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

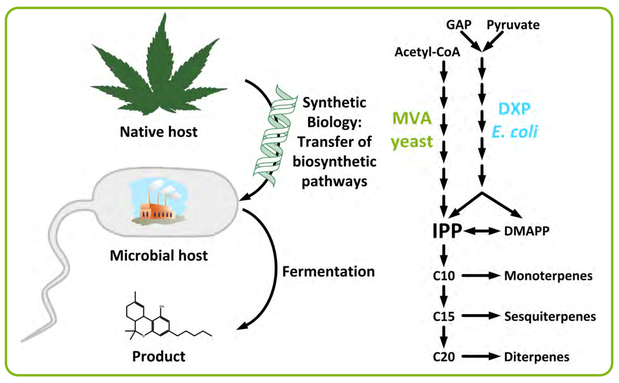
Gruchattka E., Kayser O., Schütz V.

Technische Biochemie, Fakultät Bio- und Chemieingenieurwesen, Technische Universität Dortmund
Evamaria.Gruchattka@bci.tu-dortmund.de

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

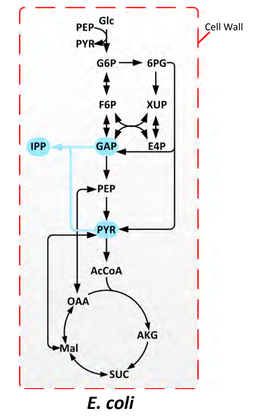
In this study *E. coli* and *S. cerevisiae* are compared by means of elementary flux mode analysis (EMA) regarding their metabolic potential to supply IPP. EMA allows the calculation of a solution space containing all steady state flux distributions of a metabolic network considering stoichiometry, topology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

Metabolic Network Analysis

Metabolic Networks

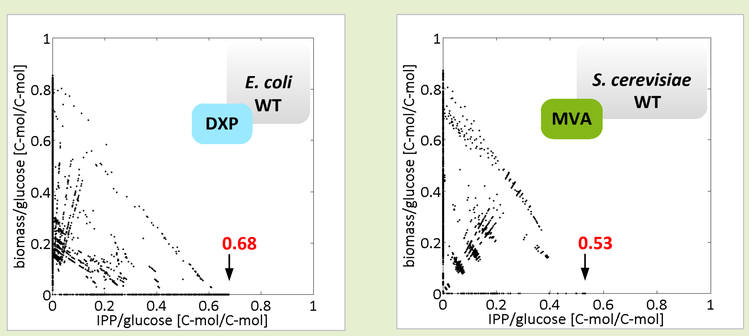
Models of the central carbon metabolism of both organisms were constructed considering the actual knowledge from genome scale models and literature [3,4,5,6,7,8]. They include glycolysis, Entner-Doudoroff pathway, gluconeogenesis, pentose phosphate pathway, citric acid cycle, anaplerotic reactions, fermentative acid production, respiratory chain and transport systems. Cell growth is described as the production of biomass considering precursors, ATP and redox equivalents and glucose is the single carbon source. The model of *S. cerevisiae* wild type further includes the compartmentalization between cytosol and mitochondrion as well as the MVA pathway while the metabolic network of *E. coli* wild type includes the DXP pathway for IPP production.

The model of *E. coli* wild type consists of 65 reactions (21 reversible, 44 irreversible) and 53 metabolites (41 internal, 12 external) whereas the model of *S. cerevisiae* wild type comprises 66 reactions (27 reversible, 39 irreversible) and 49 metabolites (41 internal, 8 external). Simplified models of the metabolic networks are shown on the right. The elementary flux modes were calculated using MATLAB R2011a and efmtool version 4.7.1 [9].



Comparison of Wild Types

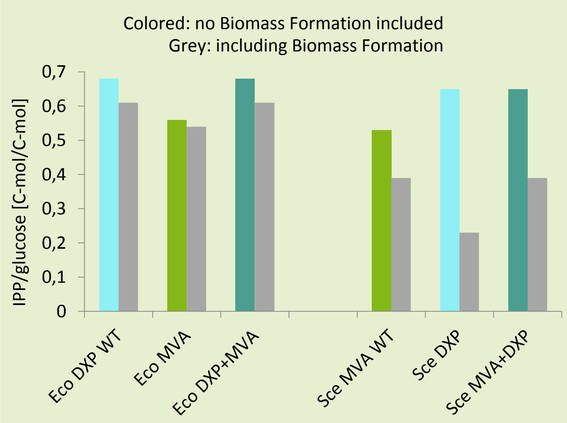
Carbon Yield for Biomass and IPP of the Obtained Elementary Modes



→ **higher potential of *E. coli*** considering theoretical maximum IPP yield compared to *S. cerevisiae*

Exchange and Combination of DXP and MVA Pathway

Theoretical Maximum IPP Yield



Exchange of terpenoid pathway

→ MVA pathway in *E. coli* lowers yield

→ DXP pathway in *S. cerevisiae* enhances yield but lowers yield considering biomass formation

Combination of both pathways

→ No benefit apart from increasing the numer of modes and thus the flexibility of the network

Number of Obtained Elementary Flux Modes

	modes in total	modes with IPP formation	modes with IPP formation and growth
***Eco* WT DXP**	35281	6365 (18 %)	2383 (7 %)
***Eco* MVA**	35969	7053 (20 %)	2764 (8 %)
***Eco* DXP+MVA**	43182	14266 (33 %)	5220 (12 %)
***Sce* WT MVA**	13751	3213 (23 %)	2937 (21 %)
***Sce* DXP**	12077	1539 (12 %)	1241 (10 %)
***Sce* MVA+DXP**	15549	5011 (33 %)	4351 (28 %)

***E. coli*:** more modes in total → network more flexible

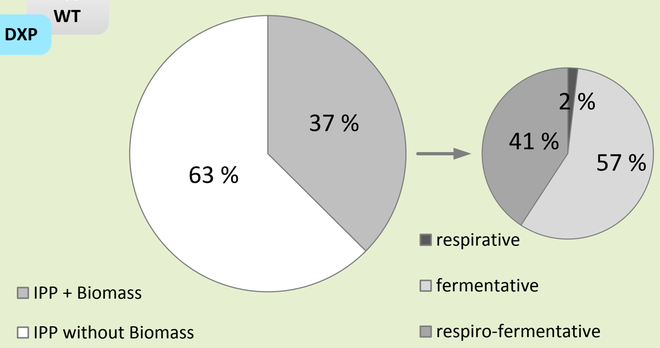
***S. cerevisiae*:** less modes in total but percentaged more modes with product (and biomass) formation

MVA pathway: more modes than DXP pathway → network more flexible, particularly considering product formation

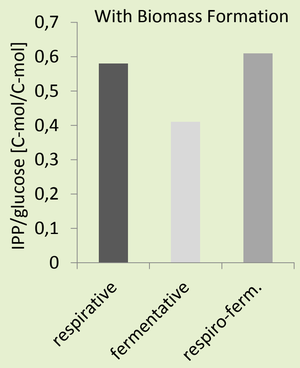
Comparison of Respirative, Fermentative and Respiro-fermentative Metabolism

***E. coli* WT**
DXP

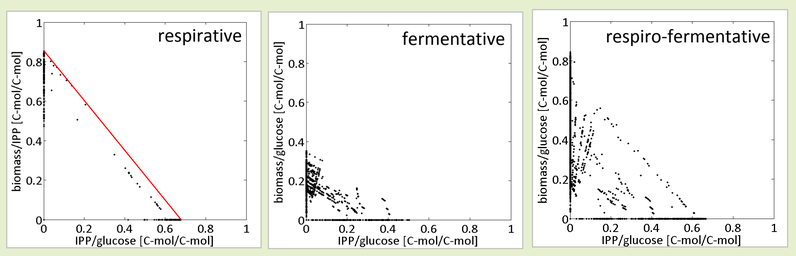
Elementary Flux Modes with IPP Formation



Theoretical Max. IPP Yield



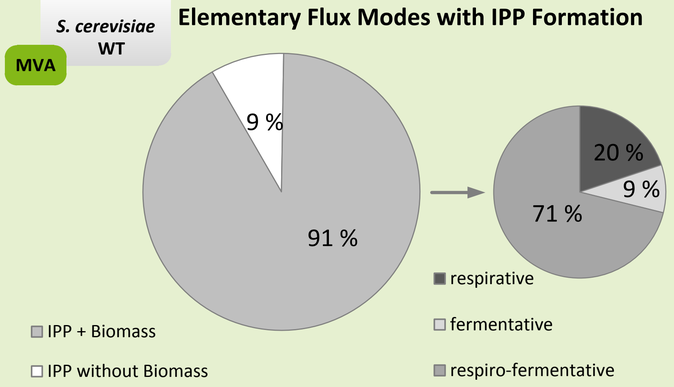
Carbon Yield for Biomass and IPP of the Obtained Elementary Modes



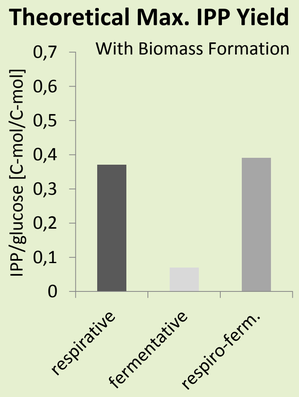
- Fermentative modes show minor IPP and biomass yields
- Least modes with respirative and most modes with respiro-fermentative metabolism → more flexibility
- Respirative and respiro-fermentative modes show similar theoretical maximum IPP and biomass carbon yields (3 % difference)
- Respirative modes lie on the optimal line, which gives the maximum IPP yield possible under different regimes of growth

***S. cerevisiae* WT**
MVA

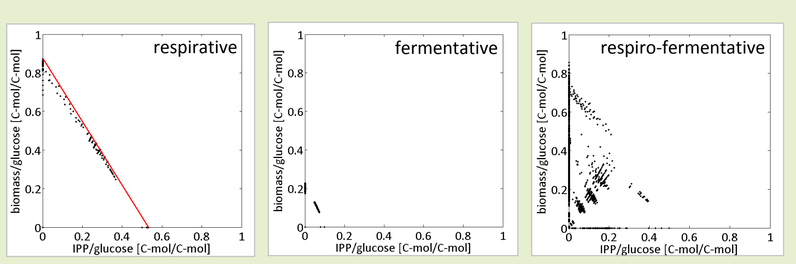
Elementary Flux Modes with IPP Formation



Theoretical Max. IPP Yield



Carbon Yield for Biomass and IPP of the Obtained Elementary Modes



- Least modes with fermentative metabolism & minor IPP and biomass yields
- Most modes with respiro-fermentative metabolism → more flexibility
- Respirative and respiro-fermentative modes show similar theoretical maximum IPP and biomass carbon yields (2 % difference)
- Respirative modes lie on the optimal line, which connects the two extreme modes for biomass and IPP formation and which gives the maximum IPP yield possible under different regimes of growth

Message

Conclusions

- Both organisms have a high potential to supply IPP, although stoichiometrically *E. coli* shows a higher potential
- Exchange of the terpenoid pathways is stoichiometrically not efficient
- The coexistence of both pathways does not enhance the IPP yield, solely the number of modes and thus the flexibility of the metabolic network is enhanced
- Respirative metabolism is most suitable for efficient IPP production in *S. cerevisiae* and *E. coli*

Future Prospects

- Investigate the influence of different carbon sources on IPP yield
- Analyze optimal flux distributions
- Identification of a combination of gene deletions as well as gene amplification targets for the efficient production of IPP
- Proof of concept using a terpenoid as an example

References

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Acknowledgements

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