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Yeast organelle engineering: a flavonoid case-study

Tom Delmulle[1], Sofie L. De Maeseneire[2] & Marjan De Mey[1]

1 Ghent University, Metabolic Engineering Group - Centre for synthetic biology
2 Ghent University, InBio.be - Centre for Industrial Biotechnology and Biocatalysis,
Coupure Links 653, Ghent B-9000, Belgium
www.csb.ugent.be
E-mail: Tom.Delmulle@UGent.be, Tel: +32 9 264 87 14

Introduction

Metabolic engineering efforts have mainly focussed on pathways located in the cytosol of production hosts. However, metabolic crosstalk and loss of intermediates still pose significant challenges. During billions of years of evolution, nature has evolved several ways to minimize this crosstalk and to separate pathways from each other. This is clearly seen in eukaroytes which possess several sub-cellular compartments, *i.e.* organelles. Organelles form specialized compartments in which their pathways and metabolites are physically separated from the remainder of the cell. Furthermore, they each have their own unique physicochemical environment. Despite several advantages, organelle engineering is not as common as engineering the cytosol, mainly due to difficulties in protein import and transport of metabolites. Recently, efficient import signals were constructed to redirect proteins to the peroxisomes of the yeast *Saccharomyces cerevisiae* (1). By using these enhanced targeting signals, this research focusses on transforming the yeast peroxisome into a production site for the flavonoid naringenin.

Results

Naringenin production was obtained by the conversion of fed p-coumaric acid (1 mM) through the consecutive action of 4-coumaric acid-CoA ligase, chalcone synthase and chalcone isomerase. Both a pathway located in the cytosol as well as a pathway located in the peroxisome were constructed. Retargeting of the naringenin production pathway to the peroxisome of *Saccharomyces cerevisiae* was achieved by adding enchanced peroxisomal targeting sequences to the different enzymes (1). Since no malonyl-CoA is present in peroxisomes, additional retargeting of the cytosolic acetyl-CoA carboxylase Acc1p was necessary to provide the pathway with this precursor (Figure 1, green arrow).

As expected, naringenin synthesis was severely affected by peroxisomal location of the pathway genes due to the absence of malonyl-CoA (Figure 2). The low naringenin titers detected in this strain are most likely a consequence of cytosolic activity of the pathway enzymes before translocation in the peroxisome. Additional import of Acc1p in the peroxisome restored naringenin synthesis to levels comparable to the cytosolic variant.

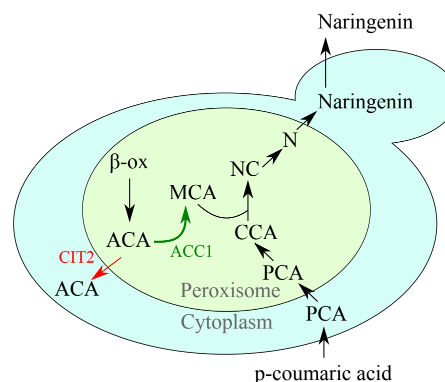
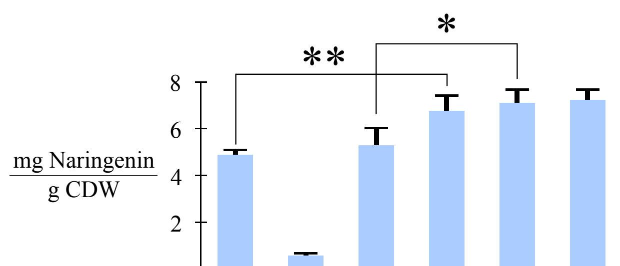


Figure 1: Schematic representation of the redirected biosynthetic pathway for naringenin from fed p-coumaric acid in *S. cerevisiae*. Retargeting of cytosolic Acc1p provides the pathway with malonyl-CoA. Strategies to eliminate acetyl-CoA removal from te peroxisome and improve its concentration in the peroxisome are indicated. β-ox: beta-oxidation, ACA: acetyl-CoA, MCA: malonyl-CoA, PCA: p-coumaric acid, CCA: coumaroyl-CoA, NC: naringenin chalcone, N: naringenin



Cytosolic pathway	+	-	-	-	-	-
Peroxisomal pathway	-	+	+	+	+	+
ACC1	-	-	+	+	+	+
Strategy 1	-	-	-	+	-	+
Strategy 2	-	-	-	-	+	+

Figure 2: Observed naringenin titers after 72 h of growth in the different constructed strains. Both strains with the pathway located in the cytosol and in the peroxisome were constructed. Additional engineering efforts were made to increase naringenin titers (indicated by the +). Data represents three biological repeats.Error bars denote standard errors. * p-value < 0.10, ** p-value < 0.05

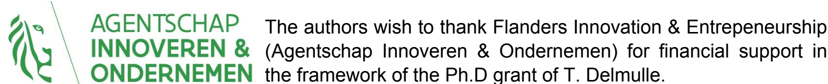
To further improve pathway efficiency, additional efforts were made to increase intraperoxisomal acetyl-CoA levels by blocking the export of acetyl-CoA form the peroxisome. This was done by deleting the CIT2 gene which converts acetyl-CoA to citrate for further conversion in the glyoxylate shunt. Since the strains were grown in medium lacking carnitine, the reaction performed by the Cit2 protein represents the only side-reaction consuming acetyl-CoA in the peroxisome. Deletion of this citrate synthase resulted in a significantly improved production compared to the pathway located in the cytosol (p-value = 0.049), however, no significant difference was observed compared to the peroxisomal pathway without the deletion of CIT2 (p-value = 0.116) (Figure 2, Strategy 1).

In a final effort to increase intraperoxisomal acetyl-CoA levels, a second strategy was executed. This new strategy clearly resulted in improved naringenin synthesis, even compared to the pathway located in the peroxisome (p-value = 0.072) (Figure 2, Strategy 2). However, combining this new strategy with the deletion of the CIT2 gene did not result in further improvements of the naringenin titer. This might indicate that acetyl-CoA is no longer a limiting factor in this strain.

References

(1) DeLoache, W. C. et al. Towards repurposing the yeast peroxisome for compartmentalizing heterologous metabolic pathways. Nat. Commun. 7:11152 doi: 10.1038/ncomms11152 (2016).

Acknowledgments



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