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Moorella thermoacetica: a chassis organism for biochemicals production?

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Moorella thermoacetica: a chassis organism for biochemicals production?



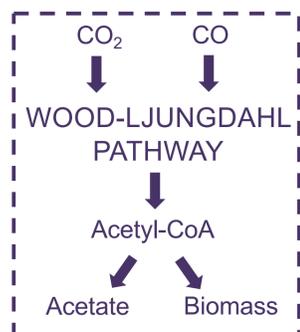
Loughborough University

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Introduction

Microbial chassis organisms are crucial for chemicals bioproduction. Indeed, modern genetic tools allow to insert or modify genes in a host organism to synthesise a desired target compound. Acetogenic bacteria, or acetogens, are promising hosts as they convert CO and CO₂ into acetate and other products during gas fermentation.



CO₂ fixation by acetogens

The acetogen *Moorella thermoacetica* is particularly interesting for industrial applications as its thermophilic requirements (55°C) limit cooling processes. Thus, this project focuses on creating synthetic pathways and experimentally implementing them in *M. thermoacetica* to produce several platform chemicals.

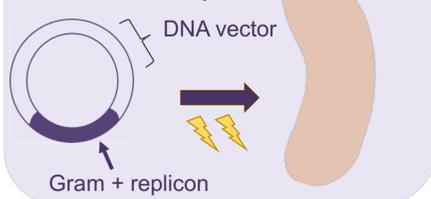
Pathway design

To expand the range of compounds produced by *M. thermoacetica*, synthetic metabolic pathways have been designed for several targets, using the computational tools From Metabolite to Metabolite¹ and Metabolic Route Explorer². These targets are widespread industrial chemicals, such as ethylene glycol or 1,2-propanediol. Once the pathways are designed, different pruning criteria, including gene availability or pathway length, are applied to select the best candidate pathways.

DEVELOPING GENETIC TOOLS

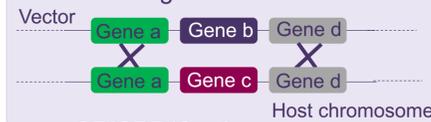
Transformation

- Vector stability
- Restriction system



Genetic modification

- Homologous recombination



- CRISPR/Cas9

Gene expression control

- Promoters
- Ribosome binding sites

CREATING SYNTHETIC PATHWAYS

Pathway Design

- From Metabolite to Metabolite
- Metabolic Route Explorer



Metabolic Route Explorer (MRE)

Pathway Analysis

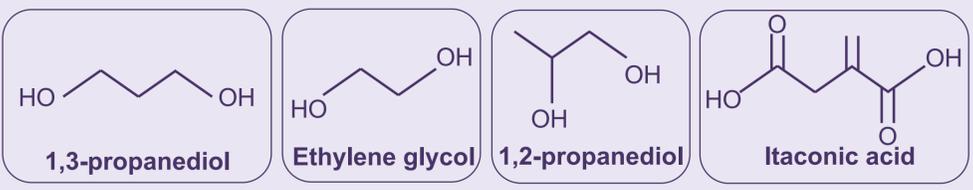
- Genome-scale and constraint-based analysis
- Flux Balance Analysis
- Thermodynamics analysis



CO/CO₂

Engineered *Moorella thermoacetica*

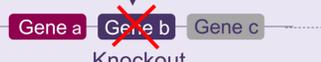
NON-NATIVE PRODUCTS



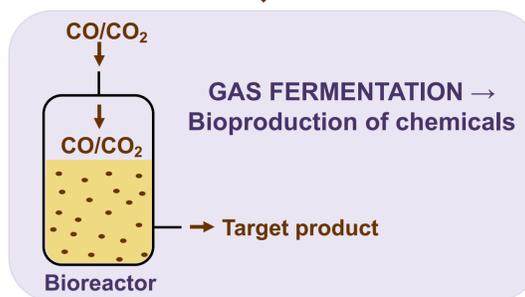
DOWNSTREAM METABOLIC ENGINEERING

- Genetic modifications to increase yield
- Computer-aided strategies (e.g. OptKnock tool)

Genome-scale analysis



Increase yield ← Modification of gene expression



INDUSTRIAL PROCESS



Demonstration plant at Shougang steel mill, LanzaTech (lanzatech.com)

Additional genome-scale analysis further excludes inappropriate pathways. For example, the constraint-based COBRA³ toolbox is used to assess pathway feasibility in the genome-scale model of *M. thermoacetica*.

Pathway Implementation

Once a feasible pathway has been selected, implementation in *M. thermoacetica* is attempted to produce the chosen target compound. However, this first requires to improve and develop genetic tools for this organism. Indeed, although some tools have been created^{4,5}, they are still limited and prevent further metabolic engineering of this promising chassis organism. While developing such genetic tools, implementation will be attempted for a candidate pathway for the production of ethylene glycol. If successful, further downstream metabolic engineering methods will be required to increase yield.

Future work

Current work focuses on developing the genetic tools necessary to implement a synthetic pathway in *M. thermoacetica*, such as identifying the control elements for strong gene expression. If ethylene glycol can be produced from the chosen pathway, further downstream engineering, such as gene deletions, will be performed to increase yield. Pathways for other target products will also be introduced in *M. thermoacetica* to further demonstrate its importance as a chassis organism. This work will also strengthen our knowledge on *M. thermoacetica*.

REFERENCE

- ¹ Chou *et al* (2009). *Nucleic Acid Research* (37)
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- ³ Becker *et al* (2007). *Nature Protocols* (2)
- ⁴ Kita *et al* (2013). *Journal of Bioscience and Bioengineering* (115)
- ⁵ Iwasaki *et al* (2017). *Applied and Environmental Microbiology*.

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