ERA CoBioTech

BioTech Research & Innovation Hack

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ERA CoBioTech Funded Projects at A Glance: SynConsor4Butanol

Sustainable Production of n-Butanol by Artificial Consortia Through Synthetic and Systems Biology Approaches

PART OF EUROPEAN BIOTECH WEEK

INNOVATION IS IN OUR GENES

SynConsor4Butanol

Sustainable Production of n-Butanol by Artificial Consortia Through Synthetic and Systems Biology Approaches

Researchers with the EU-funded SynConsor4Butanol project are using artificial twin and triplet consortia to continuously convert lignocellulosic biomass to n-butanol without releasing CO_2.

Project coordinator:

Philippe Soucaille
TBI, University of Toulouse, (France)

Consortium:

University of Nottingham, (United Kingdom)

Technical University Munich, (Germany)

University of Girona, (Spain)

BASF, (Germany)

Project duration:

01 February 2020 -
31 January 2023

Total budget: 1.2 €M

Continuous production of n-butanol without releasing CO_2

One of the greatest challenges facing society is the future sustainable production of chemicals and fuels from non-petrochemical resources while at the same time reducing greenhouse gas emissions. The use of abundant and renewable lignocellulosic materials to make chemicals and fuels can be highly valuable if the process is efficient and does not lead to CO_2 production.
In this project, SynConsor4Butanol, we will engineer synthetic consortia to convert, WITHOUT CO_2 production, the cellulosic fraction from lignocellulosic materials to a value added product, n-butanol, that can be used both as a platform chemical or a biofuel.
Engineered synthetic consortia will be derived through a combination of interdisciplinary methodologies, synthetic biology (University of Nottingham; Technical University of Munich), metabolic engineering (University of Toulouse, University of Nottingham; Technical University of Munich), systems biology (University of Girona; University of Toulouse), and fermentation development (University of Girona).
Research Innovation practices will be embedded within the program of work through the participation of dedicated Social Scientists from the Synthetic Biology Research Centre at Nottingham. Life Cycle Analysis and Techno-Economic Analysis will be undertaken by Toulouse in partnership with BASF. SynConsor4Butanol will lead to the development of new "CO_2 free" Sustainable production and conversion processes based on lignocellulosic feedstocks. This will lead to a value-added product, n-butanol, useful in the chemical industry and as a biofuel.
Ultimately, the developments made will lead to new sustainable industrial processes. Moreover, by combining resources and expertise, SynConsor4Butanol connects research partners in four different countries (Germany, UK, Spain, and France) with different but complementary scientific and technological expertise, thereby maximizing resources and sharing the risks, costs and skills.

SynConsor4Butanol's Approach

SynConsor4Butanol convenes an international team of researchers from a company and universities around Europe, joining forces to make a dream come true: the biotechnological production of n-butanol from lignocellulosic biomass without any addition of cellulolytic enzymes and without releasing CO_2. SynBio tools have been successfully developed for *Clostrdium carboxidivorans* and they have been used to produce the first metabolically engineered mutant strain. A similar approach is currently performed with *Clostridium cellulovorans*. Synthetic butanol pathways have been constructed and are currently evaluated in a *Clostrdium acetobutylicum* mutant lacking the natural butanol producing pathways. Most of the synthetic pathways have now been implemented in *C. acetobutylicum* and have been shown to be functional. Metabolically dependent engineered mutants of *C. acetobutylicum* and *C. carboxidovorans* should be available in the next quarter and evaluated both in batch and continuous cultures for n-butanol production at high yield without CO_2 release.

Main results

The SynConsor4Butanol project has already achieved several breakthroughs. By characterizing the methylome of *C. carboxidivorans,* we manage to construct a shuttle *E. coli-C. carboxidivorans* vector that could be introduced by conjugation without restriction. The base CRISPR/cas9 vector was assembled with a Cas9 encoding gene codon optimised for *C. carboxidivorans* while avoiding any RM motifs in the coding region. A base CRISPR vector was generated which allowed the knockout of *pyrE* as a proof-of-concept target. This strategy was extended to delete two of the restriction/methylation encoding genes and to obtain the first metabolically engineered strain.
A fast method for gene knockout and knockin, based on the use of CRISPR/Cas9 as a counter-selective system has also been developed for *C. acetobutylicum* and used to construct the first metabolically

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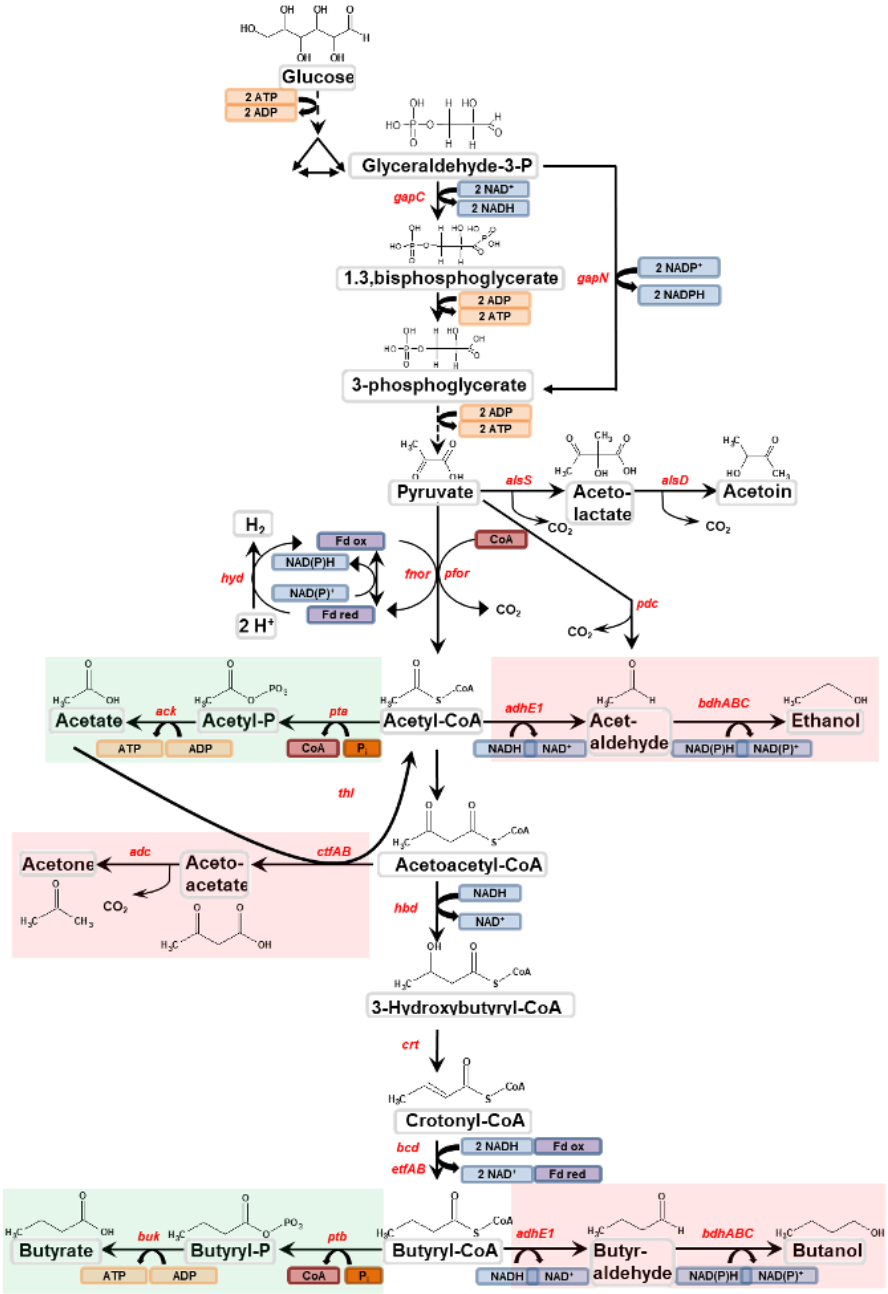
After establishment of media, carbon sources and cultivation conditions for *C. cellulovorans*, we determined the prerequisites for establishment of a method for triparental conjugation of recombinant plasmids from *E. coli* to *C. cellulovorans*. First triparental conjugation experiments have been successful. Electroporation for introduction of recombinant plasmids into *C. cellulovorans* is currently being evaluated as an alternative to triparental conjugation.

During this period, wild type strains of *C. carboxidivorans* and *C. cellulovorans* were tested in different media, and one common media was elaborated to evaluate change link to the co-cultures of the control strain. Shortly, *C. acetobutylicum* is going to be activated and evaluated.



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Central metabolism of Clostridium acetobutylicum.

The green box indicates primary products under conditions of acidogenesis, whereas the red box indicates primary products under conditions of solventogenesis. The letters in red and italics indicate the corresponding genes.

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Future prospect

During this project novel gene knockout and knockin systems have been developed for both *C. acetobutylicum* and *C. carboxidovorans*, based on the use of CRISPR/Cas9 as a counter-selective method. The gene knockout system is currently being used to engineer both *C. acetobutylicum* and *C. carboxidovorans* to rapidly obtained the first proof of concept of metabolically dependent artificial consortia for the production of n-butanol at high yield without CO_2 release.

The key enzymes to provide the additional electrons needed to produce n-butanol in *C. acetobutylicum* have been identified. This result is key to create mutant strain producing no other products than n-butanol or to expand the range of alcohols/diols that could be produced by *C. acetobutylicum* at high yield.

The developments made represent an important step in the path towards a Consolidated Bio-Process for the continuous production of n-butanol from lignocellulosic biomass without releasing CO_2.

Scientific production: publications, patents and other IPR relevant outcomes

The University of Toulouse has developed an improved and simple CRISPR/Cas9 system to more rapidly engineer *C. acetobutylicum*. Furthermore, they also identified the key enzymes to provide the additional electrons needed to produce n-butanol. The work is currently in revision in *Nature Com.*.
The Technical University of Munich develop a method to quantify two different clostridium species in a co-culture. The work is in press in *System. Appl. Microbiol*.

1. Wilding Steel T, Ramette Q, Jacottin P, and Soucaille P (2021) Improved CRISPR/cas9 tools for the rapid metabolic engineering of *Clostridium acetobutylicum*. *Int. J. Mol. Sci.* 22 : 3704.
2. Foulquier C., Rivière A., Heulot M., Dos Reis S, Perdu C., Girbal L., Pinault M., Dusséaux S., Soucaille P., Meynial-Salles I., (2021) Molecular characterization of the missing electron pathways for butanol synthesis in *Clostridium acetobutylicum*, *Nature Communication*, in revision
3. Schneider M, Bäumler M, Lee NM, Weuster-Botz D, Ehrenreich A, Liebl W (2021) Monitoring Co-cultures of *Clostridium carboxidivorans* and *Clostridium kluyveri* by Fluorescence in situ Hybridization with Specific 23S rRNA oligonucleotide probes. *System. Appl. Microbiol.*, accepted

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