In silico evolution leads to green chemical factory

Vanillin green cell factory

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Motivation

The beneficial metabolic properties of naturally occurring microorganisms are a result of an evolutionary process that increases their fitness in the natural habitat. Phenotype conferring to the fitness is often inclined towards growth and less so towards the trait of interest, e.g. high productivity of a desired compound. Random mutagenesis followed by selection pressure is a widely used way to obtain high-producing strains by exploiting the genetic plasticity. The space of possible genetic alterations, however, is too large to explore in either random or in a brute-force manner. We propose an alternative solution to this problem, by *mimicking* the Darwinian evolution process *in silico.* The motivation for this attempt lies in the availability of complete genome sequences and modeling concepts capable of simulating cellular phenotype. We explore the possibility of using such *in silico* evolution for obtaining metabolic engineering targets towards high productivity. The applicability of this approach is demonstrated with a case study of heterologous production of vanillin, one of the most popular flavor compound obtained from an *Orchidea,* in the baker's yeast – *Saccharomyces cerevisiae*.

Heterologous gene expression in *S. cerevisiae*

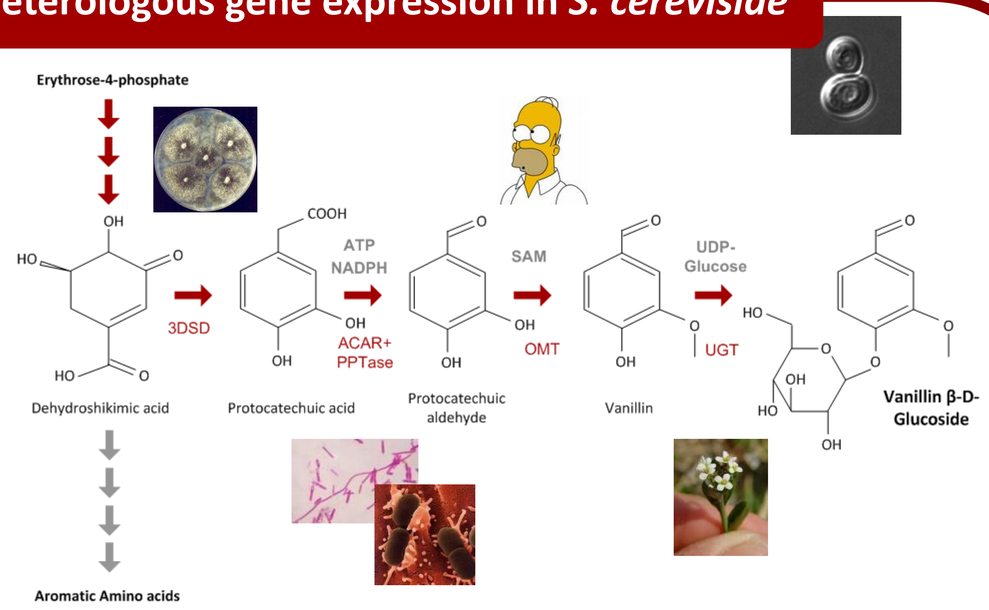

Figure 1 - Biosynthetic pathway for *de novo* biosynthesis of vanillin glucoside in *S. cerevisiae*. 3DSD: 3-dehydroshikimate dehydratase from *Podospora pausiceta*; ACAR: aromatic carboxylic acid reductase from Nocardia sp; PPTase: phophopantetheinyl transferase from *E. coli;* OMT: O-methyltransferase from *Homo Sapiens*; UGT: UDP-glycosyltransferase from *Arabidopsis Thaliana* (Hansen, et al., 2009).

In silico Darwinian evolution

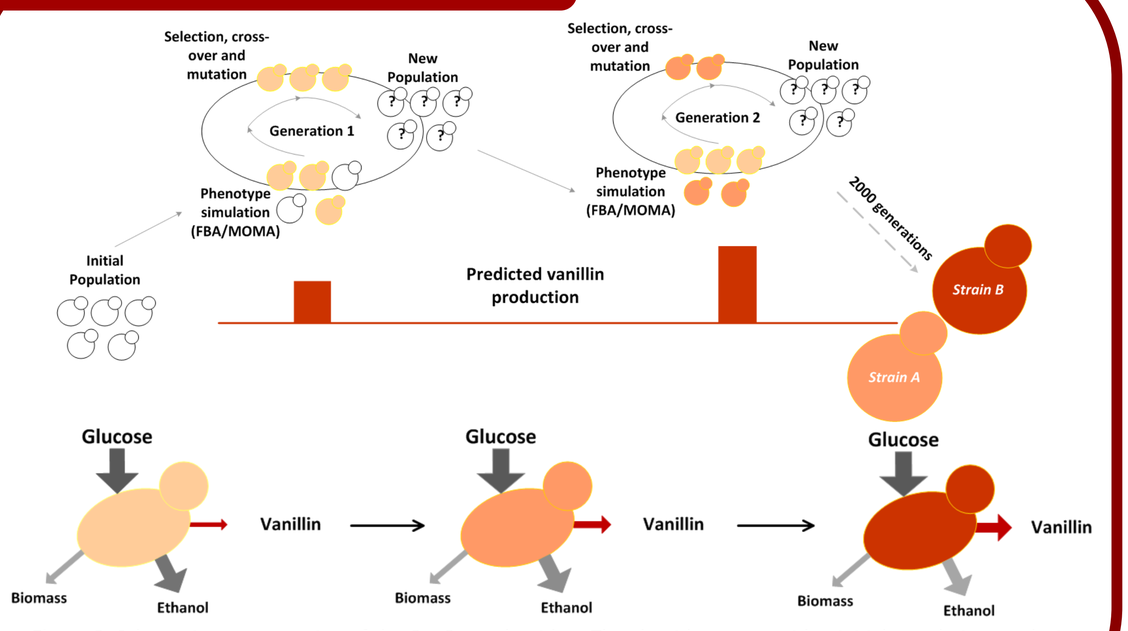

Figure 2: Schematic representation of the OptGene algorithm. The algorithm starts with a initial population producing low amount of vanillin that evolves through several generations where the best producers are progressively selected leading to the prediction of a high producing genotype.

Target selection

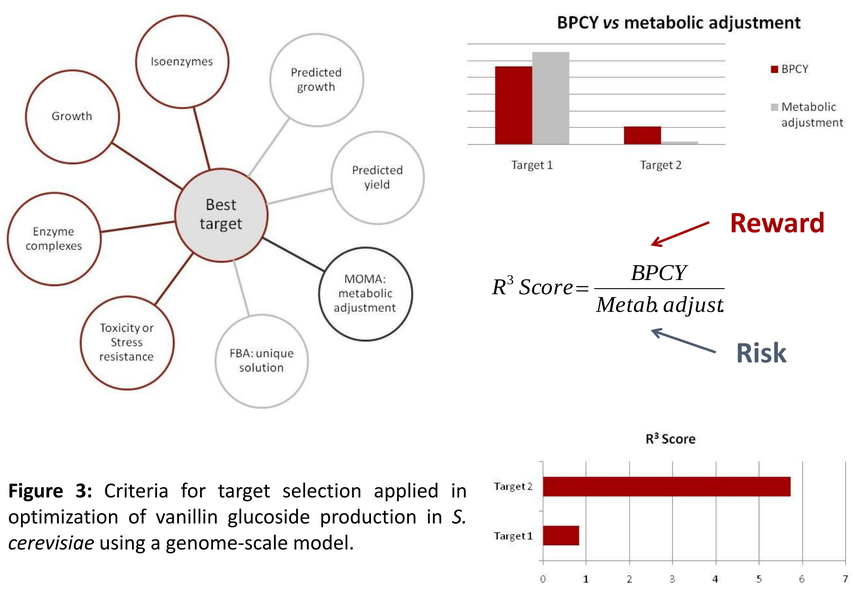

Figure 3: Criteria for target selection applied in optimization of vanillin glucoside production in *S. cerevisiae* using a genome-scale model.

In vivo results

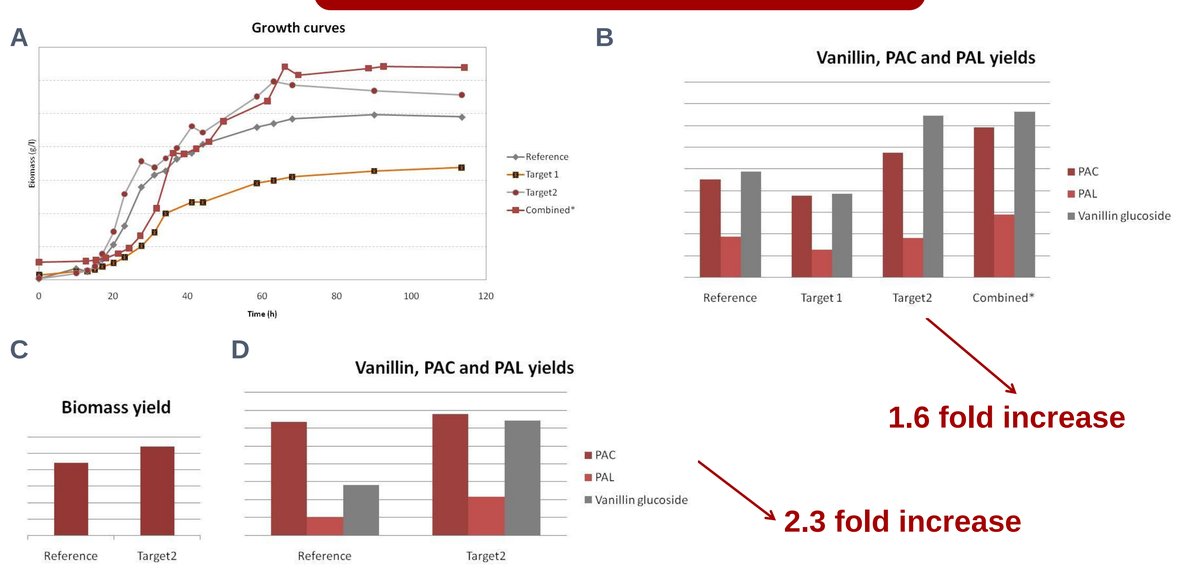

Figure 2: Physiological characterization of the metabolic engineered strains constructed in this study. **A**. Biomass profiles in batch cultivation. **B**. Substrate specific yield of vanillin glucoside, PAC and PAL in batch cultivation. **C**. Substrate specific yield for biomass production in continuous cultivation. D: Substrate specific yield of vanillin glucoside, PAC and PAL in continuous cultivation.

Conclusion and Future perspectives

A significant improvement of glucovanillin production was observed for the model driven obtained mutants. Genome-wide *omics* detailed investigation of these strains, as transcriptome and fluxome analysis will be performed and incoming results will provide an excellent platform for further strain design applying model based strategies. The resulting data may serve as a starting point for incorporating regulatory information into the yeast metabolic model. In summary, i*n silico* design by using an evolutionary approach was found to be a good platform for rational improvement of microbial cell factories – a promising step towards green chemical industry.

Hansen EH [et al.] De novo biosynthesis of vanillin in fission and baker's yeast [Journal]. - [s.l.] : Applied Environmental Microbiology, 2009.
Patil KR, Rocha I, Forster J and Nielsen, J. 2005. "Evolutionary programming as aplatform for *in silico* metabolic engineering". BMC Bioinformatics. Vol. 6, p. 308.
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