A balancing act: building the aurone pathway in *Saccharomyces cerevisiae*

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**Background**

Specialized plant metabolites like flavonoids, terpenoids and polyketides are valuable natural products. These molecules have bioactive properties of value for use in the human body, which makes the supply of these compounds really essential. Currently, the industrial production of these compounds has some inherent drawbacks like multiple reaction steps, harsh reaction conditions and the difficulty of chiral centers in chemical synthesis together with low yields using plant extraction. Production of specialized plant metabolites in micro-organisms can be a valuable alternative here, however, the development of efficient and robust production strains remains a great challenge. One of the main reasons thereof is the difficulty to tune all steps in the heterologous pathway and with the native metabolism, especially in eukaryotic hosts. Therefore, new pathway optimization tools and techniques for *Saccharomyces cerevisiae* are developed in this project, using the aurone production pathway as case study.

**Tool development**

A first set of tools required for pathway optimization are different libraries of regulating DNA parts. Therefore, upstream activating sequence (UAS) libraries, core promoter libraries and terminator libraries are generated. Finally, these libraries are tested in a combinatorial way. Second, pathway optimization techniques like Multivariate Modular Metabolic Engineering (M3ME) and Chemically Induced Chromosomal Evolution (CICHE) are assessed in *S. cerevisiae*. The evaluation of these methods and libraries is initially performed by using fluorescent reporter proteins.

**Characterization and evaluation**

Strains with different production levels are grouped and evaluated, e.g. by qPCR and sequencing analysis to detect which variations at DNA level are responsible for the differences in the production levels. Finally, the best producer will be grown on erienmeyer and reactor scale and parameters like growth rate, substrate usage, product yield and strain stability will be determined.

**DNA assembly**

A lot of DNA parts need to be combined with each other for the creation of yeast specific libraries and for the assembly of pathways. Therefore, fast and efficient DNA assembly techniques are crucial. Different assembly methods like BioBricks, Golden Gate, CPEC, Gibson assembly, SLIC and yeast *in vivo* recombination are evaluated to identify the most appropriate method.

**Aurone case study**

The tools and methods are used to assemble the aurone pathway and to create libraries of strains. The heterologous aurone pathway is split into an upstream and downstream module for the application of M3ME. The libraries are used for the optimization and balancing of these pathway modules. After choosing the optimal combination within each module, CICHE is applied to determine the ideal copy number of each pathway module. To check whether the genes are optimally balanced, a screening based on the absorption spectra of isoliquiritigenin and the end product sulfuretin is used.

**References**


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