Identifying metabolic bottlenecks that limit the production of terpenoids in the yeast *Saccharomyces cerevisiae*

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ABSTRACT

Terpenoids are natural compounds found in a wide range of plants and constitute a broad class of natural compounds with various industrial applications such as pharmaceuticals, perfumes and biofuels. Recent genetic engineering developments have enabled the cell based production of these high value compounds in prokaryotes and yeast, however process yields remain prohibitively low.

The aim of this study is to employ genome scale metabolic models (GeM) and complex numerical analysis techniques to identify metabolic bottlenecks that limit terpenoid productivity in three *Saccharomyces cerevisiae* strains that produce Valencene, Caryophyllene and Sclareol respectively.

Initially, a qualitative and quantitative assessment of available yeast GeMs was carried out to select the best model based on its ability to accurately predict intracellular fluxes. ^{13}C data from four *S. cerevisiae* strains were retrieved and used to constrain each of the four GEMs. Carbon constrained Flux Balance Analysis[1] (ccFBA) was used to simulate each of the four datasets in order to identify the most accurate GeM (Yeast8)[2].

The Yeast8 model was manually curated to reflect the metabolism of three novel strains based on the *S. cerevisiae* AM-276 strain which can achieve increased flux towards Valencene, Caryophyllene and Sclareol respectively. Each of the resulting three models was constrained with available experimental data in combination with ccFBA. FBA based methods lead to underdetermined problem formulations. Consequently, Markov Chain Monte Carlo techniques were used to sample the hyperplanes containing the gamut of feasible solutions for each model. The resulting large data-set was analyzed using dimensionality reduction (Principal Component Analysis) and unsupervised Machine Learning (Fuzzy C-means Clustering) algorithms to detect potential metabolic differences between the three strains. This led to the identification of reactions with (statistically) significant discrepancies, noting that the exogenous insertion of different terpenoid genes can result in distinct metabolic fingerprints. Finally, a bi-level Mixed Integer Linear Programming problem was solved (OptForce) to identify potential metabolic engineering strategies (knockouts, up- and down-regulations) that can lead to increased terpenoid productivity.

The combination of metabolic modeling, machine learning, and computational guided metabolic engineering strategies presented in this study offers a multifaceted understanding of the metabolic network of terpenoid-producing yeast strains.

KEYWORDS: Yeast, Flux Balance Analysis, Metabolic engineering, Machine Learning, Computational Biology

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