluxes were calculated in GAMS. A set of measured extra cellular fluxes feed the calculations. The final outcome was a metabolic flux map with all the biochemical reactions included in the model when biomass or CDA were optimized.
Comparing the metabolic fluxes when CDA was optimized with the metabolic fluxes when biomass was optimized, important changes in some fluxes were observed. A positive difference means that the flux for the reaction in CDA optimization was higher than the flux for the same reaction in biomass optimization; a negative difference means the opposite. The more important changes in the metabolic fluxes will be discussed.
The first important change observed is the flux from glucose to the Pentose Phosphate pathway. When CDA is produced an important amount of energy is required in the form of NADPH, and this is produced in the Pentose Phosphate pathway. The second change was in the flux from PGA3 (glyceraldehyde 3-phosphate) to PG2 (2-phosphoglycerate). This can be explained knowing that PG3 (3-phosphoglycerate) the precursor of PG2, is also precursor of serine and glycine, amino acids presents in CDA. A third change is in the flux from PG2 to PEP (phosphoenolpyruvate). The explanation for this is that PEP is the precursor of HPP (4-hydroxyphenylpyruvate), the precursor of one of the nonproteinogenic amino acids in CDA (HPG; L-4-hydroxy phenyl glycine). Another change is in the flux from PEP to Chor (chorismate). Chor is the precursor of Trp (L-tryptophan) another amino acid in CDA.
A negative variation is observed in the flux from PEP to Pyr (pyruvate). As was explained before, PEP is needed to produce HPG; so, the flux from PEP to Pyruvate is lower when CDA is optimized. Surprisingly, a second negative difference in flux observed is from Pyr to Mal (malate). When CDA is optimized, no flux appeared in this reaction. Equally, in our model, there is a reduction in the flux from Mal to OAA (oxaloacetate) and a reduction in the NADH production in the TCA cycle. An explanation for the reduction in the fluxes is that the carbon flux trough the Pentose Phosphate increases and the carbon flux trough the EMP pathway decreases during the S. coelicolor antibiotic production (methylenomycin); the final product of the EMP pathway is pyruvate, the precursor of malate. In addition, pyruvate is the precursor of AcCoA (acetyl-CoA), which is precursor of MaCoA (malonyl-CoA), both precursors of the fatty acid chain in CDA. Finally, the TCA cycle reduces its activity because NADH is less necessary than NADP to produce CDA. So is understandable the reduction in malate, oxaloacetate and NADH in the TCA when CDA is produced.
The last important differences in fluxes are in NADH oxidation and biomass production. The first one is explained easily; if less NADH is produced in the TCA cycle, then less NADH will be oxidized. The last difference is obvious; the biomass flux in biomass optimization is higher than the biomass flux in CDA optimization.

References

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