Poster J-39

Metabolic flux balance model as a potentially approach for the design and optimization of Cosmomycin production by Streptomyces olindensis ICB20

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Short Abstract: Cosmomycin is an anti-tumor drug produced by Streptomyces olindensis. In this work a computational model based on metabolic flux balancing methodology was developed to investigate some strategies for the selection of media, precursor addition, and genetic engineering targets in order to direct and optimize the production of the product.

Long Abstract:
The organism model studied in this work is the mutant strain of Streptomyces olindensis ICB20 which produces the antibiotic cosmomycin classified as anthracycline. Anthracyclines are potent drugs antitumour globally used in the treatment of several neoplasias. Even though more than 2000 anthracyclines have been isolated or synthesized in laboratories throughout the world only eight new drugs have been introduced into clinical practice. In the case of chemotherapy treatments, the main used anthracyclines is the doxorubicin. However, its used is limited by collateral effects such as cardiotoxity. Another important anthracycline is the cosmomycin. It has similar structure and physical and chemical properties compared to doxorubicin. The low production of cosmomycin by the strain Streptomyces olindensis DAUFPE 5622 associated with the cardiotoxic collateral effects, make it unviable for commercialization purposes. Recently, some studies were accomplished with the strain mutant ICB20 aiming to enhance antibiotic production in bioreactor as well as to obtain higher purity products and less toxicity. cosmomycin is an aromatic polyketide antibiotic complex produced by the condensation of one propionyl-CoA with 9 acetate molecules derived from malonyl-CoA forming a polyketide structure by the action of enzymes known as minimal Polyketide Synthetases (minimal PKS). The structure of Cosmomycin consists of one tetracycline aglycon (β-rhodomycinone) and six deoxysugars (two dTDP-L-2-deoxifucose, two dTDP-L-rodosamine and two dTDP-L-rodinose).
Several research groups have been studying process in bioreactors in order to optimize the cosmomycin production. However, there is still a lack of research in the study of metabolic flux balance. The aim of this work is to develop a computational model based on metabolic

flux balancing methodology for the primary metabolism of Streptomyces olindensis and the biosynthetic reactions of cosmomycin so that metabolic shifts in directing metabolism from growth to cosmomycin production can be investigated. The in silico metabolism is reconstructed involving more than 200 stoichiometrically balanced metabolic reactions in matrix formalism using the information from the literature and databases. The model includes reactions from carbon metabolism, nitrogen metabolism, and nucleotide biosynthesis and other important reactions; as well as macromolecular components, biomass and cosmomycin biosynthesis. The main pathways included are glycolysis, pentose phosphate (PP), trycarboxylic acid (TCA), glyoxylate, anaplerotic reactions, storage reactions, amino acid biosynthesis, pyrimidine and purine biosynthesis, nitrate reduction, sulphate assimilation and folic acid reactions. To complete the biochemical model, some assumptions were made: The amount of precursors and polymerisation energies to produce one gram of protein, RNA and DNA were assumed to be the same as E. coli. Fatty acids in phospholipids were assumed to be iso-C15, anteiso-C15, iso-C17, anteiso-C17, C16 and iso-C16, each 15%; as well as C18 and iso-C18, each 5%. Biomass was assumed composed of protein, RNA, DNA, phospholipids, and carbohydrates. The amount of these macromolecules in the dry biomass of S. coelicolor was assumed equal to E. coli. P/O ratio was assumed to be 1.9 in NADH oxidation reactions and 3/5 of this amount for FADH2 oxidation reactions. Then, computational metabolic flux balancing method is used in order to obtain fluxes of all the metabolic reactions with linear programming and optimisation in GAMS environment (general algebraic modeling system). The objective function of optimisation is either the maximisation of the specific growth rate or the maximisation of the specific cosmomycin production rate. The experimental specific glucose uptake and growth rates are used as the model constraints, as appropriate. The solution of the metabolic model gives the specific growth and/or product formation rates as well as the specific rates of all 200 metabolic reactions.

The comparison of internal metabolite fluxes between the maximisation of specific growth and Cosmomycin production reveals important changes in fluxes related to energy production and redox balances (NADH, FADH2, and ATP), as well as oxygen consumption and CO2 generation when the metabolism shifts from growth to cosmomycin production. In addition, there is an important change in the fluxes of the TCA intermediates. This indicates that during growth maximisation more energy is required, so the TCA cycle is complete, more active and the aerobic respiration rate is increased. Another important observation is in the Acetyl- CoA flux; during growth maximisation, this metabolite enters the TCA cycle but during cosmomycin maximisation all Acetyl-CoA goes to Malonyl- CoA, the precursor of this antibiotic. The TCA cycle is not complete in this case and depends on the anaplerotic reactions and 3-phosphoglycerate. The sensitivity analysis performed in GAMS also confirms this. This also highlights the reason for the inclusion of casamino acids in the culture medium in the experiments. Another important change is observed in the Glucose-1P flux because during growth maximisation it goes to carbohydrate biosynthesis and during cosmomycin production it goes to this product.

The results of the model and the experiments for growth and cosmomycin production are in reasonable agreement which means that this model can be used in a predictive mode, in order to investigate the effects of different genetic or environmental conditions on growth and product formation. This should indicate strategies for the selection of media (type of carbon and nitrogen sources), precursor addition, batch versus fed-batch operation, and genetic engineering targets in order to direct the metabolism from growth to secondary metabolism and achieve more efficient production of the desired product. This research should have generic applications for novel chemotherapeutics production in streptomycetes.