

Title: Genetic manipulation of *Cutaneotrichosporon oleaginosus* for improved lipid accumulation

Group: Systems and Synthetic Biology

Project type: MSc thesis

Credits: 36

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Description:

Type of thesis: Experimental work.

Description:

Fatty acids are often used to provide beneficial properties to cosmetics, personal-care, chemical products. These oils and fatty acids are mostly gathered from plants (i.e. palm, coconut, olive, sunflower) however the increasing demand leads to severe consequences on the ecosystem. Oleaginous yeasts have great potential for the sustainable production of lipids. *Cutaneotrichosporon oleaginosus* ATCC 20509 (*C. oleaginosus*) is a well-known oil-producing yeast and able to accumulate oils up to 40-60% of its biomass under nutrient-limited conditions (N-limitation) [1,2]. On the other hand, its natural capacity is limited to develop an economically feasible production process. Therefore, obtaining enhanced lipid accumulation on a systematic way is essential. One of the strategies to establish *C. oleaginosus* as a microbial cell factory is manipulating some identified genes that have shown potential to increase lipid accumulation and growth.

Previous investigations on *C. oleaginosus* resulted in some overexpression targets. These genes were overexpressed successfully and new strains were characterized. Within the scope of this project, the promising overexpression targets will be combined in one construct and transformed into *C. oleaginosus*. After confirming the increased expression levels of these genes, new transformants will be characterized through shake flask experiments [3]. Samples will be collected to identify the biomass composition obtained via engineered strains and at different fermentation conditions. After the initial characterization, the cultivation medium's carbon-to-nitrogen ratio will be optimized for the best performer strain.

References:

- [1] F. Bracharz, T. Beukhout, N. Mehlmer, T. Brück, Opportunities and challenges in the development of *Cutaneotrichosporon oleaginosus* ATCC 20509 as a new cell factory for custom tailored microbial oils, *Microb. Cell Fact.* 16 (2017) 1–15. <https://doi.org/10.1186/s12934-017-0791-9>.
- [2] N. Pham, M. Reijnders, M. Suarez-Diez, B. Nijssse, J. Springer, G. Eggink, P.J. Schaap, Genome-scale metabolic modeling underscores the potential of *Cutaneotrichosporon oleaginosus* ATCC 20509 as a cell factory for biofuel production, *Biotechnol. Biofuels.* 14 (2021) 1–17. <https://doi.org/10.1186/s13068-020-01838-1>.
- [3] M. Duman-Özdamar, Zeynep Efsun; Martins dos Santos, Vitor A.P.; Hugenholtz, Jeroen; Suarez-Diez, Tailoring and optimizing fatty acid production by oleaginous yeasts through the systematic exploration of their physiological fitness, (n.d.). <https://doi.org/https://doi.org/10.1101/2022.06.20.496586>.

Used skills:

- Advanced molecular biology techniques
- Biochemistry and analytic techniques

Requirements:

- Basic microbiology (cell culture, transformation)
- Molecular biology (PCR design, cloning)