Creation of a fungal production platform for biosurfactants by P450 engineering

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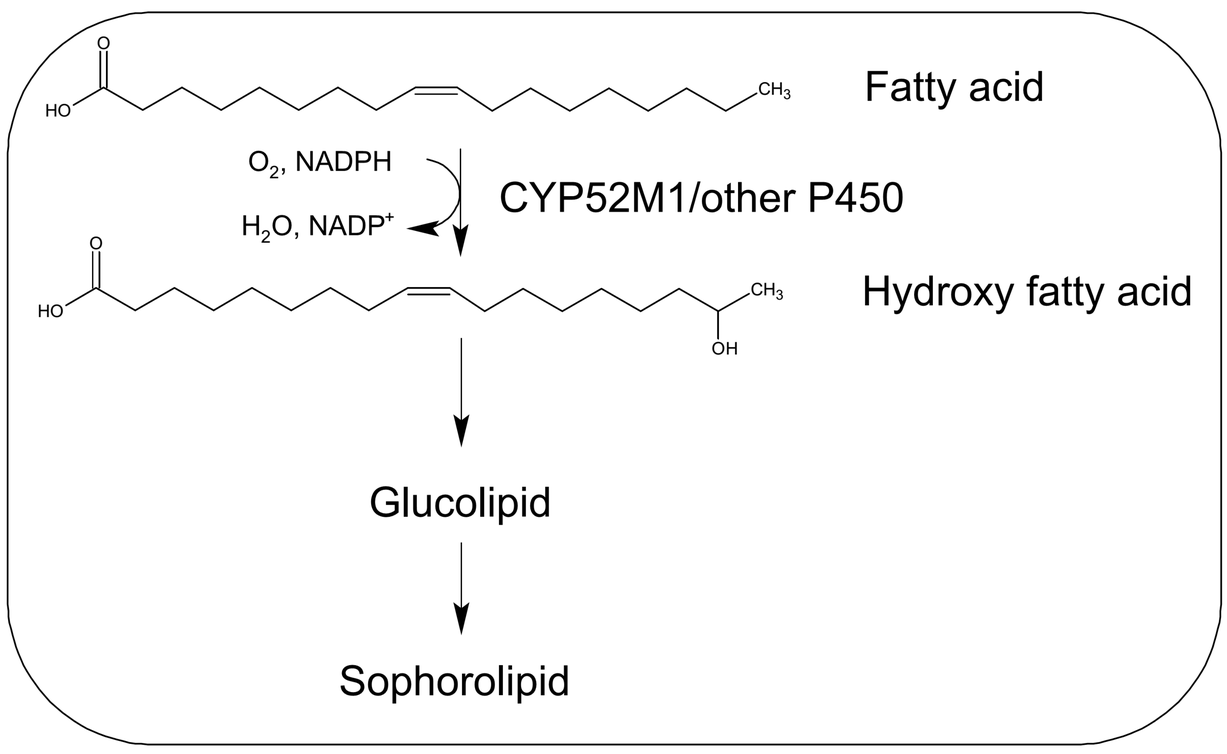
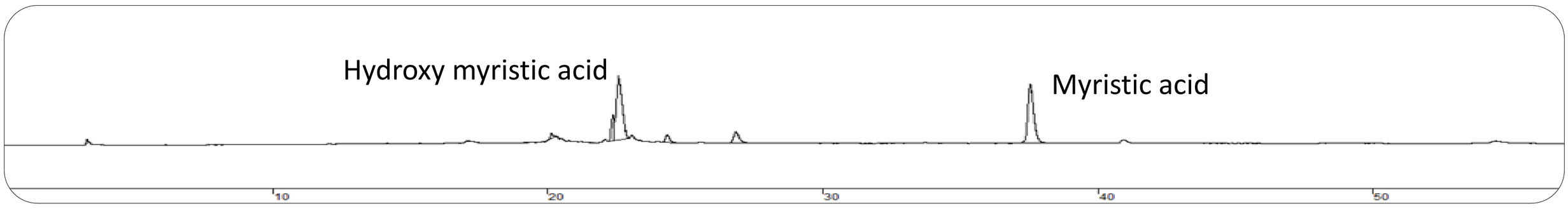
The yeast *Starmerella bombicola* is known for the efficient production of sophorolipids, a kind of biosurfactant. Even though concentrations up to 400 g/L can be achieved, the long fermentation times hamper further cost reduction of these molecules. Several cytochrome P450s have been identified in its genome. One of them, CYP52M1, is necessary for the biosynthesis of the sophorolipids by performing the first step in the pathway. 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. This way, a less complex system with potential higher activity is created. By fusing the *S. bombicola* CYP52M1 with a suitable reaction partner, a higher flux through the pathway may be achieved. It also creates a potential platform for the introduction of other P450s and thereby altering the fatty acids being incorporated.

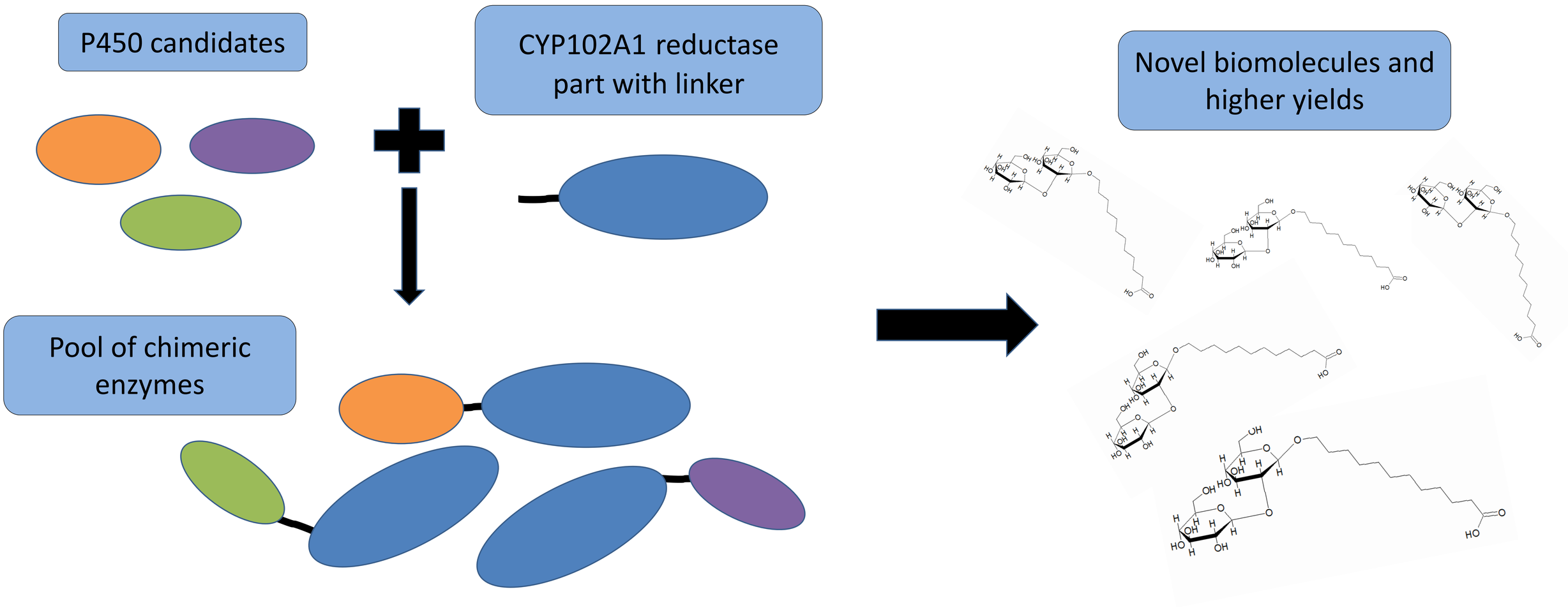


Previous research showed that it is possible to express (self-sufficient) P450 enzymes with interesting properties in *S. bombicola*. The ones tested were CYP102A7 from *Bacillus licheniformis* and $P450_{Foxy}$ from *Fusarium oxysporum*. Even though they produced subterminal hydroxylated acids with varying chain length, no sophorolipids were produced due to stereochemical constraints. Recently, by introducing another P450, sophorolipids with a palmitic acid tail were produced in a more pure form.



Chromatogram of myristic acid and the products formed by CYP102A7

To generate chimeric self-sufficient P450s, several constructs were designed with several P450s. These are both unaltered wild type sequences and truncated variants. All of them are combined with the reductase part of CYP102A1, the most studied self-sufficient P450 from *Bacillus megaterium*. Preliminary results with CYP52M1 variants show productivity of these kinds of enzymes.



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