Enhanced microbial lipid production with genetically modified yeast and fungus

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- Cross-disciplinary technological and business expertise
- A not-for-profit and impartial research centre

VTT offering covers everything from strain constructions to the end product testing

- Host selection
- Pathway evaluation with metabolic modelling and bioinformatics
- Enzyme discovery, characterization, engineering, synthetic enzymes
- Genetic engineering, synthetic biology
- Systems biology tools for identifying key bottle necks
- Analytics, automation and HTS
- Bioprocess development
- Application testing
Industrial Biotechnology at VTT

- Engineering of broad range of production organisms to the desired product, from PoC to industrial use
- Expertise in non-conventional production organisms: plant, algae, fungi, bacteria

Product portfolio
- Various alcohols
- Organic acids; lactic acid, xylonic acid, arabinioic acid, glycolic acid, galactaric acid, galactonic acid, etc.
- Terpenes, volatile hydrocarbons
- Triacylglycerides and other lipid derivatives
Triacylglycerol (TAG) is raw material for biodiesel and renewable diesel

- Vegetable (and other bio-based) oils are mainly triacylglycerols (Oil = lipid = TAG)
- Triacylglycerol (TAG) contains three fatty acid residues esterified with a glycerol molecule

R1, R2, R3 denote aliphatic fatty acid chain (saturated or unsaturated). Usually >15 carbons. E.g. oleic acid.

- TAG is used as raw material for biodiesel (transesterified lipids) and renewable diesel (hydrogenated alkanes)
- Composition of vegetable oils is similar to composition of microbial oils => microbial oils can replace vegetable oils
Microbial lipid production

- Some microbes (yeast, filamentous fungi, algae) can produce lipids over 20% of their biomass, i.e. they are **oleaginous** microbes.
- The produced lipids are mainly triacylglycerols (TAGs).
- Lipid production requires a lot of carbon and cofactors (especially NADPH). At most **32 g of TAG** can be produced from **100 g glucose** (assuming that all glucose is used for TAG production). **In practise, the max yield has been approx. 22%**.

\[ \text{100 g glucose} \rightarrow \text{32 g TAG} \]
Triacylglycerol biosynthesis

Glycolysis

Production of cytosolic acetyl-CoA

Synthesis of fatty acids

Synthesis of triacylglycerol
Production of cytosolic acetyl-CoA differs in oleaginous and non-oleaginous microbes

Cytosolic acetyl-CoA is produced in oleaginous microbes via citrate and in non-oleaginous microbes via acetaldehyde.

With high C/N ratio high lipid production

Under nitrogen limited conditions decrease of AMP results in decrease of IDH activity → citrate accumulates.
Engineering of microbial strains for oil production

*Cryptococcus curvatus* (yeast) and *Mucor circinelloides* (fungus) were genetically engineered by over-expressing four genes in different combinations. The codon optimized genes were expressed under endogenous promoters and terminators.

**Pathway:**

1. Glucose / xylose
2. Pyruvate
3. Acetyl-CoA
4. Acyl-ACP/CoA
5. Glycerol + 3 x acyl-CoA
6. Triacylglycerol

**Expression:**

- PDC1, ALD6, ACS2 expression
- PDAT expression

PDAT = phospholipid-diaclyglycerol acyltransferase
Bioreactor cultivations with *C. curvatus* transformants with C/N ratio of 80

TAG titre (max 21.8 g/l)

Yield on biomass (max 50.0%)

![Graphs showing TAG production and yield on biomass for glucose and xylose]
Bioreactor cultivations with *C. curvatus* transformants with C/N ratio of 80

Yield on substrate (max 23.3%)

![Yield on substrate graph]

Production rate (max 0.18 g/l/h)

![Production rate graph]
Bioreactor cultivations with \textit{C. curvatus} transformants with C/N ratio of 80

• The ALD+PDAT and the ALD+ACS+PDAT transformants produced more triacylglycerol than wild type \textit{C. curvatus} from glucose or xylose.

• The yields of triacylglycerol on both biomass and substrate were higher in the transformants than in wild type \textit{C. curvatus}, but the rate of triacylglycerol production was lower.
  • Titre and yield on substrate were improved by approximately 25\% by addition of the \textit{ALD6} and \textit{PDAT} genes to \textit{C. curvatus}

• The ALD+PDAT transformant was a better triacylglycerol producer than the ALD+ACS+PDAT transformant

• The improvements were generally larger on glucose than on xylose.
Bioreactor cultivations with *M. circinelloides* transformants with C/N ratio of 60

TAG titre (max 11.5 g/L)

Yield on substrate (max 26.0%)

- TAG titre (% change relative to control)
- Yield on substrate (% change relative to control)

Glucose | Xylose
---|---
 PDAT | ALD+ACS+PDAT

- Glucose
- Xylose

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Bioreactor cultivations with *M. circinelloides* transformants with C/N ratio of 60

Yield on biomass (max 62.6%)

Production rate (max 0.10 g/l/h)
Bioreactor cultivations with *M. circinelloides* transformants with C/N ratio of 60

- The PDAT and the ALD+ACS+PDAT transformants generally produced more triacylglycerol, at higher yields, than wild type *M. circinelloides* from glucose.
- The PDAT transformant also produced more triacylglycerol, at higher yield, than wild type *M. circinelloides* from xylose.
- The rate of triacylglycerol production was lower than wild type *M. circinelloides* on glucose, but improved on xylose with the PDAT transformant.
Conclusions

- Targeted genetic modifications to both *C. curvatus* and *M. circinelloides* can improve not only the triacylglycerol content of the cells and the titre of triacylglycerol produced, but also the yield of triacylglycerol from carbohydrate.

- Both *C. curvatus* and *M. circinelloides* use xylose as well as glucose as a substrate for triacylglycerol production.

- Non-conventional, oleaginous yeast and fungi can be genetically engineered.
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