Towards a platform organism for terpenoid production—

in silico comparison of metabolic networks of E. coli and S. cerevisiae as potential hosts

Gruchatka E., Kayser O., Schütz V.

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

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 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 metabolic network are analyzed.

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 network 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

Compared to S. cerevisiae, E. coli shows a higher potential of IPP considering the theoretical maximum IPP yield.

Exchange and Combination of DXP and MVA Pathway

Theoretical Maximum IPP Yield

IPP + Biomass
IPP without Biomass

Exchange of terpenoid pathways:
- MVA pathway in S. cerevisiae lowers yield but increases yield considering biomass formation.
- Combination of both pathways:
  - No benefit apart from increasing the number of modes and thus the flexibility of the network

Number of Obtained Elementary Flux Modes

Please refer to the table for detailed information.

Comparison of Respiratory, Fermentative and Respiro-fermentative Metabolism

Elementary Flux Modes with IPP Formation

 IPP without Biomass
IPP + Biomass

Elementary modes with IPP and biomass formation have a different distribution between S. cerevisiae and E. coli.

Theoretical Max. IPP Yield

IPP/glucose [C-mol/C-mol]

Theoretical maximum IPP yield indicates a higher potential for E. coli.

Carbon Yield for Biomass and IPP of the Obtained Elementary Modes

IPP/glucose [C-mol/C-mol]

Carbon yield for biomass and IPP demonstrates a higher potential for E. coli.

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.
- Respiratory 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


Acknowledgements

Thanks to Torsten Tobias Arndt for MATLAB support.

Graduate Cluster Industrial Biotechnology