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Towards a platform organism for terpenoid production – *in silico* comparison of *E. coli* and *S. cerevisiae* as potential hosts

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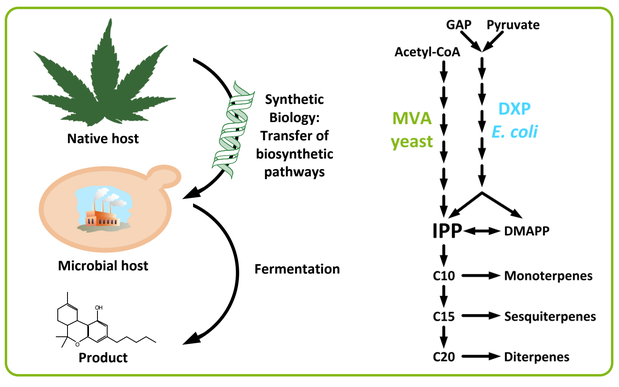
Terpenoids

Synthetic Biology for Terpenoid Production

Terpenoids are one of the largest classes of natural products and they possess important medicinal and industrial properties. The heterologous production of plant terpenoids in microorganisms is a concept to overcome supply problems and high purification costs as several compounds are rare and produced only in low amounts in plants [1]. Our focus is on the development of a platform organism for the efficient supply of isopentenylpyrophosphate (IPP), the biosynthetic precursor of all terpenoids. *E. coli* and *S. cerevisiae* are potential hosts that use two different pathways to produce IPP.



Objectives

In this study *E. coli* and *S. cerevisiae* are compared *in silico* by means of elementary flux mode analysis (EMA) regarding their metabolic potential to supply IPP. EMA allows the calculation of a solution space containing all steady state flux distributions of a metabolic network considering stoichiometry, topology and thermodynamics [2]. The theoretical maximum IPP yield is calculated, which can be used for the estimation of the potential efficiency of a process. Exchange and combination of the DXP and MVA pathway as well as optimal flux distributions are analyzed providing a basis for rational strain design.

Metabolic Networks

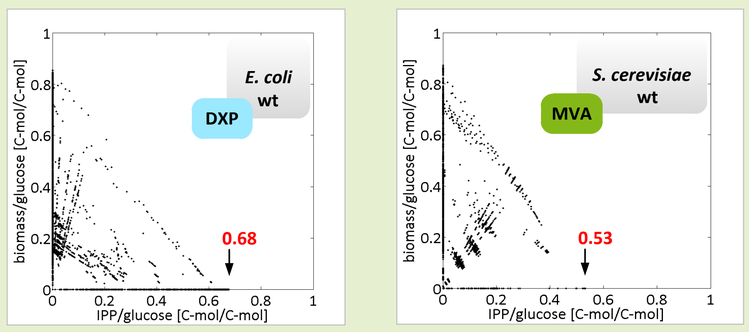
Models of the central carbon metabolism were constructed considering the actual knowledge from genome scale models and literature [3,4,5,6,7,8]. Elementary flux modes were calculated using MATLAB R2011a and efmtool version 4.7.1 [9].

***E. coli* model:** 65 reactions (21 reversible) and 53 metabolites (12 external) including DXP pathway

***S. cerevisiae* model:** 66 reactions (27 reversible) and 49 metabolites (8 external) including MVA pathway and compartmentalization between cytosol and mitochondrion

Comparison of Wild Types

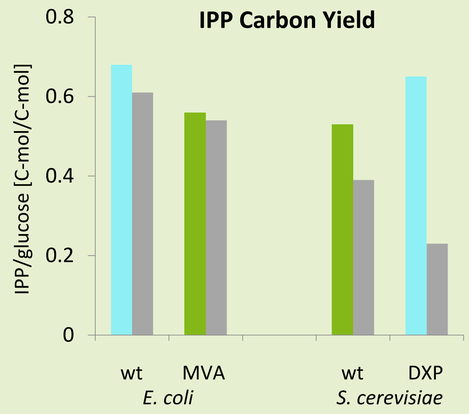
Carbon Yield for Biomass and IPP



→ **Higher potential of *E. coli*** considering theoretical maximum IPP yield.
Why? Due to terpenoid pathway or host-specific central carbon metabolism?

→ **Exchange of terpenoid pathways...**

Exchange of DXP and MVA Pathway



Colored: no biomass formation included
Grey: including biomass formation

Exchange of terpenoid pathways:
Stoichiometrically not efficient (considering biomass formation)

Coexistance (not shown):
IPP yield not enhanced
Number of modes/flexibility is enhanced

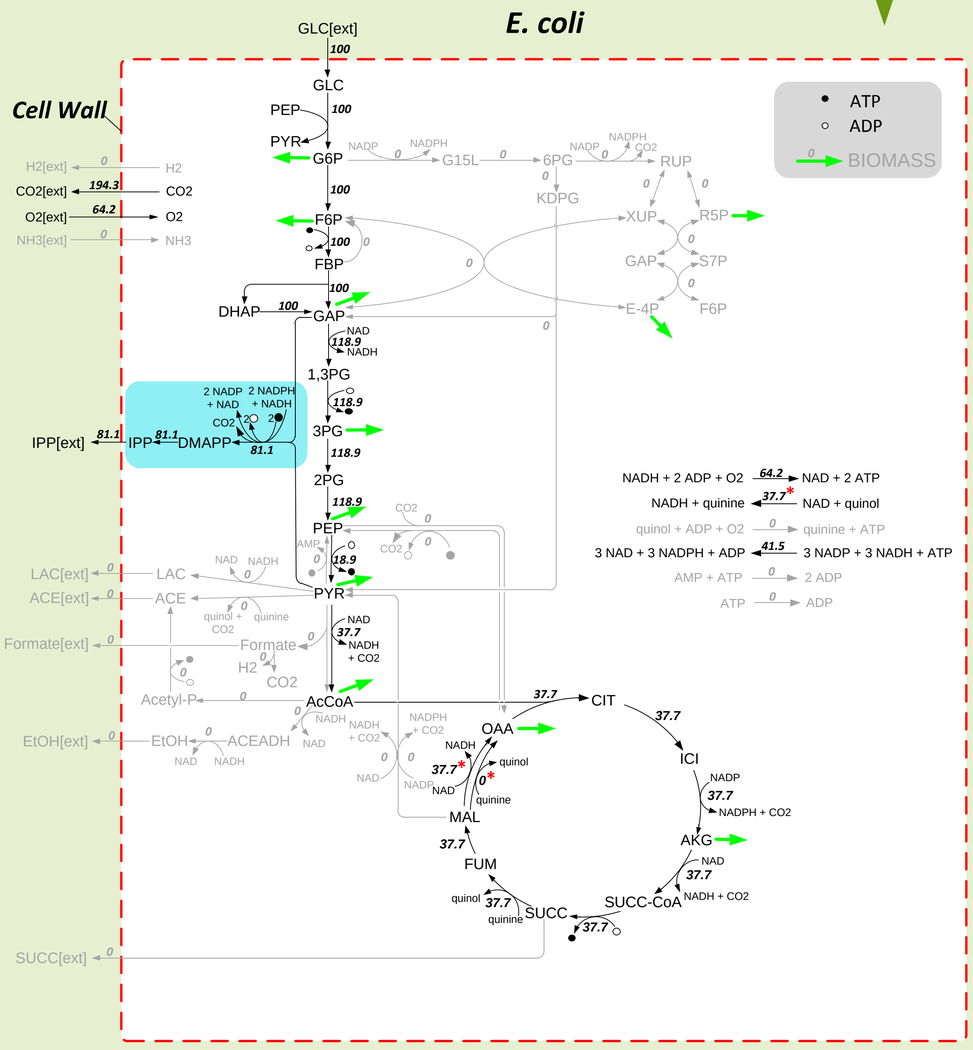
Differences are due to:

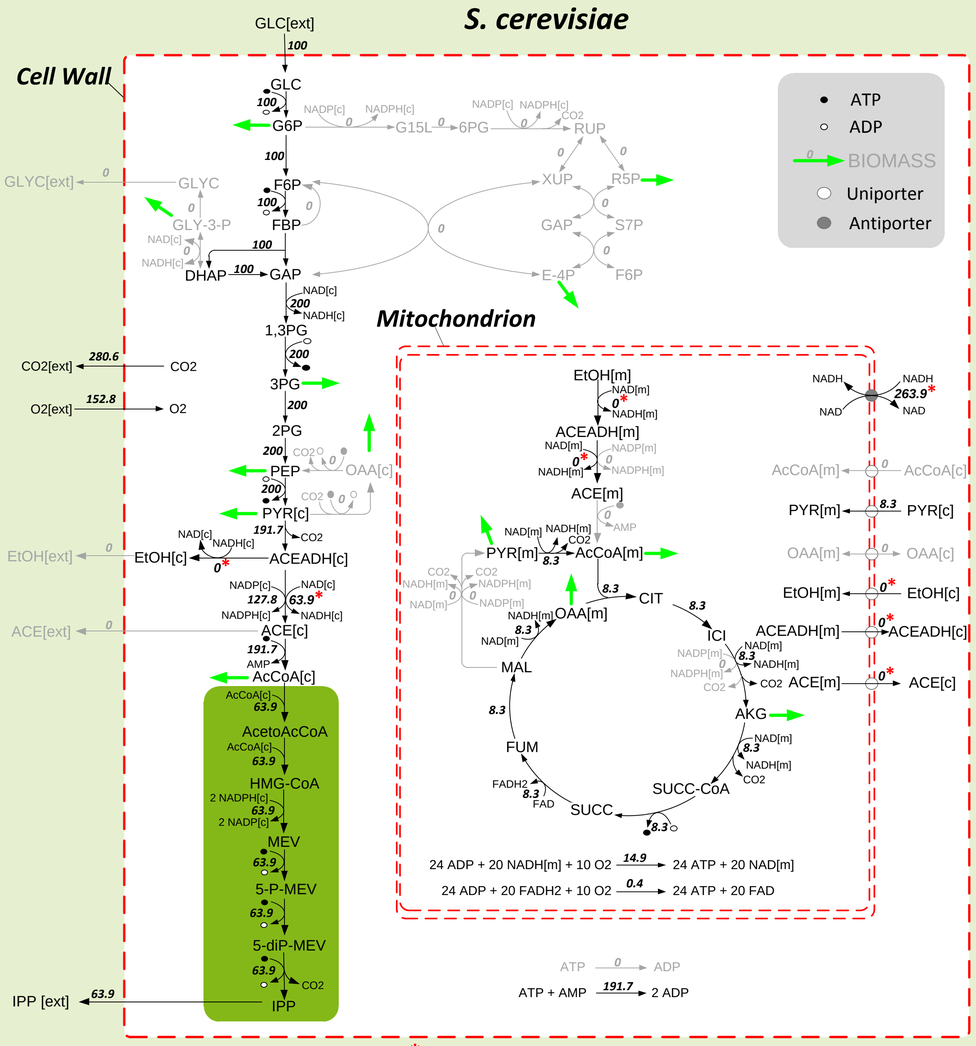
- **Terpenoid pathway**: DXP pathway is stoichiometrically more efficient, MVA uses AcCoA as precursor whose production involves a CO_2 loss
- **Host specific central carbon metabolism**: But why? Which host specific reactions are responsible?

→ **Analyze optimal flux distributions...**

Metabolic Network Analysis

Comparison of Optimal Flux Distributions





One mode out of 2 (*E. coli*) and 5 (yeast) optimal modes (max. IPP yield) are shown, variable fluxes are indicated (*)

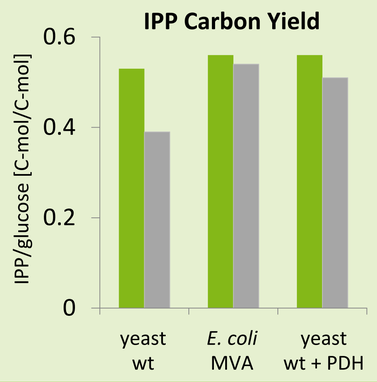
Key Difference in Central Carbon Metabolism

E. coli: Cytosolic pyruvate dehydrogenase complex (requires 1 NAD^+)

Yeast: Mitochondrial pyruvate dehydrogenase complex (requires 1 NAD^+) and cytosolic pyruvate dehydrogenase bypass (requires 1 NAD/P^+ and 2 ATP per AcCoA)
AcCoA cannot be exported from the mitochondrion into the cytosol
→ Pyruvate dehydrogenase bypass (energy-consuming) has to be used for terpenoid production in *S. cerevisiae* wild type.

→ ***In silico* introduction of cytosolic pyruvate dehydrogenase complex into yeast...**

Potential Target for Metabolic Engineering



Result:
Besides the terpenoid pathway, lack of a cytosolic pyruvate dehydrogenase complex (PDH) is primarily responsible for lower yield in yeast

→ **Cytosolic pyruvate dehydrogenase complex is a promising target to enhance terpenoid production in yeast!**

Colored: no biomass formation included
Grey: including biomass formation

References

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Acknowledgements

Thanks to Torsten Tobias Arndt for MATLAB-support
E. Gruchattka is supported by a fellowship from the CLIB-Graduate Cluster Industrial Biotechnology

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