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Faculteit Bio-ingenieurswetenschappen
Faculty of Bioscience Engineering

UNIVERSITEIT GENT

SEVENTH FRAMEWORK PROGRAMME

Genetic engineering of *Starmerella bombicola* for the production of cellobiose lipids

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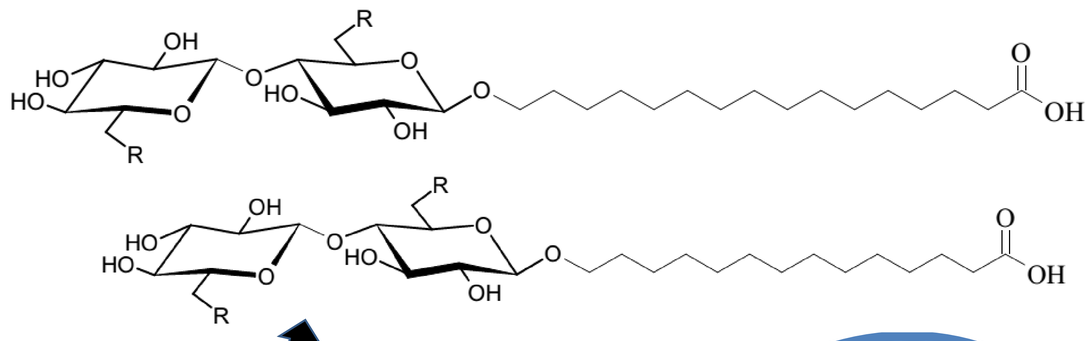
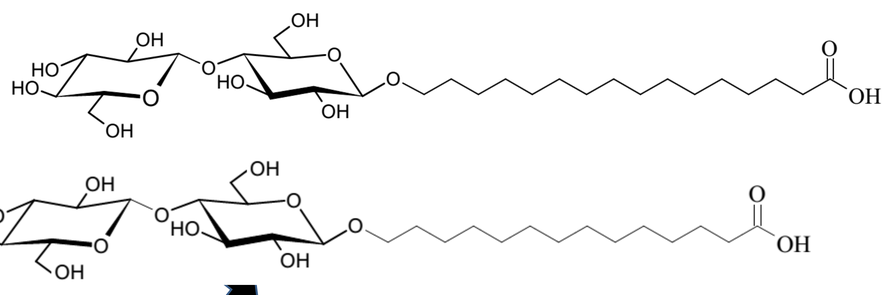
Glycolipids are an interesting kind of biosurfactants. They have valuable properties like low toxicity, good biodegradability and excellent surface activity. Unfortunately, commercialization of these molecules has been hampered by low yields and low structural variety. Replacing the different wild type producers by a platform organism is a possible strategy to solve these problems. *Starmerella bombicola* is well known for the production of sophorolipids. Exceptional yields (400g/l) and high tolerance towards glycolipids make this yeast an interesting candidate. Attempts at engineering this organism to create a production platform for cellobiose lipids have been successful but further optimization is required.

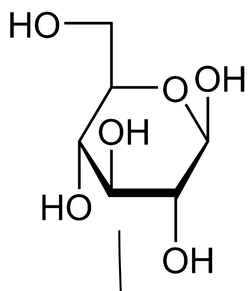
Accumulation of cellobiose lipids in the cytosol can trigger regulatory effects, resulting in low production levels. To eliminate this problem, transport is needed. *S. bombicola* already has a transporter for the sophorolipid molecules, but the possibility remains that the newly produced cellobiose lipids are not recognized. Several transporters will be tested to circumvent this.

Products ready for downstream processing



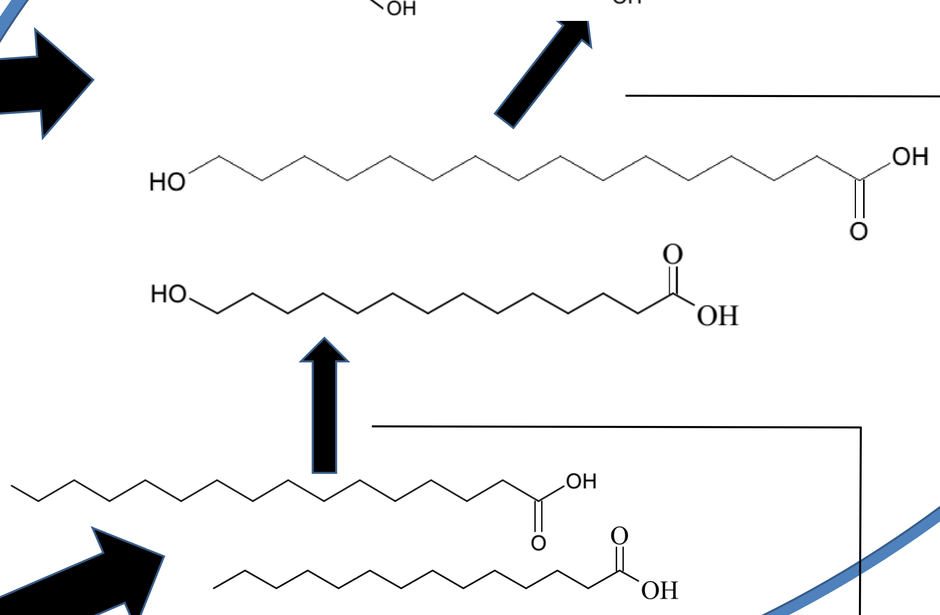
Starmerella bombicola





Glucose

Vegetable oil



Glycosylation of the processed fatty acids is done by a single enzyme, consuming two molecules of UDP-glucose in this reaction. Even though wild type cellobiose lipids only contain a lipid tail with 16 carbon atoms, the possibility remains that other fatty acids can be used as a substrate. Several candidates will be tested to see if higher yields and/or novel molecules can be created.

Incorporation of fatty acids in cellobiose lipids requires the terminal hydroxylation of these molecules. This hydroxylation is catalyzed by P450 enzymes. By knocking in P450 enzymes with interesting substrate specificities, different fatty acids can be shunted in the pathway.

Combining the different genes can result in several interesting strains. Not only higher yields can be achieved, also new-to-nature cellobiose lipids with altered fatty acid tails are a possibility. For the moment, 4 strains with different combinations of transporters and glucosyltransferases have been created and are being tested for their production capacity by shake flask fermentations. During these and other experiments, several hydrophobic substrates will be tested as well to see whether or not they have an influence on the production levels or structural diversity of the molecules being produced. Interesting vegetable oils with high ratios of medium chain fatty acids like lauric, myristic and palmitic acid are coconut oil, palm kernel oil and palm oil. The knowledge gathered during this project can be used for further engineering of *S. bombicola* for the production of other tailor-made glycolipids.

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