ERA CoBioTech

BioTech Research & Innovation Hack

2021

# ERA CoBioTech Funded Projects at A Glance: SYCOLIM

## Synthetic microbial communities for the production of limonene derived products

PART OF EUROPEAN BIOTECH WEEK

INNOVATION IS IN OUR GENES

# SYCOLIM

**Bioproduction of limonene and its derivatives, such as perillyl alcohol, in *Yarrowia lipolytica* and *Pseudomonas putida* microbial communities.**

***Limonene can be easily produced in microorganisms through the MEV or MEP pathways adding an extra enzyme. Perillyl alcohol is produced from limonene through 3 metabolic steps. SyCoLim partners are working on microorganism engineering and metabolic modelling in order to optimize these processes.***

## The huge potential of synthetic microbial communities to overcome metabolic engineering drawbacks and enhance bioproduction of limonene and its derivatives.

The bioproduction of compounds of interest for industry (as pharmaceuticals, nutraceuticals, cosmetics, etc.) is currently replacing the chemical synthesis, as usually the process is cheaper and more respectful with the environment. A division of labor approach is able to reduce detrimental effects due to genetic and metabolic burden, resulting in an increase of the production yield in such artificial communities. Therefore, the aim of the SyCoLim project is to build an optimized consortia of yeast (*Yarrowia lipolytica*) and/or bacteria (*Pseudomonas putida*) for the implementation of a metabolic pathway that leads to the production of limonene (d-limonene and l-limonene) and perillyl alcohol for relevant industrial applications. In this project, construction of yeast and bacterial strains that produce those compounds is combined with an in silico approach for simulating synthetic microbial consortia to study the producer microorganisms behaviour. Engineered strains of *Y. lipolytica* that produce limonene and perillyl alcohol are already available. Moreover, some of the engineered *P. putida* strains have already been developed and the consortium is working on some more. Additionally, strains able to bind each other will be developed with the aim to stabilize communities where every strain provides a part of the pathway. Furthermore, different growth media are being checked for the different strains to find the best carbon source that can lead to higher yields. On the other side, a general mathematical model is available to guide experimental design, as well as to provide new theoretical knowledge in the organization and co-culture stability of microbial communities.

### Classical and cutting-edge techniques at the interface between metabolic engineering and synthetic biology

Production of limonene and perillyl alcohol can become more profitable for industry due to the bioproduction approach proposed in the SyCoLim project. To date, these compounds are extracted from lemon peelings, so they are obtained as a side product from the lemon market and this is dependent on the availability of lemon wastes. Using microorganisms for the synthesis of limonene and derived compounds will allow a more stable production based on commercial needs. To reach such an objective, classical molecular biology methods but also cutting-edge technologies will be used. As a result, we will obtain strains of *Y. lipolytica* and *P. putida* that are able to produce limonene in its two versions and perillyl alcohol. Strains that are able to specifically bind to each other will be built at the end of the project. Additionally, analytic data will be generated to understand how these compounds are produced in both organisms. Our general mathematical model could be applied to alternative pathways and organisms by changing some of its parameters in a user-friendly manner, therefore representing an important result applicable to this and further studies. Bottlenecks are expected to be where the major of metabolic engineering problems appear: toxicity due to metabolite accumulation, balance of enzymes, yield, stability, scalability, etc. However, all the previous drawbacks are expected to be addressed and overcome in the SyCoLim project.

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## Current results

The SyCoLim project has already achieved some important breakthroughts in the field. For example, Y. lipolytica strains able to produce d-limonene, l-limonene and perillyl alcohol are already available and are currently used to study the optimization of metabolite bioproduction. In the process, some knowledge has been acquired about the functioning of central carbon metabolism and derived pathways. This will be applied to further applications that will be required for this and other metabolic engineering problems. Furthermore, *P. putida* strains with surface display devices that make possible their attachment to *Y. lipolytica* strains or other *P. putida* strains are currently available, which is something convenient for the proper balance of a microbial community co-culture. A general mathematical model is available to study production of limonene and derived compounds in both *P. putida* and *Y. lipolytica*. Results achieved during the project will be properly disseminated to a bigger audience in relevant and targeted scientific events.

Regarding the developed technologies that are being applied in the SyCoLim project, the list below shows the work that has been already published:
https://pubs.acs.org/doi/pdf/10.1021/acssynbio.1c00227

**Project website:** https://sycolim.eu/

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