Co-localization of enzymes for improved production of flavonoids in *Saccharomyces cerevisiae*

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Introduction

Flavonoids comprise a class of secondary plant metabolites with potential health benefits. In order to fully investigate these benefits, they have to be available in sufficient amounts with a high degree of purity. Current production methods like plant extraction and chemical synthesis are inadequate to provide to this need. Microbial production might offer a promising alternative. However, production quantities are currently too low to be economically feasible. New and improved strategies are therefore needed to increase product titers. One such strategy is to co-localize the different enzymes to allow for substrate channeling, thereby improving the overall efficiency. This research focuses on applying different co-localisation strategies for the production of naringenin, a key molecule in flavonoid biosynthesis.

A first step towards this goal is the construction of a reference strain without co-localization and the development of a high-throughput screening method.

Results

In order to obtain a reference strain, the genes necessary for the production of naringenin from p-coumaric acid were codon harmonized for use in *S. cerevisiae*, inserted in an expression vector and transferred to the BY992 strain. In order to allow for naringenin production, p-coumaric acid (1 mM) was supplied to the culture medium. This strain was able to convert p-coumaric acid to naringenin as seen by UPLC analysis (Figure 1). Further improvements in production were made by overexpressing a feedback insensitive mutant of the native ACC1 gene (ACC1**) under control of the strong TEF1 promoter (Figure 2). Finally, a high-throughput screening method for naringenin was developed based on a complexation reaction with Cu²⁺-ions (Figure 3).

Conclusion and perspectives

Naringenin was produced in *S. cerevisiae* using p-coumaric acid as a substrate. Production titers reached 14.95 ± 0.9 mg/L after 2 weeks of incubation. Further improvements were made by overexpression of a feedback insensitive mutant of the native ACC1 gene which improves the availability of the malonyl-CoA precursor (27.89 ± 7.74 mg/L). In order to further improve the yield, different co-localization strategies will be implemented and compared to each other.

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