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UNIVERSITEIT GENT

SEVENTH FRAMEWORK PROGRAMME

IWT

Creation of a fungal production platform for biosurfactants by P450 engineering

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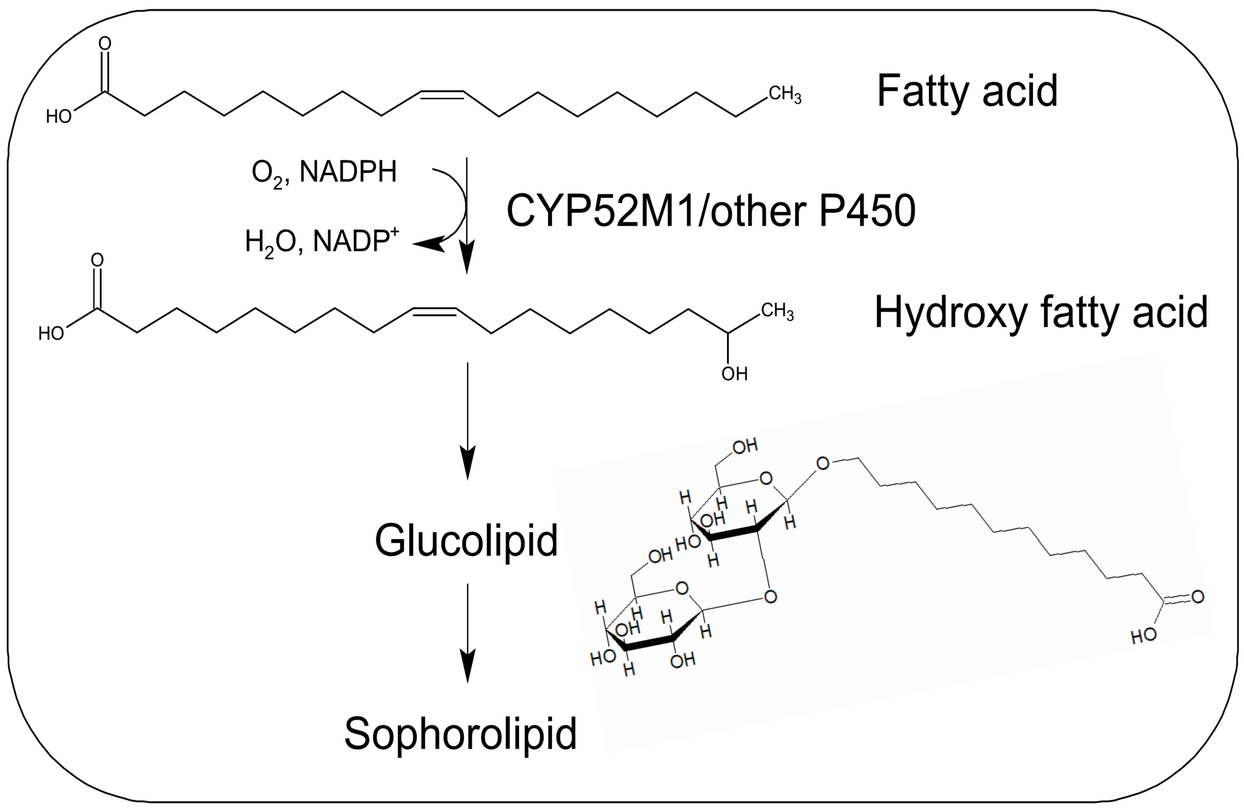
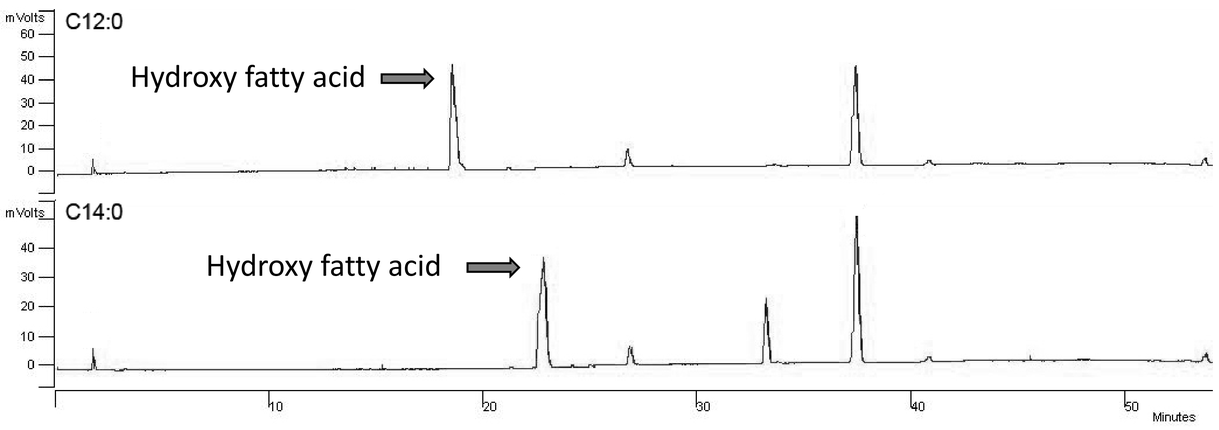
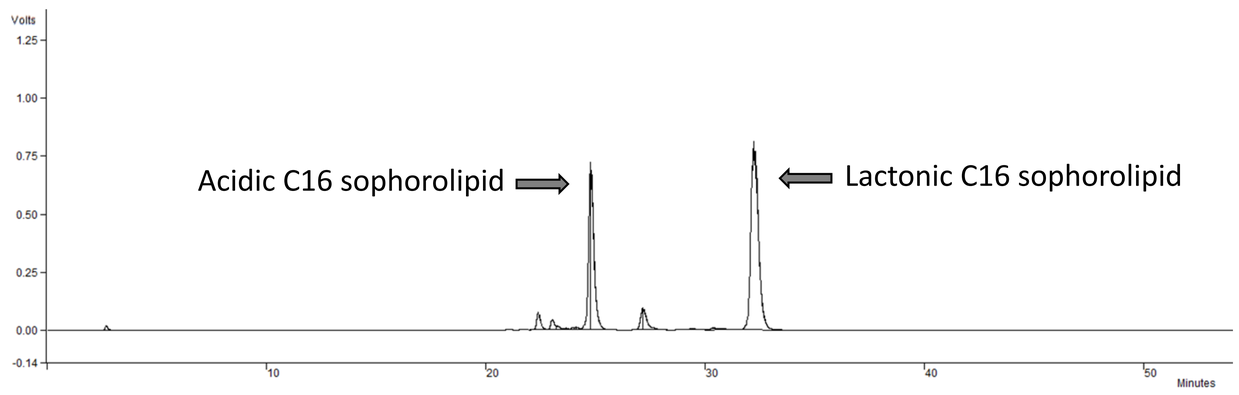
The yeast *Starmerella bombicola* is known for the efficient production of sophorolipids, a kind of biosurfactant. Even though concentrations up to 400 g/L can be achieved, the long fermentation times and limited molecular diversity hamper further market penetration. In the sophorolipid biosynthesis, the P450 monooxygenase CYP52M1 performs the first step in the production pathway. It also controls the size of the fatty acid being incorporated. Most P450s rely on a reaction partner to donate the electrons necessary for the catalytic reaction. In nature, several classes of P450s have their reaction partner fused to the P450, acting as a single self-sufficient protein controlling the entire chain from electron donor to product. By introducing a chimeric P450 enzyme coupled to its own reductase in the sophorolipid pathway, a simpler system is created with potentially higher production titers and/or novel biosurfactants.

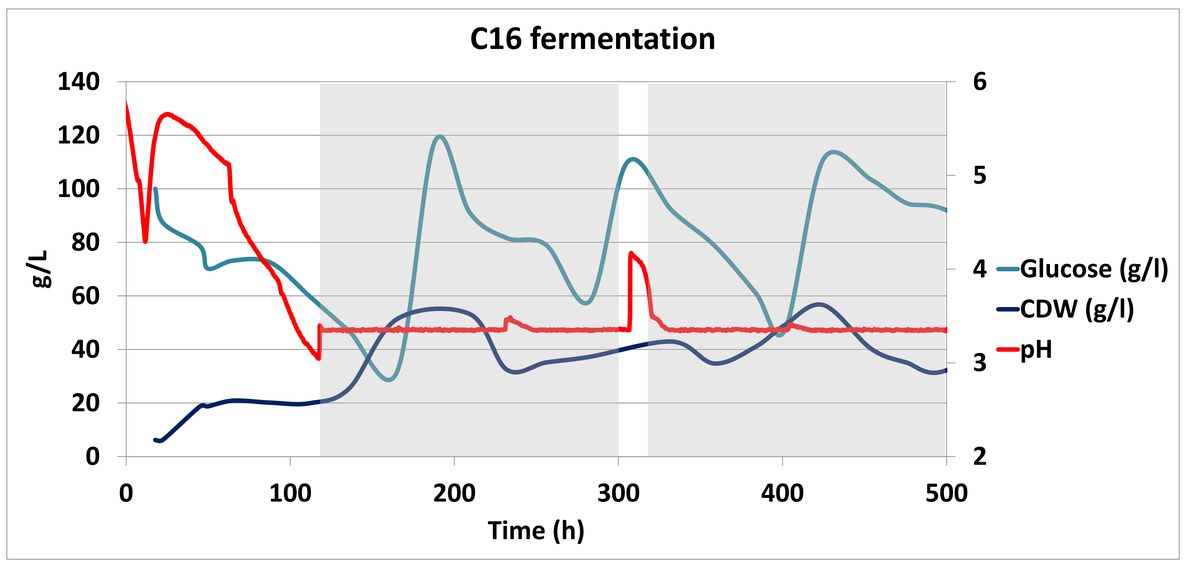




Previous research showed that it is possible to express (self-sufficient) P450 enzymes with interesting properties in *S. bombicola*. The ones tested were CYP102A7 from *Bacillus licheniformis* and $P450_{Foxy}$ from *Fusarium oxysporum.* Both enzymes show high subterminal hydroxylation activity towards lauric and myristic acid, fatty acids with respectively 12 and 14 carbon atoms. Even though the enzymes are active, no sophorolipids could be detected due to stereochemical constraints of the hydroxy fatty acids being produced.

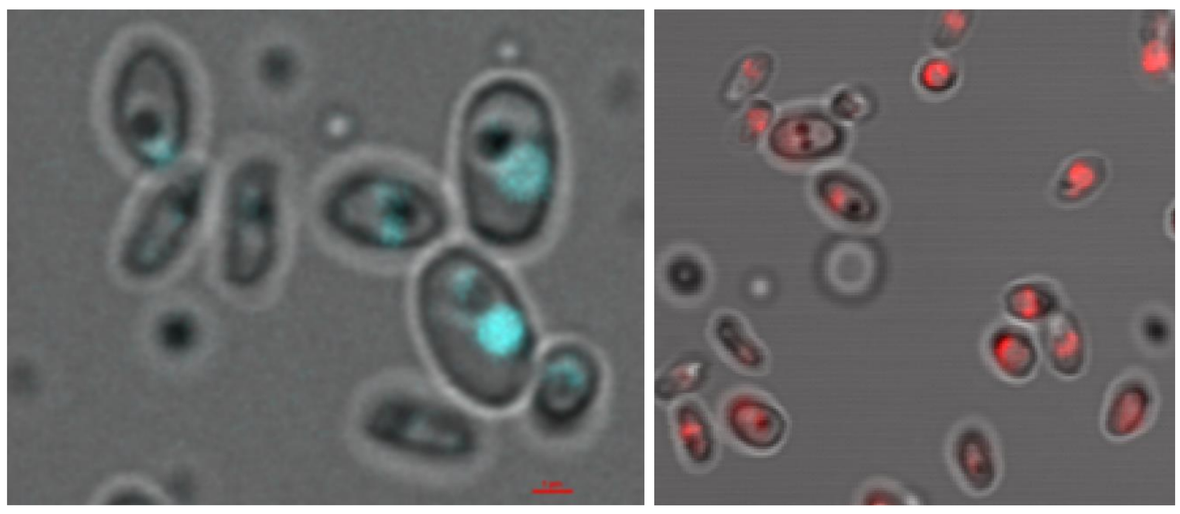
Introduction of chimeric P450s proved to be a valuable tool for engineering *S. bombicola*. Not only were the P450s active but they proved to exhibit interesting properties. One of the chimeric P450s tested hydroxylated only one specific fatty acid without overoxidating them into dicarboxylic acids. It is the first time that sophorolipids with a fatty acid tail consisting out of 16 carbon atoms are being produced without other variants present.





Upscaling the production process from Erlenmeyer flask to bioreactor proved to be a valuable step in optimising the production process. A cyclic fermentation with a total time of approximately 20 days delivered several grams of both acidic and lactonic C16 sophorolipids. Even though the first cycle lasted 12 days, it only delivered 1.5 grams while the second one delivered just above 5 grams in 8 days. This can easily be explained by the long exponential phase lasting 120 hours during the first cycle. The second cycle resulted in a very short exponential phase of only 24 hours.

The strategy of introducing chimeric P450s proved to be successful. Not only were new kinds of molecules produced, it also provided a platform technology to produce specific types of sophorolipids. Furthermore by coupling fluorescent proteins to CYP52M1, localisation of the enzyme inside the cell was possible. In total three different fluorescent proteins were used and all were expressed without significantly influencing the production capacity of the sophorolipids. This offers a powerful tool for fundamental studies of the sophorolipid biosynthetic pathway in *S. bombicola*.



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