Cell factory design for the valorisation of local substrates into food supplements

Summary

The transition towards a bioeconomy requires novel processes that use sustainable substrates, have improved life cycle assessments, and, hence, require less energy to produce. Oleochemicals are a wide range of molecules that can be used as biofuels, cosmetics, plastics, surface coatings, but also as food and feed supplements. Microbial lipids (ML) are one high-potential feedstock for the production of these oleochemicals. Production of MLs does not require land, compete with arable land, and is not affected by weather. Non-conventional yeast Rhodotorula toruloides has recently been defined as a promising workhorse for biotechnological applications. This yeast grows on various substrates, including lignocellulosic hydrolysates and food waste, accumulates over 70% of its biomass as lipids (g/gdw), and grows to high cell densities. R. toruloides is also a natural producer of high-value compounds, such as carotenoids and enzymes. In the current project, novel synthetic biology tools for R. toruloides will be utilized and further developed to improve the uptake of hemicellulose hydrolysates and convert them into value-added food supplements like specialty lipids and antioxidants.

Research field:	Chemistry and biotechnology
Supervisors:	Prof. Dr. Petri-Jaan Lahtvee Dr. Nemailla Bonturi
Availability:	This position is available.
Offered by:	School of Science Department of Chemistry and Biotechnology
Application deadline:	Applications are accepted between November 16, 2020 00:00 and December 16, 2020 23:59 (Europe/Zurich)

Description

Background

The transition towards bioeconomy requires novel processes that use sustainable substrates, have improved life cycle assessments, and, hence, require less energy to produce. Oleochemicals are a wide range of molecules that can be used as biofuels, cosmetics, plastics, surface coatings, among others [1]. Microbial lipids (ML) are a potential feedstock for oleochemical production [2]. MLs are mostly triacylglycerides (TAGs) produced by oleaginous microorganisms [3]. Production of MLs does not require land, compete with food production, and is not affected by weather [4]. The yeast *Rhodotorula toruloides* has recently been defined as a high-potential workhorse for biotechnological applications [5]. This yeast grows on various substrates [6], including lignocellulosic hydrolysates [7], accumulates over 70% of lipids (g/gdw), and grows to high cell densities [8]. *R. toruloides* is also a natural producer of high-value compounds, such as carotenoids and enzymes [5].

In our previous research, we have sequenced the *R. toruloides* strain, developed metabolic engineering tools (CRISPR/Cas9, Golden Gate Assembly), and developed a systems biology platform with a genome-scale model and omics data analysis pipelines.

In the current thesis project, we aim to engineer this promising non-conventional yeast to accumulate and excrete high levels of specialty lipids and optimize their metabolism to regenerate limiting redox factors as well as to reduce the excretion of the main byproduct – CO2.

Plan

For creating an efficient platform for specialty lipid production, we will focus on three main strategies: (i) secretion of fatty acids, (ii) decoupling of biosynthesis and cofactor regeneration, and (iii) improved fixation of CO2.

Production of specialty lipids and Fatty acid secretion

Lipids and fatty acids (FA) are accumulated intracellularly limiting their production (directly coupled to growth) and their extraction represents a significant cost in downstream processing. Secreting lipids and FA is a paramount strategy to overcome the aforementioned drawbacks.

In our approach, we will use transcriptomics and proteomics and identify the transporters involved in FA excretion by the oleaginous yeast *Yarrowia lipolytica* [9] and, further, transfer these transporters to *R. toruloides*. In order to

improve FA production, the genes FAA1 (long-chain fatty acid CoA ligase 1, FAA2 (long-chain fatty acid CoA ligase 2), DGA (diacylglycerol O-acyltransferase 1), and LRO1 (phospholipid:diacylglycerol acyltransferase) will be deleted and the native Tes1/ACOT8 (acyl CoA thioesterease 8) will be overexpressed. For specialty lipid production we aim to control the lenght of the FA chain and this will be done by fusing FAS2 (fatty acid synthease alpha subunit) with diferent non native thioesterases: EcTesA, *E. coli* TesA (Thioesterase 1); EcYbgC, *E. coli* YbgC (Acyl-CoA thioester hydrolase); UcBTE, *Umbellularia californica* BTE/FATB (dodecanoyl-[acyl-carrier-protein] hydrolase), AcTesA, *Acinetobacter baylyi* TesA (Thioesterase 1).

The project will be carried out in collaboration with Prof. Ledesma-Amaro lab at Imperial College London, UK, and will result in at least two publications.

Decoupling growth and external cofactor regeneration

NADPH regeneration represents the main limiting step reaching maximal lipid production yields.
Therefore, our aim is to test environments, where cofactors can be regenerated independently from growth or biosynthesis.
Previously, we have developed yeast-laden hydrogels, where living cells are 3D printed into nutrientpermeable matrixes. In this project, we aim to functionalize a conductive hydrogel and the current of electrons will be generated by highly efficient light-harvesting indium phosphide nanoparticles. Therefore, we aim to create a system, where light-induced decupling of growth from cofactor regeneration will take place in a novel cell immobilization platform.
The project will be carried out in collaboration with Dr. Tarmo Tamm from the University of Tartu and Prof. Neel S. Joshi from Northeastern University and will result in at least two publications.

CO2 consumption

CO2 represents (one of) the most abundant unwanted byproduct of bioprocesses while being an attractive substrate due to its low cost and abundance in the atmosphere. *R. toruloides* has a native CO2-fixing pathway – acetyl-CoA carboxylase. Based on proteomics analysis, we have identified the presence of the pathway under glucose and xylose growing conditions, and additionally, the created genome-scale models have predicted its activity. In the project, we intend to overexpress the pathway and evaluate its effect on the growth of *R. toruloides*. The work will result in at least one publication.
The applicant will also be involved in RITA Bioeconomy project ‘LLTTI18211 "Maximising added value and efficient use of raw materials in the bioeconomy and its sectors in Estonia"’, a project to create an input for the development of the Estonian Bioeconomy roadmap.

References

[1] Yang X, Sun W, Shen H, Zhang S, Jiao X, Zhao ZK (2018) Expression of phosphotransacetylase in: *Rhodosporidium toruloides* leading to improved cell growth and lipid production. RSC Adv 8:24673–24678.
[2] Unrean P, Khajeeram S, Champreda V (2017) Combining metabolic evolution and systematic fedbatch optimization for efficient single-cell oil production from sugarcane bagasse. Renew Energy 111:295–306.
[3] Ratledge C, Wynn JP (2002) The Biochemistry and Molecular Biology of Lipid Accumulation in Oleaginous Microorganisms. Adv Appl Microbiol 51:1–51.
[4] Koutinas AA, Chatzifragkou A, Kopsahelis N, Papanikolaou S, Kookos IK (2014) Design and technoeconomic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production. Fuel 116:566–577.
[5] Park YK, Nicaud JM, Ledesma-Amaro R (2017) The Engineering Potential of *Rhodosporidium toruloides* as a Workhorse for Biotechnological Applications. Trends Biotechnol 36:304–317.
[6] Shen H, Gong Z, Yang X, Jin G, Bai F, Zhao ZK (2013) Kinetics of continuous cultivation of the oleaginous yeast *Rhodosporidium toruloides*. J Biotechnol 168:85–89.
[7] Bonturi N, Crucello A, Viana AJC, Miranda EA (2017) Microbial oil production in sugarcane bagasse hemicellulosic hydrolysate without nutrient supplementation by a *Rhodosporidium toruloides* adapted strain. Process Biochem 57:16–25.
[8] Li Y, Kent Z, Bai F (2007) High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. 41:312–31.
[9] Ledesma-Amaro, R., Dulermo, R., Niehus, X., & Nicaud, J. M. (2016). Combining metabolic engineering and process optimization to improve production and secretion of fatty acids. Metabolic engineering, 38, 38-46.

Responsibilities and tasks:

- Develop a promoter library for the non-conventional yeast *R. toruloides*
- Design *R. toruloides* cells capable of secreting fatty acids
- Design *R. toruloides* cells to accumulate and secrete poly-unsaturated fatty acids

- In collaboration with UT and NU, test a platform for *R. toruloides* immobilization and external redox balance regeneration
- Overexpress native pathways in *R. toruloides* for improved CO2 fixation

Qualifications:

- Proficiency in yeast synthetic biology
- Proficiency in 3D printing of living materials
- Proficiency in bioprocess optimization
- Strong background in analytical quantification of small molecules

The applicants should fulfill the following requirements:

- MSc degree in synthetic biology, biotechnology, microbiology, or similar field
- Previous experience in working with microbes
- Previous experience in metabolic engineering
- Previous experience with yeast is a plus
- Previous experience handling bioreactors is a plus
- Good English language skills (oral and written)
- Fluency in Estonian is a plus

To get more information or to apply online, visit https://taltech.glowbase.com/positions/177 or scan the the code on the left with your smartphone.