Acknowledgements

P-knockout mutant CO [EtOH cMCS NAD [ ] 0.32

GLYC Native host 1 O [Ethanol ] 0.53 ext

Wall Cytosol GLC GLC Maximal IPP carbon yield

Elementary modes

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]

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 malonate 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 on carbon stoichiometry

1.5 GLC (C6) 3 PYR (C3) 3 AcCoA (C2) MVA IPP (C5)

Taking ATP and redox equivalents into account

1.5 GLC (C6) 3 PYR (C3) 3 AcCoA (C2) MVA IPP (C5)

Elementary mode analysis of metabolic network

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

Possible solutions: alternative pathways with less ATP usage or less CO2 loss

Cytosol Terpenoids

Mitochondrion

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

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

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

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


cMCS knockout mutant

Wild type

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)

Constrained minimal cut sets: enhanced terpenoid production 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

Predicted results

<table>
<thead>
<tr>
<th>Terpenoid</th>
<th>Wild type</th>
<th>cMCS knockout mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental modes</td>
<td>18,844</td>
<td>19,142</td>
</tr>
<tr>
<td>Minimal IPP carbon yield</td>
<td>0</td>
<td>0.27-0.33</td>
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<tr>
<td>Maximal IPP carbon yield</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>Maximal biomass yield</td>
<td>0.87</td>
<td>0.32-0.4</td>
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</table>

Summary/Conclusion

- Heterologous enzymes/pathways with decreased energy consumption or less CO2 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 patchouli as a reporter (a sesquiterpenoid and fragrance compound used in perfume industry)

References


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