Direct fermentation route for the production of acrylic acid

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Outline

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Acrylic Acid (AA)

- AA and its amide and ester derivatives are principle materials in the manufacture of polymeric products

- Over 4.2 million t/a of AA is used for the manufacture of various products

- Examples for products: superabsorbent polymers (SAP), water treatment chemicals, coatings (decorative, automotive, paper) and adhesives

- Currently AA is produced by petrochemical process
Conventional production of AA and alternative routes

AA is commercially produced through the oxidation of propylene or propane

Problem: - high CO$_2$ emission affects global climate changes
  - limited and not renewable petrochemical carbon sources

Alternative routes involve one fermentation step to produce precursor of AA

Problem: - high amounts of energy are required
  - cost-intensive separation and purification of chemical catalysts
Solution

Replacement of petrochemical production of AA by ecofriendly process

Development of a direct pathway from glucose to acrylic acid in recombinant E. coli by

→ Usage of genetically modified E. coli strain for the production of 3-hydroxypropionate (3-HP)

→ Extensive screening for key enzymes for the conversion of 3-HP into AA

→ Designing a novel metabolic pathway for AA
Pathway design for the biosynthesis of 3-HP from glucose

- Integration of the genes *dhaB*, *gdrAB* and *gabD4* into genomic DNA of *E. coli* W3110 → SE001
- *dhaB* gene from *Klebsiella pneumoniae* codes for glycerol dehydratase
- *gdrAB* gene from *Klebsiella pneumoniae* allows the expression of reactivating factor, which participates in the reactivation of the coenzyme B$_{12}$
- *gabD4* from *Cupriavidus necator* codes for aldehyde dehydrogenase for the synthesis of 3-HP
• Elimination of the two genes *ackA-pta* and *yghD* to reduce the formation of by-products

• *ackA-pta* and *yghD* are responsible for the decreasing level of acetyl-CoA and 3-hydroxypropionaldehyde (3-HPA) as a result of formation of acetic acid and 1,3-propanediol

• Enhanced 3-HP production in the recombinant *E.coli* strain SE002
• Integration of the genes *gpsA* and *GPP2* into genomic DNA of the *E.coli* strain SE002

• *gpsA* gene from *E.coli* encodes glycerol-3-phosphate dehydrogenase, which converts glucose into glycerol-3-phosphate

• *GPP2* gene from *S.cerevisiae* encodes glycerol-3-phosphatase, which synthesize glycerol

• the genes were cloned into the vector and transformed into the SE002 strain to yield SE003
Screening for key enzymes

Since there is no known direct route for the enzymatic dehydration of 3-HP into AA, a detour path was designed

Three enzymatic steps → CoA attachment to 3-HP by CoA-transferase
→ dehydration of 3-HP-CoA to Acryloyl-CoA
→ detachment of CoA from AA-CoA by CoA-hydrolase

Transferase-, dehydratase- and hydrolase-coding genes were amplified by PCR and cloned into vectors, which were transformed into \textit{E.coli} parental strain W3110

The selection of appropriate enzymes could be realized by in vitro enzymatic activity tests
After enzyme expression and purification on a Ni-NTA agarose column the enzyme activity was analyzed.

15 transferase, 67 dehydratase and 7 hydrolase candidates were analyzed. Best coding gene candidates were:

- **YdiF** from *C. necator* for CoA transferase
- **Aflv_0566** from *Anoxybacillus flavithermus*
- **yciA** from *E. coli* for CoA hydrolase
Complete synthesis of acrylic acid from glucose

The final step was the integration of the selected genes for AA production from 3-HP into the DNA of the SE003 *E.coli* strain

The resulting strain SE004 was able to produce 0,12 g/l of AA after 15 h
Conclusion and forecast

Successful development of the first direct fermentative route for producing 0.12 g/l of AA from glucose via 3-HP, 3-HP-CoA and Acryloyl-CoA

To increase the amount of AA several technical hurdles need to be overcome

non-specific CoA transferase and hydrolase can convert key metabolic intermediates 3-HP-CoA and AA-CoA into undesired by-products

Increased toxicity of acrylic acid compared to 3-HP can negatively affect enzymatic activity in *E.coli*

Enzyme engineering such as site-directed and random-mutagenesis can be applied to improve substrate specificity of the enzymes

Metabolic engineering enzymes that improves the substrate selectivity of CoA transferase and CoA hydrolase by balancing the pool of free CoA and gene expression

Optimization of the fermentation conditions for oxygen supply
Sources


Thank you for your attention!

Any questions?