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Towards a platform organism for terpenoid production – *in silico* analysis of *Saccharomyces cerevisiae* as potential host

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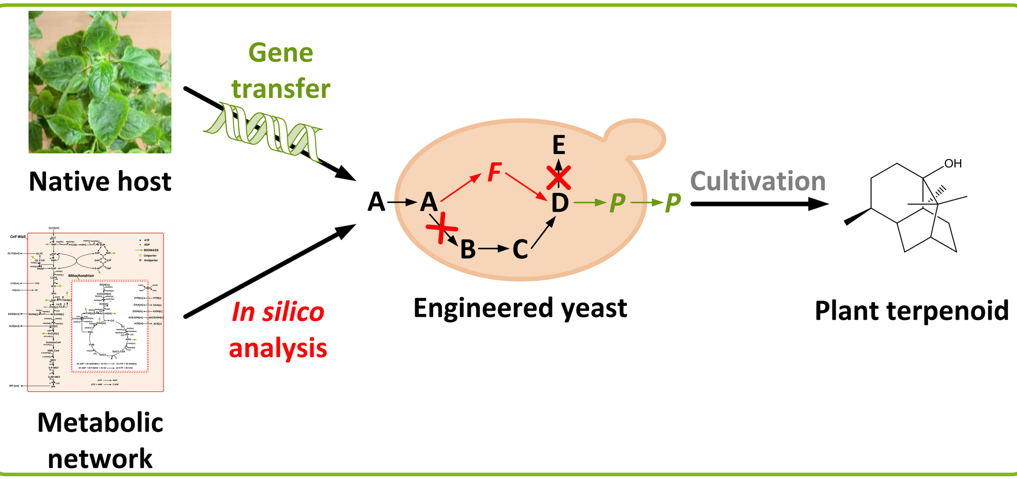
Introduction

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 isopentenyl diphosphate (IPP), the biosynthetic precursor of all terpenoids using *in silico* modelling
- *S. cerevisiae* is analyzed by means of elementary mode analysis [2] regarding the metabolic potential to supply IPP and to identify overexpression candidates
- Knockout-strategies for an enhanced terpenoid yield are identified using constrained minimal cut sets [3]

Method

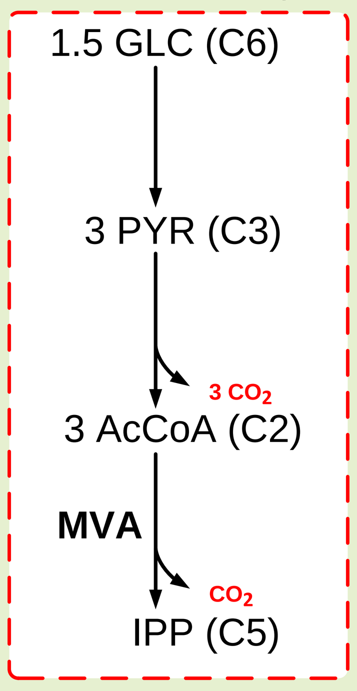
Metabolic Network & Computations

- Construction of central carbon metabolism model considering the current knowledge from genome scale models and literature [4-7]
- Model consists of 69 reactions (30 reversible) and 60 metabolites (8 external) including mevalonate pathway (MVA) and compartmentalization between cytosol and mitochondrion
- Elementary modes and constrained minimal cut sets were computed using the software package *CellNetAnalyzer* [8]

Results: Metabolic Network Analysis

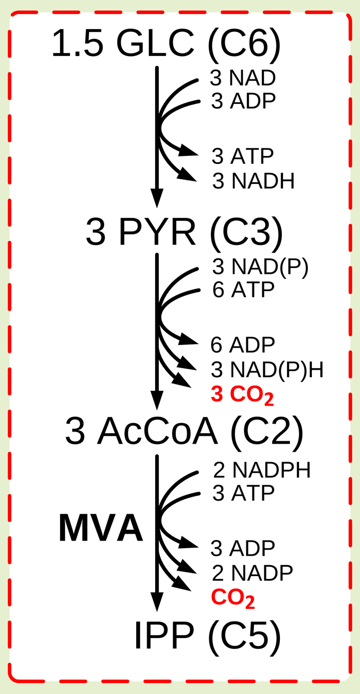
Analysis of Terpenoid Pathway and Central Metabolic Network: Identification of Limitations and Solutions

Based only on carbon stoichiometry



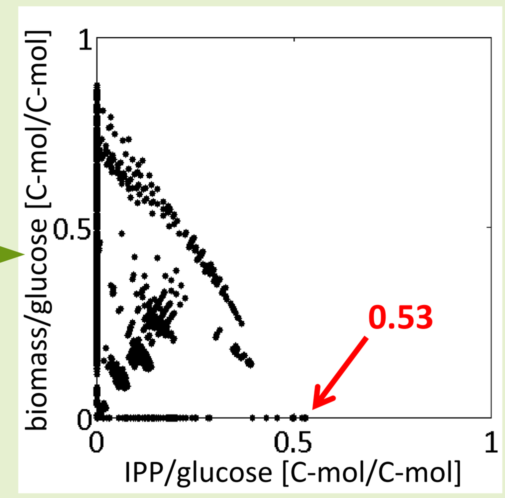
IPP/glucose: **0.56 C-mol/C-mol**
Reason: **CO_2 loss**

Taking ATP and redox equivalents into account



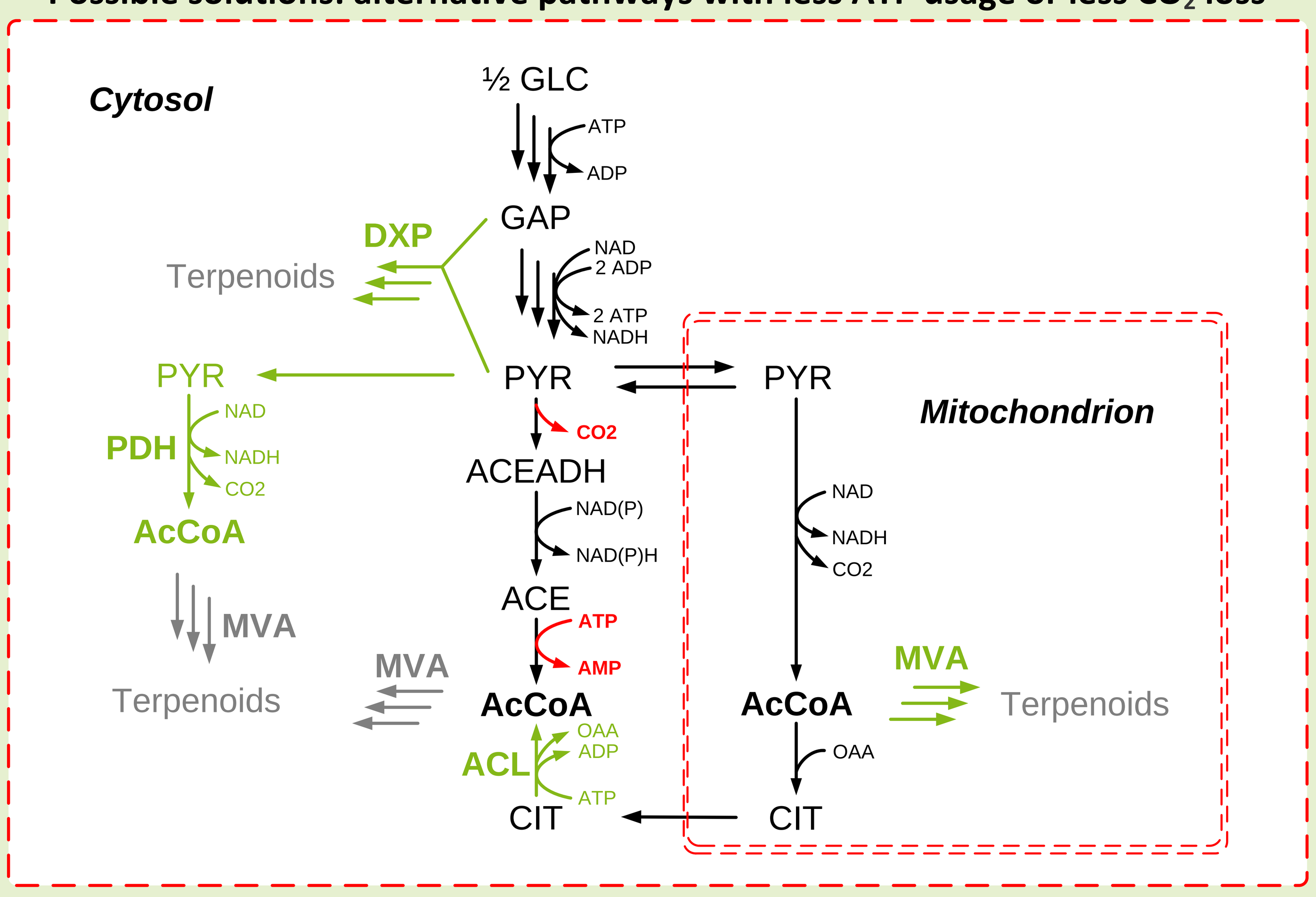
4 NAD(P)H produced
6 ATP needed
→ have to be balanced

Elementary mode analysis of metabolic network



Balancing leads to reduction in theoretical maximum IPP yield to **0.53 C-mol/C-mol**

Possible solutions: alternative pathways with less ATP usage or less CO_2 loss



Heterologous ATP-citrate-lyase (ACL) e.g. from *Yarrowia lipolytica*
- Maximal IPP/glucose: 0.55 C-mol/C-mol

Transfer of MVA to mitochondria
- Maximal IPP/glucose: 0.56 C-mol/C-mol

Heterologous pyruvate dehydrogenase complex (PDH) e.g. from *E. coli*
- Reduces potential production of acetate (ACE) or ethanol (via ACEADH)
- Maximal IPP/glucose: 0.56 C-mol/C-mol

Heterologous DXP pathway (non-MVA pathway, e.g. from *E. coli*)
- Maximal IPP/glucose: 0.64 C-mol/C-mol

[13]

Constrained Minimal Cut Sets (cMCS): Enhanced Terpenoid Production

IPP yields in wild type yeast in C-mol/C-mol

C-source	Theoret. max.	Experimental *
Glucose	0.53	0.003-0.01 [9-11]
Galactose	0.53	0.004 [12]
Fructose	0.53	-
Xylose	0.53	-
Glycerol	0.56	-
Ethanol	0.68	0.19 [11]

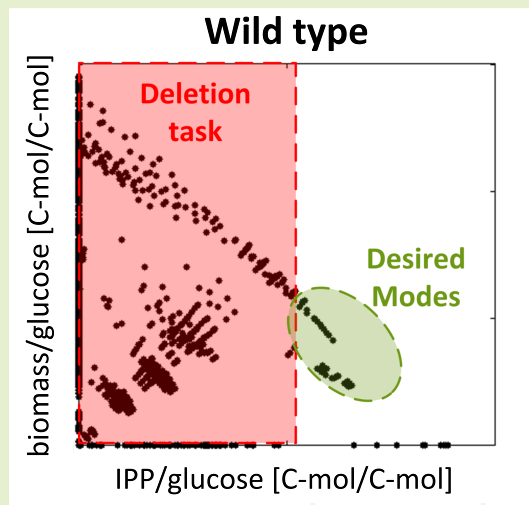
* yeast strains optimized within terpenoid pathway

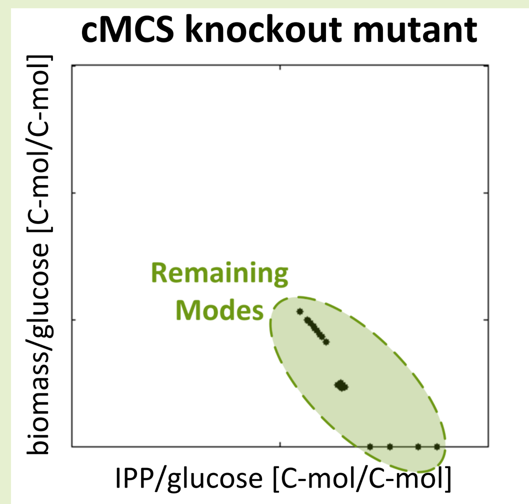
Experimental IPP yields are very low

→ Change flux distribution to force the cell to produce high yields

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

cMCS for enhanced terpenoid yield on glucose

Feasible set of interventions

1. Prevention of acetate secretion
2. Prevention of ethanol secretion or production (alcohol dehydrogenases)
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

Predicted results

Feature	Wild type	cMCS knockout mutant
Elementary modes	9,844	19-142
Minimal IPP carbon yield	0	0.27-0.33
Maximal IPP carbon yield	0.53	0.53
Maximal biomass yield	0.87	0.32-0.4

[13]

Message

Summary/Conclusion

- Heterologous enzymes/pathways with decreased energy consumption or less CO_2 loss are identified leading to a higher theoretical maximal IPP yield
- Knockout strategies are identified for a coupling of biomass production to a minimal IPP yield which is higher than published yields to date

Future Prospects

- *In vivo* validation of predicted results using patchoulol as a reporter (a sesquiterpenoid and fragrance compound used in perfume industry)

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Acknowledgements

E. Gruchattka is supported by a fellowship from the CLIB-Graduate Cluster Industrial Biotechnology
Thanks to Dr. Steffen Klamt and Oliver Hädicke (MPI Magdeburg)

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