Moorella thermoacetica: a chassis organism for biochemicals production?

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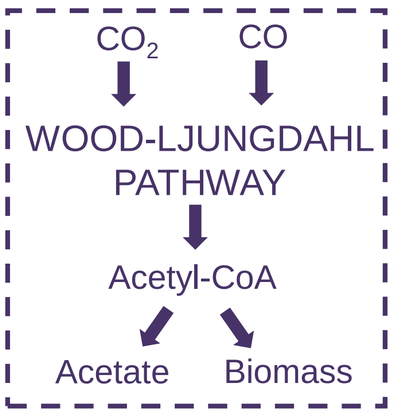
Introduction

Microbial chassis organisms are crucial for chemicals bioproduction. Indeed, modern genetic tools allow to modify or insert genes in these organisms to synthesise desired target compounds. Acetogenic bacteria, or acetogens, are promising hosts as they **convert CO and CO_2** into acetate and other products during **gas fermentation**.



CO_2 fixation by acetogens

The acetogen ***Moorella thermoacetica*** is interesting for industrial gas fermentation as its thermophilic requirements limit cooling processes. Thus, this project focuses on **creating synthetic pathways** and **implementing them** in *M. thermoacetica* to produce platform chemicals.

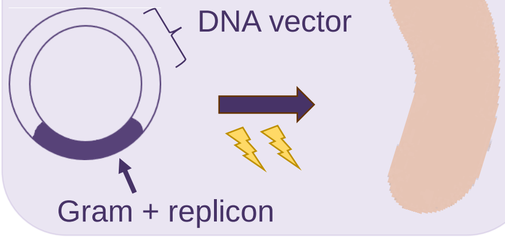
Pathway design

To produce non-native compounds, **synthetic metabolic pathways** have been designed using the **computational tools** From Metabolite to Metabolite[1] and Metabolic Route Explorer[2]. The chosen targets are **widespread industrial chemicals**, such as ethylene glycol or 1,2-propanediol. After pathway design, different **pruning criteria**, including gene availability or pathway length, are applied to select the best candidate pathways.

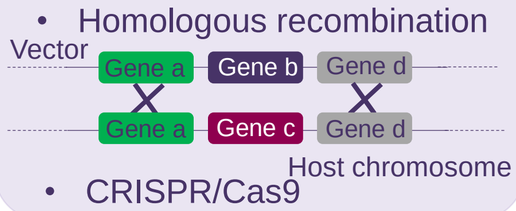
DEVELOPING GENETIC TOOLS

Transformation
- Vector stability
- Restriction system

Moorella thermoacetica



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

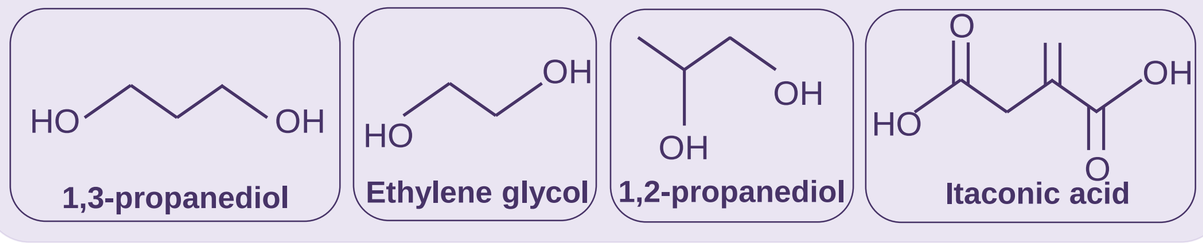
Metabolic Route Explorer (MRE)

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

CO/CO_2

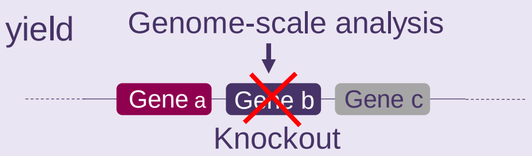
Engineered ***Moorella thermoacetica***

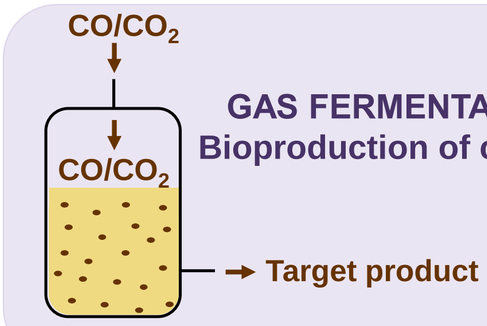
NON-NATIVE PRODUCTS



DOWNSTREAM METABOLIC ENGINEERING

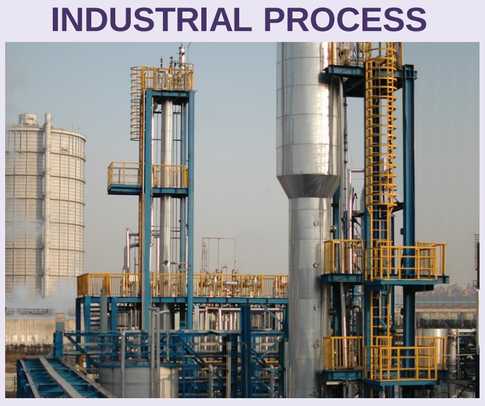
- Genetic modifications to increase yield
  - Computater-aided strategies (e.g. OptKnock tool)





GAS FERMENTATION → Bioproduction of chemicals

INDUSTRIAL PROCESS



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

Further **genome-scale analysis** excludes inappropriate pathways. For example, the constraint-based COBRA[3] toolbox is used to assess **pathway feasibility** in the genome-scale model of *M. thermoacetica*.

Pathway Implementation

Once feasible pathways have been selected, **implementation in *M. thermoacetica*** is attempted to produce the chosen target compounds. 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 metabolic engineering of this promising organism. While developing such genetic tools, implementation is currently being attempted for a candidate pathway for the **production of ethylene glycol**.

Future work

Current work focuses on **developing the genetic tools necessary** for metabolic engineering in *M. thermoacetica*. 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* and its metabolism.

REFERENCE

[1] Chou *et al* (2009). *Nucleic Acid Research* (37)
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