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In vivo validation of *in silico* predicted metabolic engineering strategies for terpenoid production in yeast

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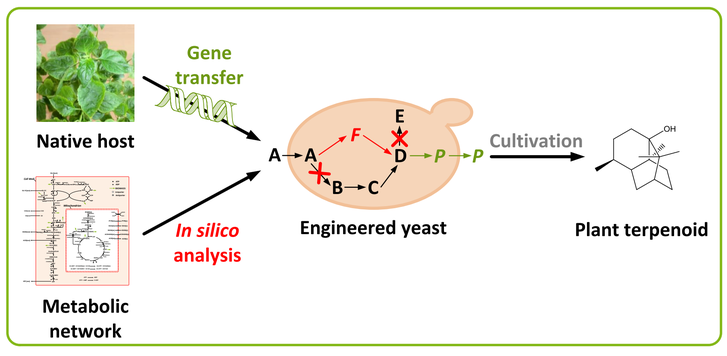
Introduction

Terpenoids

- One of the largest classes of natural products
- Possess important medicinal and industrial properties
- Some are rare and produced in low amounts in plants
- Heterologous microbial production may help to overcome supply problems and high purification costs
- Necessity of optimization of yield and productivity in yeast e.g. via metabolic engineering [1]



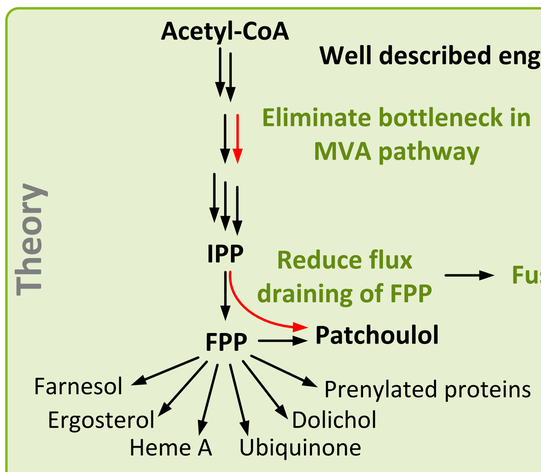
Objectives

- Development of a platform organism for the efficient supply of biosynthetic precursors for the production of terpenoids using an *in silico* stoichiometric metabolic network analysis
- In a previous study, we compared *E. coli* and *S. cerevisiae* as heterologous hosts and identified promising metabolic engineering strategies using elementary mode analysis and constrained minimal cut sets [2,3]
- In a follow-up, we identified considerations for practical realization and validated selected strategies *in vivo* [4]

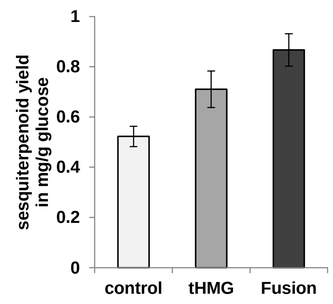
Prerequisites and *in vivo* validation of *in silico* predicted strategies

Step 1: Engineering the mevalonate pathway (MVA)

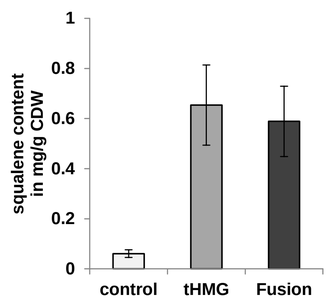
Theory



Results



➢ Increased terpenoid yield



➢ Increased squalene content (triterpenic intermediate to ergosterol)

[4]

Step 2: Choice of carbon source

Theory

Theoretical terpenoid yields in wild type yeast in C-mol/C-mol (based on elementary mode analysis)

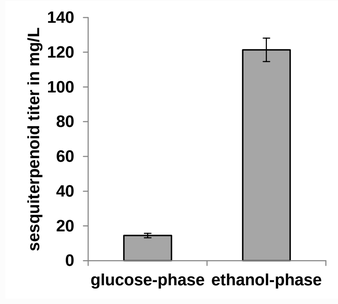
C-source	Theoretical maximum
Glucose	0.53
Galactose	0.53
Fructose	0.53
Xylose	0.53
Glycerol	0.56
Ethanol	0.68

➢ Different carbon sources have different theoretical potential

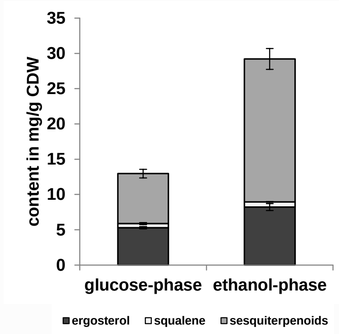
➢ Glycerol and especially ethanol are more promising than sugars

[3]

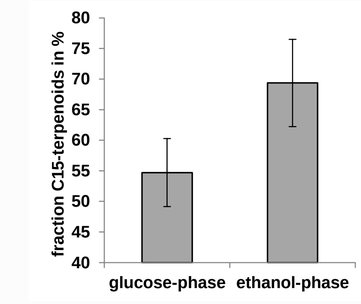
Results



➢ Increased sesqui-terpenoid titer



➢ Increased (total) terpenoid content



➢ Increased fraction of sesquiterpenoids in total terpenoids

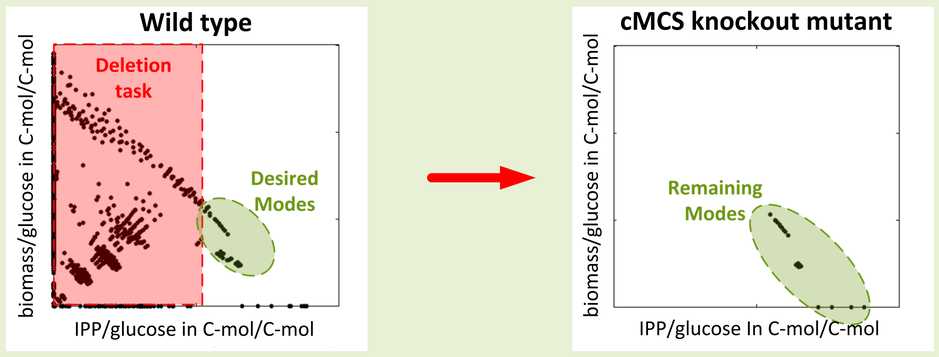
[4]

Step 3: Validation of *in silico* predicted metabolic engineering strategies: knockout in citric acid cycle

Theory

***In silico* method: constrained minimal cut sets - theory**

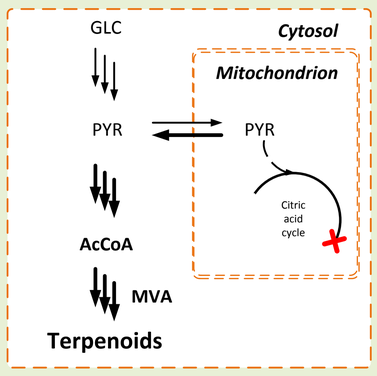
- Minimal set of structural interventions (gene knockouts)
- Repressing a certain functionality (deletion task: low product yield)
- Preserving a certain functionality (desired modes: high product yield)



➢ Coupling of a specified minimum terpenoid yield to growth

[2]

cMCS for enhanced terpenoid yield: feasible set of interventions



1. Prevention of acetate secretion
2. Prevention of ethanol secretion or production
3. Partial disruption of citric acid cycle, e.g.:
- mitochondrial α-ketoglutarate dehydrogenase
- mitochondrial succinyl-CoA ligase

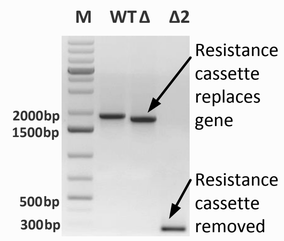
Consequence for flux distribution
- Carbon cannot be oxidized completely to CO_2
- Carbon flux is redirected towards AcCoA

[3]

Disruption of α-ketoglutarate dehydrogenase gene (*KGD1*) of α-ketoglutarate dehydrogenase complex in citric acid cycle

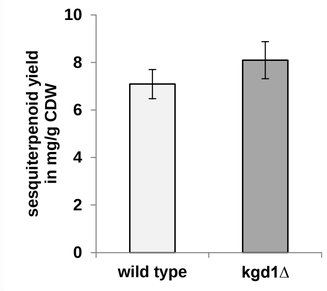
Results

Gene disruption



➢ Cre-loxP mediated gene disruption successful

Growth on glucose-phase

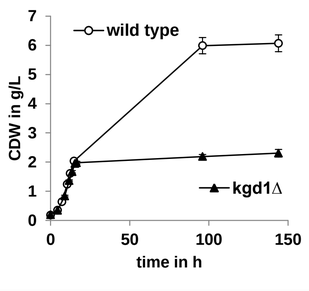


➢ Hardly any effect on terpenoid production

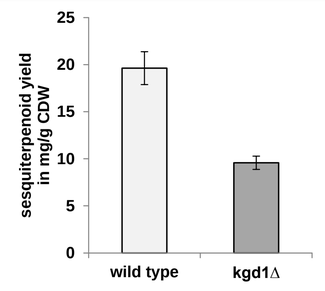
➢ No effect on physiological parameters

➢ Reason: citric acid cycle activity not sufficient

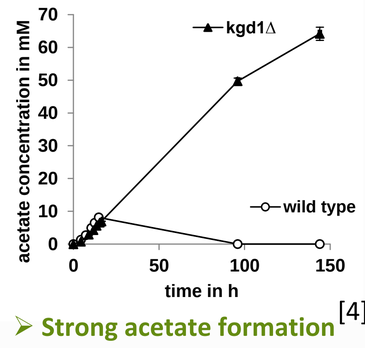
Growth on ethanol-phase



➢ Reduced growth



➢ Reduced terpenoid production



➢ Strong acetate formation

[4]

Future prospects

➢ Acetate formation needs to be prevented (neg. effect on growth and terpenoid production)
➢ If acetate would be efficiently converted to terpenoids, titers in the g/L range would be possible

Acknowledgements and References

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[1] Chang, M.C.Y. and Keasling, J.D. (2006) *Nat. Chem. Biol.* 2: 674-681
[2] Hädicke, O. and Klamt, S. (2011) Metab. Eng. 13:204-213
[3] Gruchattka, E. *et al.* 2013, Microb Cell Fact, 12:84
[4] Gruchattka, E. *et al.* 2015, submitted

CLIB Graduate Cluster Industrial Biotechnology

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