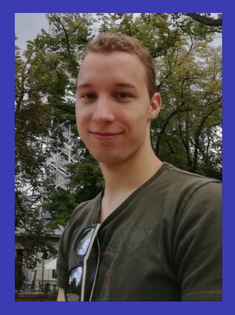
Genetic engineering of the yeast *Starmerella bombicola* for efficient production of biosurfactants from waste streams



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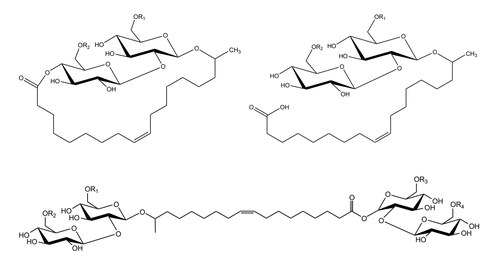
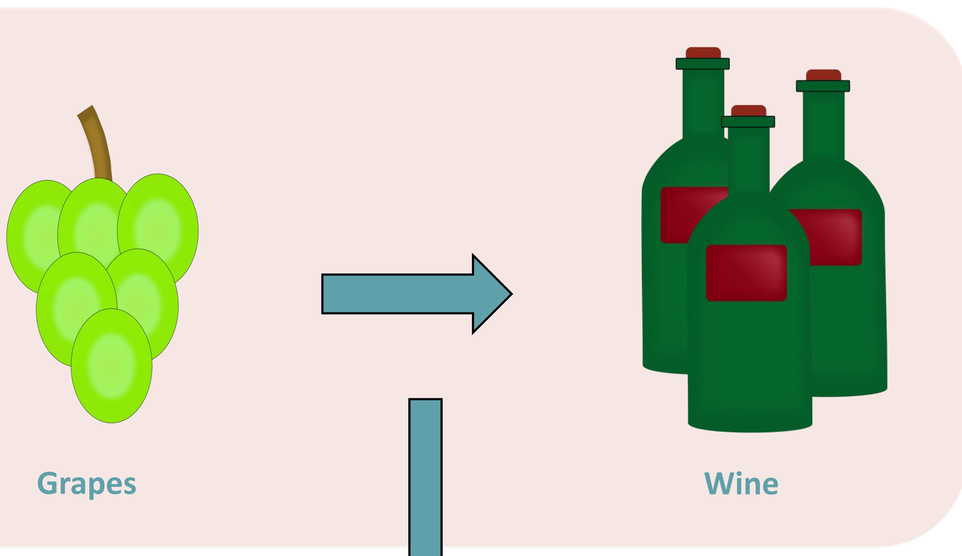
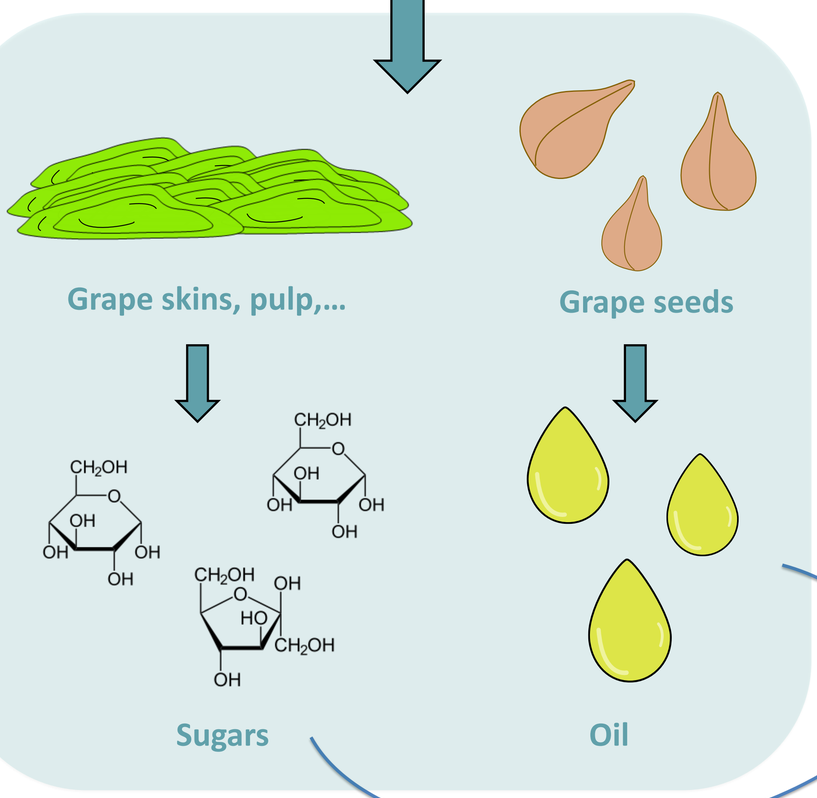
Introduction

Surfactants are important chemicals used in diverse applications from detergents to pharmaceuticals. However, as most of them are fossil fuel based, their continued use is not sustainable and this is where microbial biosurfactants offer a solution. The yeast ***Starmerella bombicola*** is a natural producer of the biosurfactant class **sophorolipids**, which consist of a hydrophilic sophorose group attached to a hydrophobic fatty acid tail. In order to make the production competitive with traditional surfactants and as **sustainable** as possible, this research aims to start from agricultural **waste streams** for the growth medium of *S. bombicola* and to simultaneously create genetically engineered strains with **enhanced productivities**.



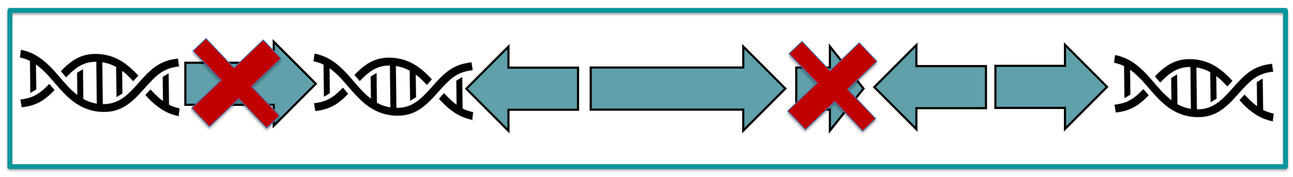
Sophorolipids

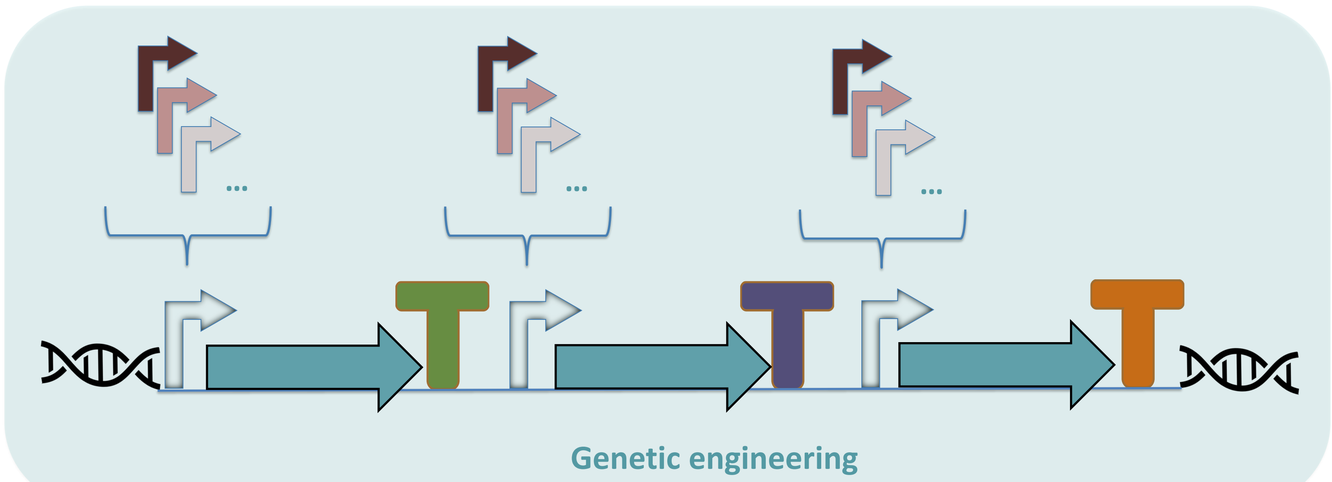


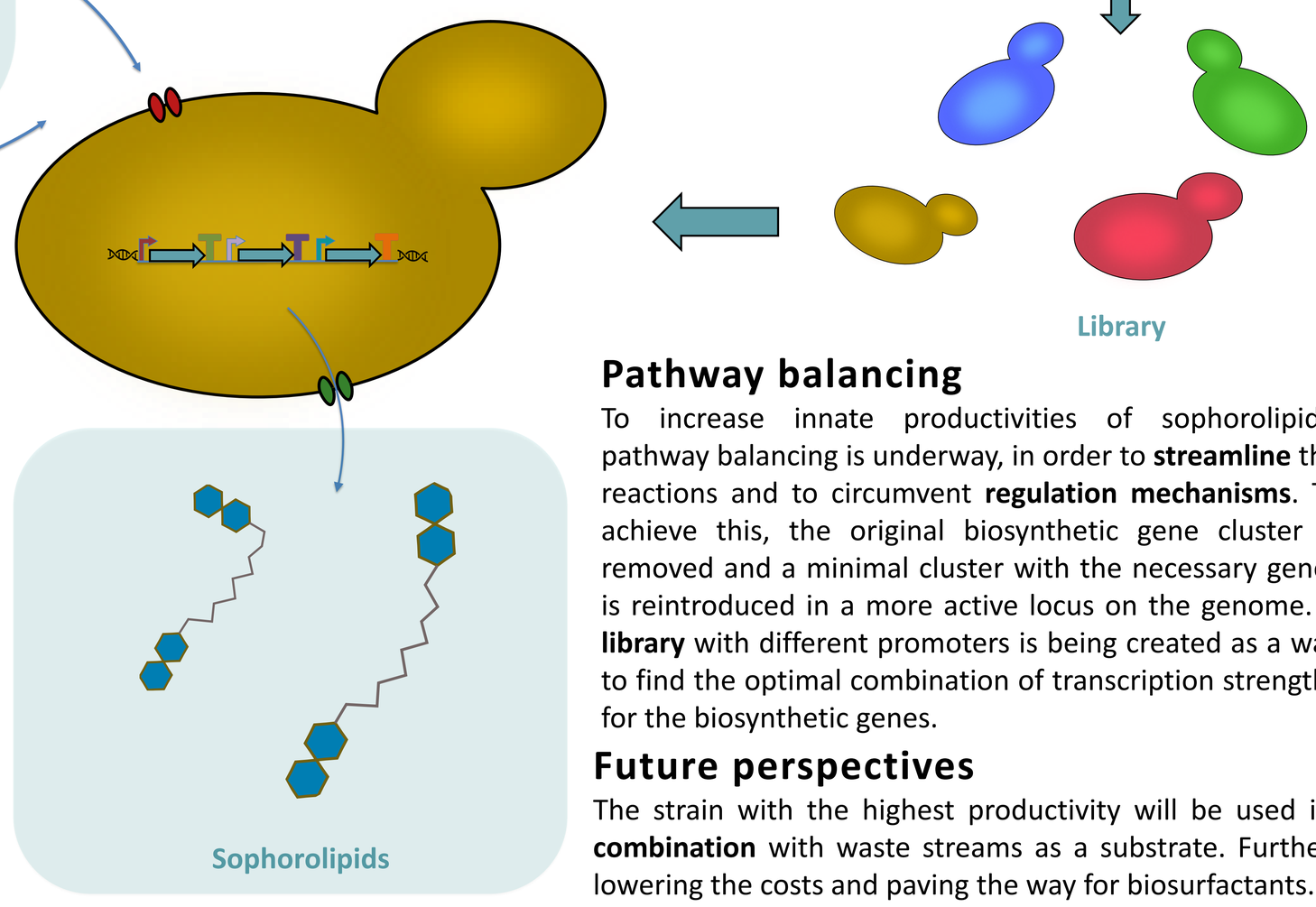


Biosynthetic cluster

S. bombicola's genes for the production of sophorolipids are **clustered** together in the genome. This cluster consists of a **cytochrome oxidase** for the hydroxylation of fatty acids, two **glycosyl transferases** that attach glucoses groups to the hydroxy-fatty acid, an **acetyl transferase** for the acetylation of the glucoses, a sophorolipid **transporter** and a **lactone esterase** for the lactonization of excreted sophorolipids. A **KO** in the lactone esterase and the acetyl transferase genes creates a strain that produces bolaform sophorolipids.







Wine Pomace

During wine production, substantial amounts of wine pomace are generated as a **by-product**, containing the pulp, seeds, skins and stalks of the grapes. As this pomace still contains a lot of valuable compounds and since the substrate is an important contributor to the final biosurfactant production cost, **extraction** of sugars and oil was performed. The sugar extract was tested as a possible substrate for the growth of ***S. bombicola*** and subsequent production of sophorolipids, together with the lipid extract form the grape seeds. *S. bombicola* was able to **grow** on the waste derived substrates and **production** of biosurfactants was established. These results will not only make biosurfactant production more **sustainable** but also more **economically competitive** with fossil-based surfactants, while providing a **valorisation** route for a significant waste stream.

Pathway balancing

To increase innate productivities of sophorolipids, pathway balancing is underway, in order to **streamline** the reactions and to circumvent **regulation mechanisms**. To achieve this, the original biosynthetic gene cluster is removed and a minimal cluster with the necessary genes is reintroduced in a more active locus on the genome. A **library** with different promoters is being created as a way to find the optimal combination of transcription strengths for the biosynthetic genes.

Future perspectives

The strain with the highest productivity will be used in **combination** with waste streams as a substrate. Further lowering the costs and paving the way for biosurfactants.

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