Colorful Microbial Cell Factories - DTU Orbit (04/10/2019)

**Colorful Microbial Cell Factories: Biosynthesis of plant natural products in yeast**

Yeast cell factories are powerful tools used for the production of high-value natural compounds otherwise not easily available. Many bioactive and industrially important plant secondary metabolites can be produced in yeast by engineering their biosynthetic pathways into yeast cells, as these both possess the cellular functions to synthesize, express and fold the eukaryotic genes and proteins, as well as many of the precursors needed as substrates for biosynthesis of most classes of plant natural products.

Natural colorants represent an important class of food ingredients in industry, as they have desirable properties compared to chemically synthesized artificial dyes; one of the most prominent being their health-promoting properties. Several problems in the form of low concentrations in host tissues, seasonal availability, and chemical instability exist for plant pigments, such as the desirable anthocyanins.

Yeast cell factories present a platform to circumvent the problem of low yields of interesting molecular structures in plant tissues, as hand-picking of desired enzyme activities allows for specific biosynthesis of the precise pigment of interest, as well as choosing more stable structures for heterologous biosynthesis is possible. In cell factories, great improvements in yields can be achieved through molecular engineering of flux from endogenous yeast precursors, e.g. by elimination of by-product formation, and by genetic optimization of pathway components, such as fine-tuning of expression levels.

The genetic background of the yeast strain chosen as host for genetic engineering efforts has proven to have great influence on the final yields of produced metabolites. This is in great part due to extensive genetic variations between yeast strains that confer changes to the regulation of the cellular metabolism. A deeper understanding of how and why one strain is a better cell factory than another is important to ensure industrially relevant yields in the future.

In this study, a simple model pathway for biosynthesis of p-coumaric acid was engineered in the two popular yeast strain backgrounds CEN.PK and S288C, and through physiological characterization and chemical analysis of their metabolite profiles their respective potentials for plant-derived hydroxycinnamic acid production was evaluated. Under batch cultivations, the S288C-based cell factory gave about twice as high yields as the CEN.PK-based cell factory, thus, underlining the importance of giving consideration into the choice of the yeast chassis strain before commencing in genetic engineering endeavors. For the biosynthesis of compounds derived from the shikimate pathway S288C should, thus, be the chassis strain of choice for cell factory engineering.

The CEN.PK and S288C strains have great genetic differences, and CEN.PK contains great numbers of single nucleotide polymorphisms (SNPs) in many genes that lead to non-synonymous amino acid substitutions, as compared to S288C. In this study, two of these were investigated. The SNPs present in the CEN.PK-derived ARO3 gene from the shikimate pathway was not shown to have any effect on the activity of the protein. Expression of the adenylate cyclase (CYR1) from CEN.PK in an S288C-based cell factory, however, was shown to increase production yields of p-coumaric acid 2-fold in shake flask cultivations. The data presented here suggests that the reason for the increase in production titers was due to changes in cellular growth responses to the availability of glucose. Thus, CYR1 was identified as a promising step for improvement of metabolite yields in yeast.

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