Engineering of Saccharomyces cerevisiae for production of resveratrol and its derivatives

Resveratrol is a natural potent antioxidant with multiple beneficial effects on human health and is therefore used in medical, food, and cosmetic areas. In my PhD thesis I describe how I engineered yeast cell factory Saccharomyces cerevisiae for production of resveratrol by fermentation of cheap carbon sources. I adopted rational metabolic engineering design strategies, synthetic biology techniques and system biology approaches to engineer and analyse yeast cell factories. There are two biosynthesis pathways of resveratrol, starting from tyrosine or phenylalanine, which in this thesis are defined as the TAL pathway and PAL pathway respectively. For the TAL pathway, I collaborated with Christian Bille Jendresen, Steen Gustav Stahlhut to screen fourteen diverse heterologous tyrosine ammonia lyases (TALs) for their activity in yeast and E. coli. I expressed the 14 TALs in S. cerevisiae and analysed the resulting strains for production of p-coumaric acid. Two of the screened TALs, one from Herpetosiphon aurantiacus and another from Flavobacterium johnsoniae, were highly active and selective, i.e., they did not have a side reaction of converting phenylalanine to cinnamic acid. These results were published in Applied and Environmental Microbiology (Jendresen et al., 2015). I then constructed the TAL pathway to resveratrol by expressing the two TAL genes in combination with 4-coumaryl-CoA ligase (4CL) from Arabidopsis thaliana and resveratrol synthase (VST) from Vitis vinifera in S. cerevisiae; the best combination resulted in 11.66±0.57 mg l⁻¹ and 2.74±0.05 mg l⁻¹ resveratrol on minimum medium in the presence or absence of 5 mM tyrosine respectively. In order to improve resveratrol production, I increased the flux towards tyrosine precursor by de-regulating the aromatic amino acids biosynthesis pathway (overexpression of feedback-inhibition resistant versions of Aro4pfbr and Aro7pfbr) and the supply of malonyl-CoA precursor by de-regulating acetyl-CoA carboxylase (overexpression of Acc1p S659A, S1157A) to avoid inactivation by the global regulator Snf1p. The first strategy resulted in 78% improvement of resveratrol titer and the second in 31% improvement. Combining the two strategies further improved resveratrol titer to 6.40±0.03 mg l⁻¹. I hypothesized that the activity of resveratrol biosynthetic enzymes was limiting the flux towards resveratrol. To test this hypothesis I integrated the resveratrol pathway genes into Ty-4 retrotransposon regions, which resulted in integration of up to 8 copies of the pathway genes. Indeed my hypothesis was correct as this strategy boosted the elements was revealed to be the key limiting factor for resveratrol biosynthesis and sharply improved resveratrol production 36-fold and gave 235.57±77.00 mg l⁻¹ resveratrol in batch fermentation. I fermented the final strain in controlled fed-batch reactors with glucose or ethanol feed and obtained 415.65 and 531.41 mg l⁻¹ of resveratrol respectively. The results were published in Metabolic Engineering (Li et al., 2015). I also engineered the PAL pathway for resveratrol production by introducing PAL2 encoding phenylalanine ammonia lyases, C4H encoding cinnamate-4-hydroxylase, and 4CL2 from A. thaliana and VST1 from V. vinifera in S. cerevisiae. To enhance the activity of C4H, a notorious cytochrome P450 enzyme, I overexpressed NADPH-cytochrome P450 reductase (ATR2) from A. thaliana and S. cerevisiae cytochrome b5 (CYB5). Ty-4 element-mediated multiple integration of PAL pathway and overexpression of ARO4pfbr and ARO7pfbr together with ACC1S659A, S1157A resulted in 201.72±7.91 mg l⁻¹ resveratrol. In order to further improve the precursor supply, I knocked out ARO10 encoding phenylpyruvate decarboxylase and overexpressed a post-translational unregulated variant of acetyl-CoA synthetase (ACS1L641P) from Salmonella enterica, which improved resveratrol production to 272.64±1.34 mg l⁻¹. Finally, I obtained 811.50 mg l⁻¹ and 754.70 mg l⁻¹ resveratrol in fed-batch fermentation of the engineered strain using glucose or ethanol feed respectively. I further integrated heterologous methyltransferases into the resveratrol platform strain and hereby demonstrated for the first time de novo biosynthesis of two resveratrol derivatives, pinostilbene and pterostilbene, which have better stability and uptake in the human body compared to resveratrol.

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