ERA CoBioTech

BioTech Research & Innovation Hack

2021

ERA CoBioTech Funded Projects at A Glance: C1Pro

Microbial conversion of C1 to value-added products by integrated systems and synthetic biology

PART OF EUROPEAN BIOTECH WEEK

INNOVATION IS IN OUR GENES

C1Pro

Development of microbial cell factories that can convert the sustainable raw material methanol into medically and industrially relevant compounds

This project uses the methanol-consuming bacterium Bacillus methanolicus as a chassis to develop cell factories that can convert methanol into four utterly different, commercially valuable chemicals. Our research brings sustainable solutions to the industrial biotechnology sector.

Project coordinator:

Trygve Brautaset
Norwegian University of Science and Technology (NTNU), (Norway)

Consortium:

Bielefeld University, (Germany)

INSA - Institut National des Sciences appliquées de Toulouse - (France)

BASF SE, (Germany)

SINTEF Materials and Chemistry, (Norway)

Acies Bio d.o.o., (Slovenia)

Project duration:

01 April 2018 - 30 September 2021

Total budget: 1.3 €M

Methanol as an alternative and sustainable feedstock for biotechnology

Society needs sustainable production processes for key platform chemicals with various industrial applications. Industrial biotechnology uses sugar-based feedstocks to feed microbial cell factories for bioproduction of a wide variety of chemicals and compounds. Sugars rely on use of cultivable land and therefore represents an undesired competition with food and feed sector. Methanol is a one-carbon (C1), non-food feedstock and so far underutilized in bio-industry. Methanol is an attractive raw material for biotechnological processes because of its chemical properties, relatively low price and availability from both fossil and renewable sources. The overall objective of C1Pro project is to establish *B. methanolicus* as a platform host for the production of value-added products from methanol. The secondary objective is to use a combined and integrated systems and synthetic biology approach for *B. methanolicus* strain engineering, including genetic engineering, metabolic network modelling, and omics analyses, resulting in recombinant strains with desired properties. Those objectives have been well reached and addressed in the C1Pro project.

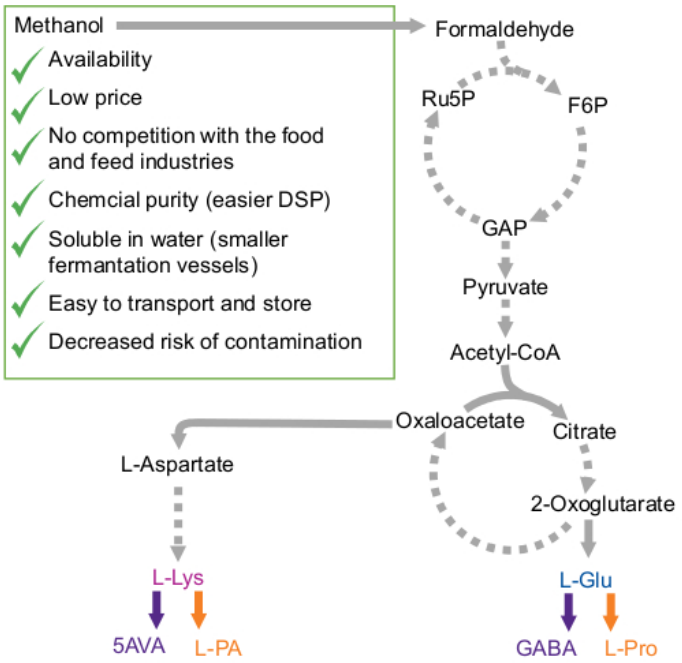

Figure 1: From methanol to value added products
Schematic view of biosynthesis pathways of 5AVA, L-PA, GABA and L-Pro from the sustainable feedstock methanol. GAP, glyceraldehyde 3-phosphate; F6P, fructose 6-phosphate; Ru5P, ribulose-5-phosphate.

This project has received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No [722361]

The C1Pro project has established a sustainable platform for methanol-based production of several value-added products with proven industrial applications: γ-aminobutyric acid, 5-aminovaleric acid, L-proline and L-pipecolic acid (GABA, 5AVA, L-Pro and L-PA, respectively). Our project paves the way for implementing methanol as alternative feedstock in biotechnology.

Multi-omics, systems and synthetic biology approaches for the development of targeted microbial cell factories

The natural methylotrophic and thermophilic bacterium *B. methanolicus* was chosen as model organism in this project for several reasons: it utilizes methanol as raw material for growth and energy, it grows at elevated temperatures (50 °C), it naturally overproduces L-glutamate, and its classical mutants have demonstrated a high potential to overproduce L-lysine. In addition, the project partners have accumulated experience in systems and synthetic biology research on this organism. Regarding the products we aimed for: the ω-amino acids GABA and 5AVA can be cyclized by lactamization, while L-Pro and L-PA are cyclic amino acids. The targeted products serve as building blocks of polymers or precursors of pharmaceuticals and other biologically active substances. Systems and synthetic biology approaches were key to the strain and process development, which is facilitated by common biosynthesis pathways. Novel genetic tools simplifying regulation of gene expression on different levels via CRISPR interference for gene repression and riboswitches for regulatory circuits, have been developed and used. Pathway design was guided by the genome-scale metabolic model constructed in this project and iteratively fine-tuned based on experimental results. Best-performing bacterial strains were characterized in a multi-omics approach.

Main results

We have demonstrated bioproduction of all four different target products from methanol by genetically engineered *B. methanolicus* strains, fulfilling our overall objective (see above). The production levels of the compounds vary and for some of them will have to be further improved to be commercially relevant. The results and achievements have been published in peer review articles and presented elsewhere. Along the way to obtain these results, we have generated new genetic tools and techniques highly useful for all future genetic engineering of this organism, including adjustable expression vectors, techniques for genome modification based on CRISPR/Cas9 or counterselection markers, fermentation and cultivations techniques, development and refinement of genome scale metabolic model. We have applied different omics technologies in a combined synthetic and systems biology approach for the in-depth analysis of production strains. All the results and achievements have heavily relied on the broad and diverse competences of the project partners brought together into a close collaboration through C1Pro consortium.

Future prospect

Much of the efforts, competences, knowledge, genetic tools and technologies generated in C1Pro was taken further into a new application for the ERACoBioTech 3rd call and this has resulted in a new funded 3-year project MCM4SB (to be presented elsewhere) which started in April 2021. MCM4SB assembles some of the C1Pro members NTNU, ACIES Bio and UNIBI as partners, and adds new partner from University of Marmara Turkey on board. MCM4SB is a systems and synthetic biology project with its new goals and objectives and it will build on and further develop generic tools and technologies developed in C1Pro, including GSM (Genome Scale Model), CRISPR/Cas9-based genome modification system, genetic tools and cultivation technologies. In addition, several of the strains constructed in C1Pro are currently central model organisms in ongoing or to be started Master projects among C1Pro project partners, hopefully resulting in improved production titers and new understanding of metabolic background of production strains.

C1Pro has been very prolific with respect to generation of peer review publications, many of them with co-authors representing several project partners. The project partners have also been active presenting project results on conferences and workshops as well as other more popular scientific arenas. We have an active website: https://www.ntnu.edu/web/c1pro/c1pro where all dissemination activities are constantly monitored and announced. So far, no patents have been generated but we have one industrial partner that actively builds valuable knowledge and technology experiences from C1Pro useful for their commercial profile.

C1Pro website: https://www.ntnu.edu/web/c1pro/c1pro

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