CHANIA 2023

Strain resolved metagenomics applied to biogas upgrading

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21/06/23

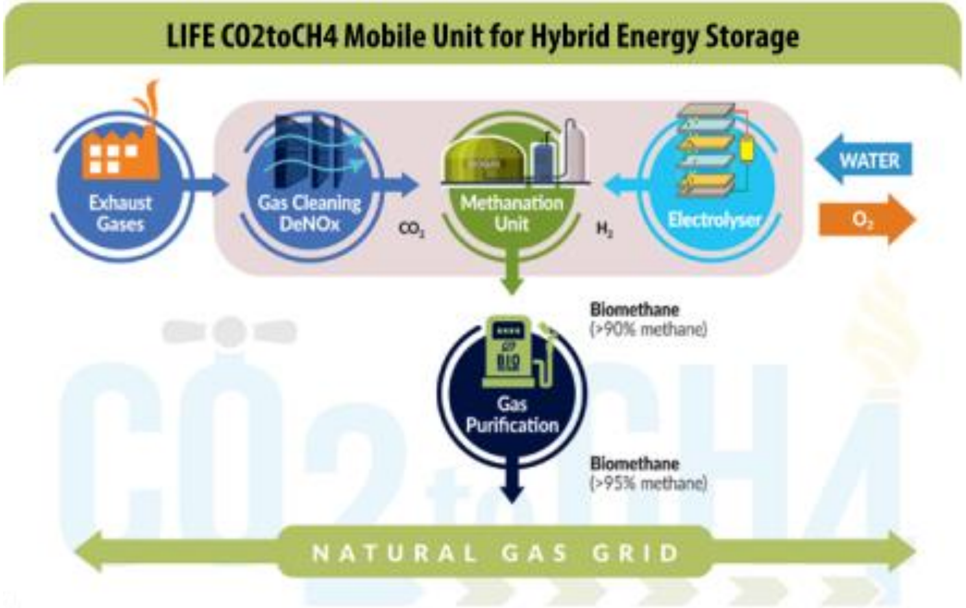
Agenda

01. Introduction

02. Methods

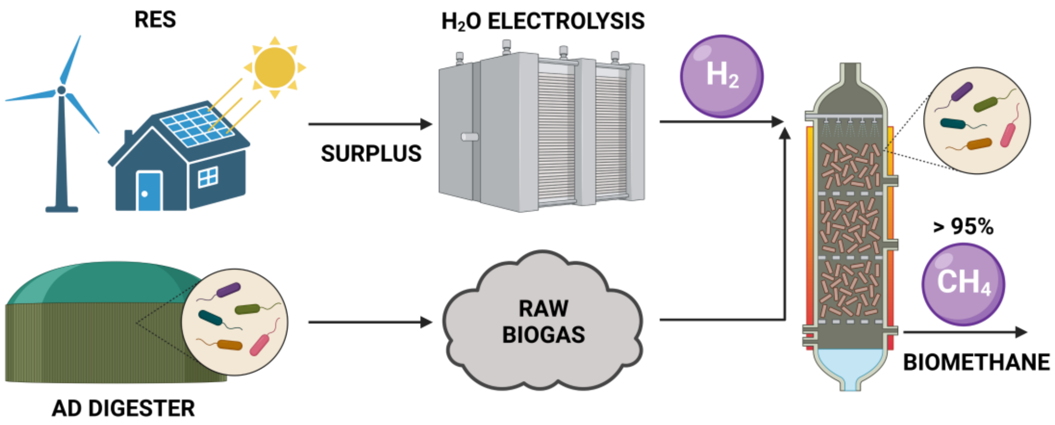
03. Results

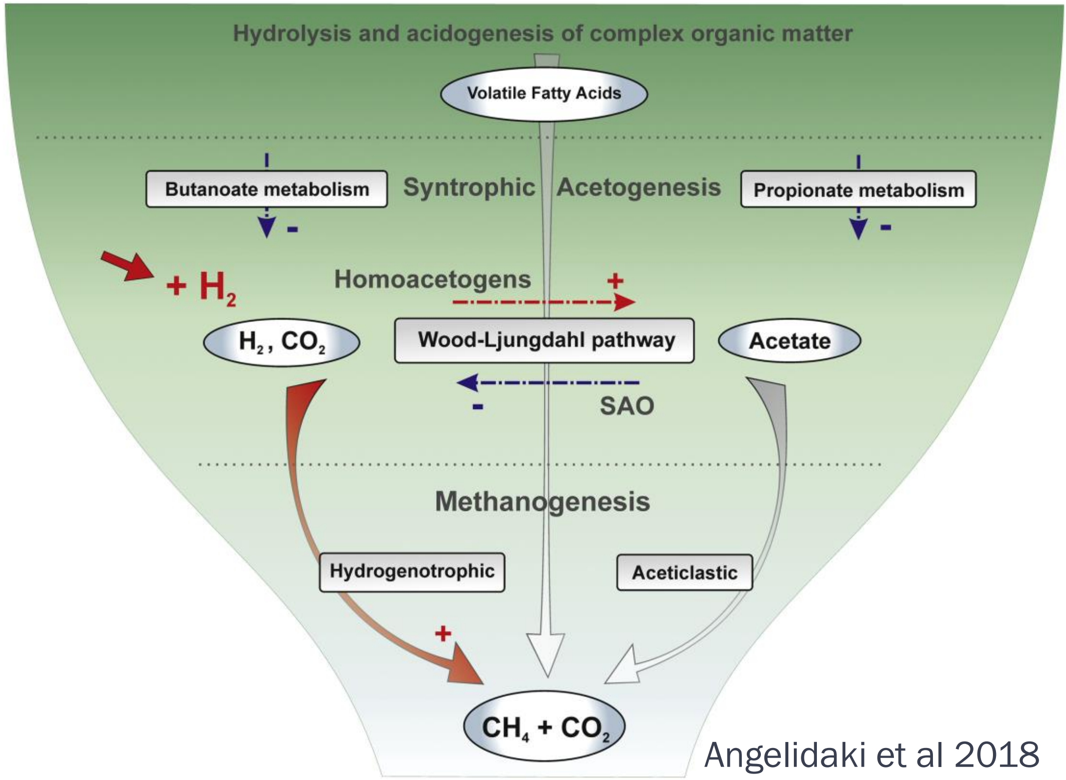
04. Take home message



Biogas Upgrading (BU)

- Raw biogas consists of 50-70% of CH_4 and 30-50% of CO_2
- **BU** is used to consume the residual CO_2, producing biomethane (≥95% CH_4)
- **Biological fixation of CO_2** with the use of external H_2 can follow different metabolic routes:
    - **Hydrogenotrophic** methanogenesis
    - **Acetoclastic** methanogenesis
- Microbiome involved has been already deciphered at the species level (Campanaro et al 2016)



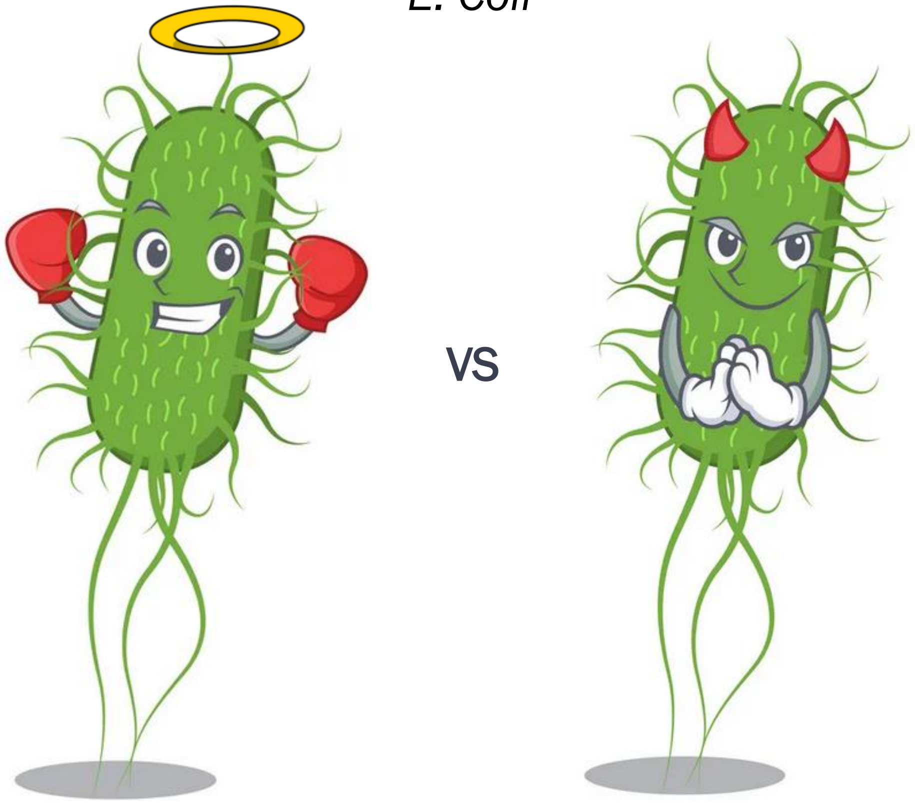


Angelidaki et al 2018

Why strain-level?

- Microbes are characterized by high **genetic heterogeneity**
- Differences in **gene content** are important for understanding microbial evolution, adaptation, and the gain of specific metabolic functions.
- Strain-level analysis allows a **higher resolution**

E. Coli



VS

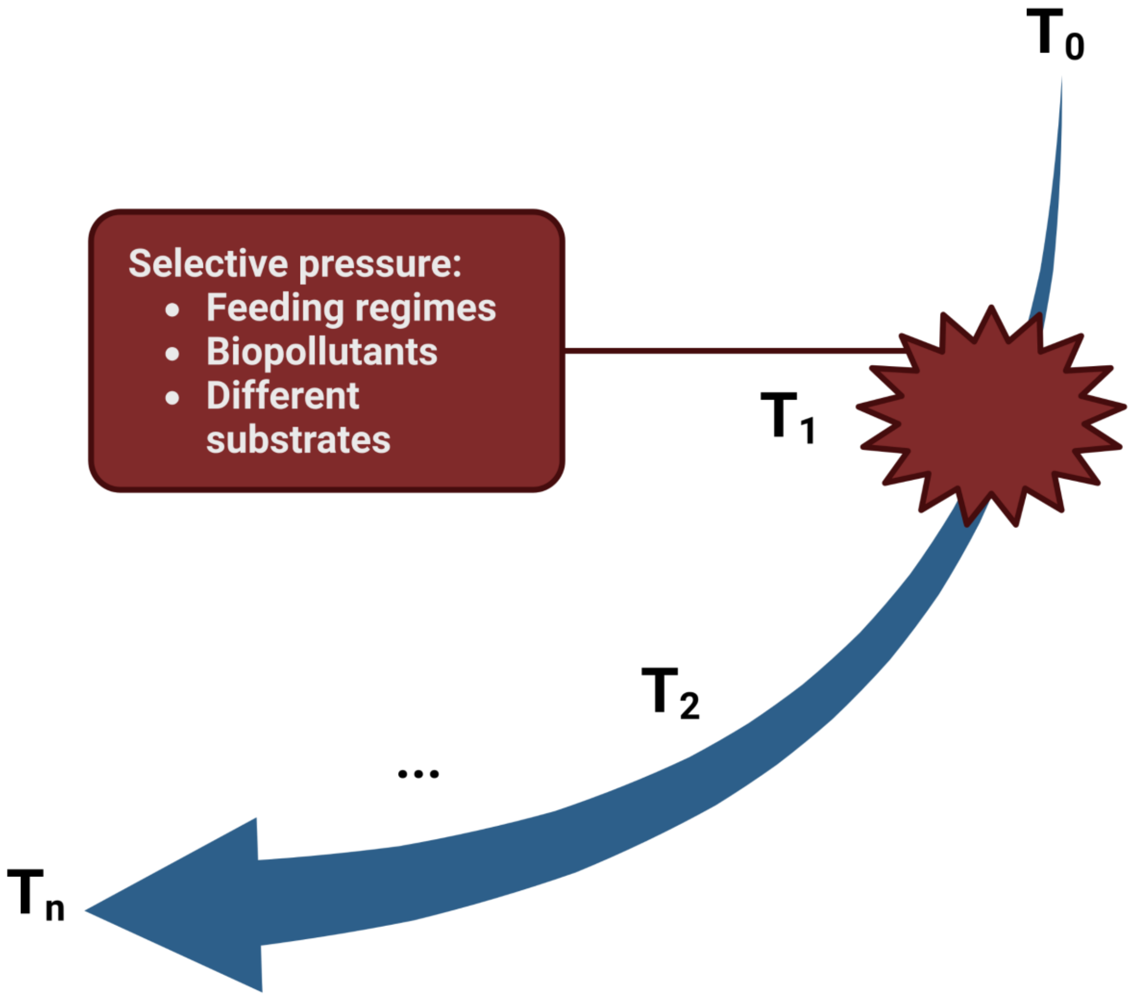
Harmless commensal or pathogen?

↓

Same specie, but different strains

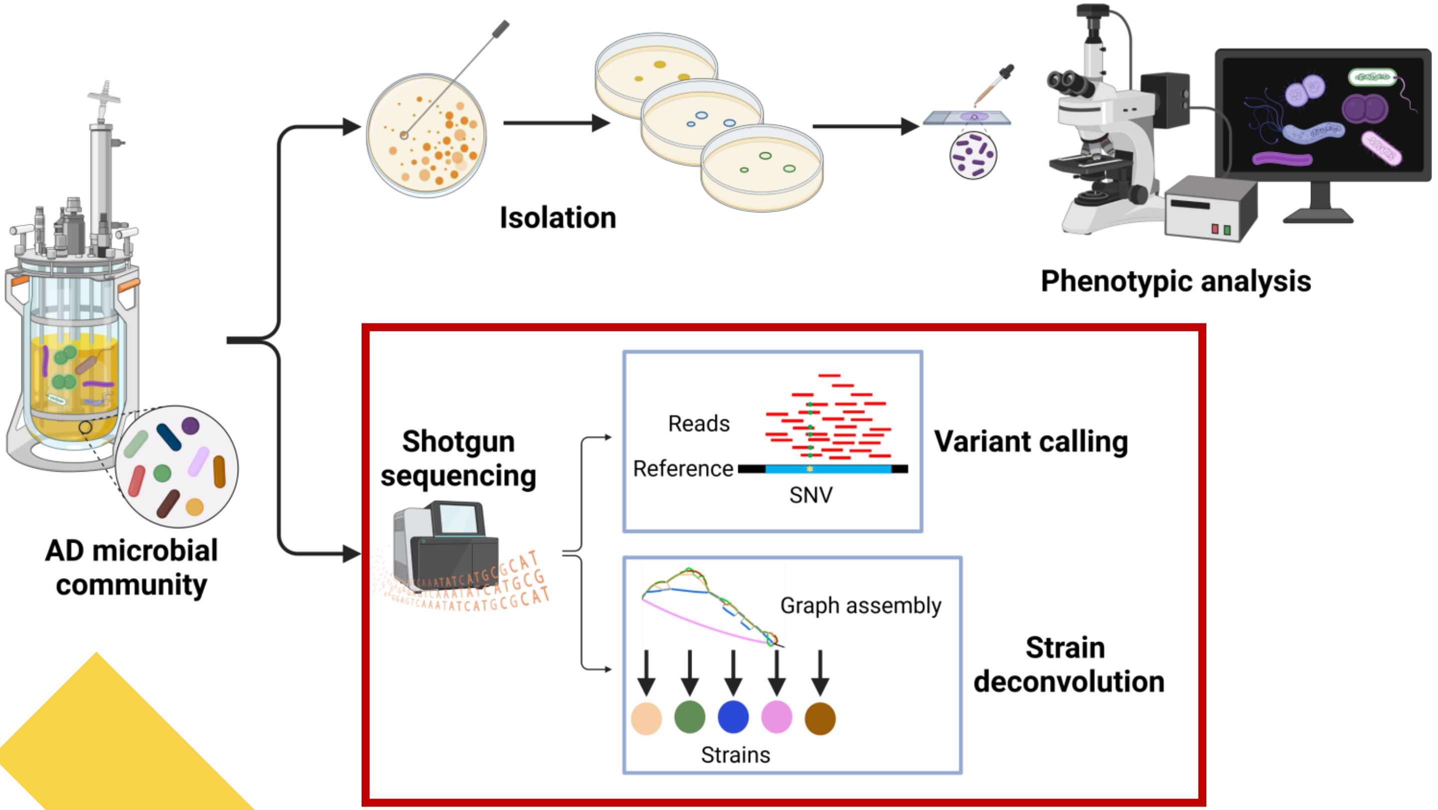
Selective pressure shapes microbiomes

- Performances and stability of the process are linked with the **fitness** of the microbiome
- A **selective pressure** is capable of shaping the microbial community
- Genomic variants fixed through time give a **phenotypic advantage**
- Strain **selection** can occur



Selective pressure:
- **Feeding regimes**
- **Biopollutants**
- **Different substrates**

Strain-resolved metagenomics



- Old strategy:
  - isolation
  - phenotypic analysis
- Metagenomics offers new opportunities
- New strategy:
  - **variant analysis**
  - **strain deconvolution**
- Extremely challenging to study

Bioinformatic workflow

Metagenomics

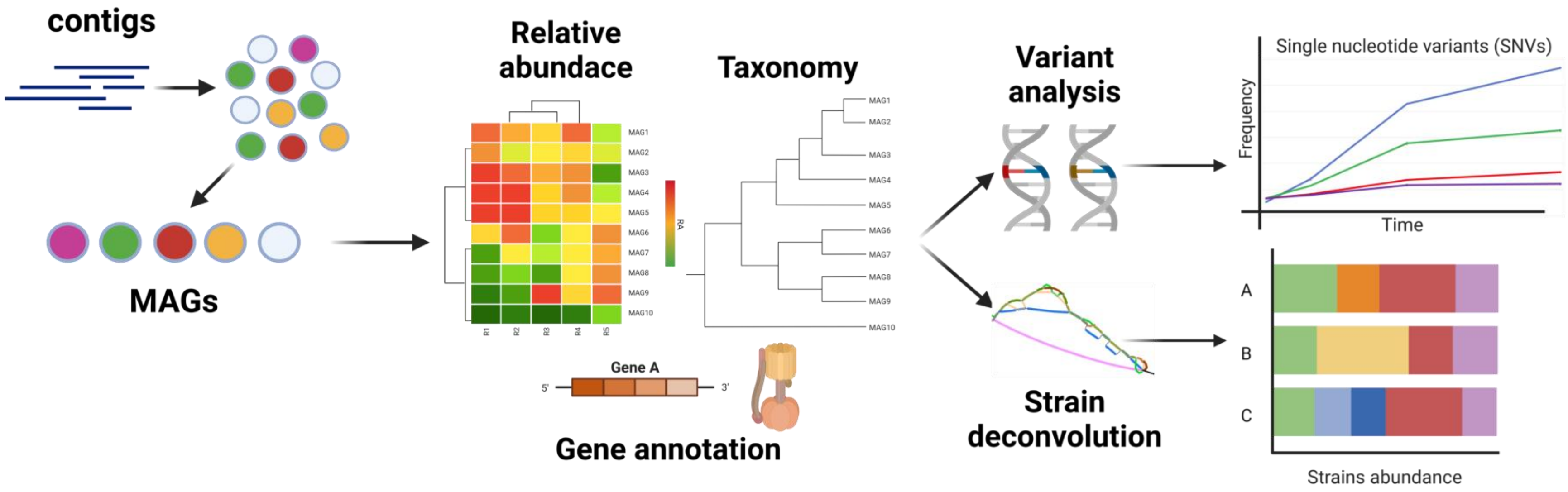
- Shotgun sequencing
- Assembly and binning
- Phylogenetic analysis
- Gene annotation

Analysis of variants

- Variant calling (InStrain) on MAGs
- Quality filtering
- Clustering based on frequency
- Map variants on genes

Strain deconvolution

- Retrieve number of strains (STRONG)
- Calculate the strain's abundance
- Link variants to strains

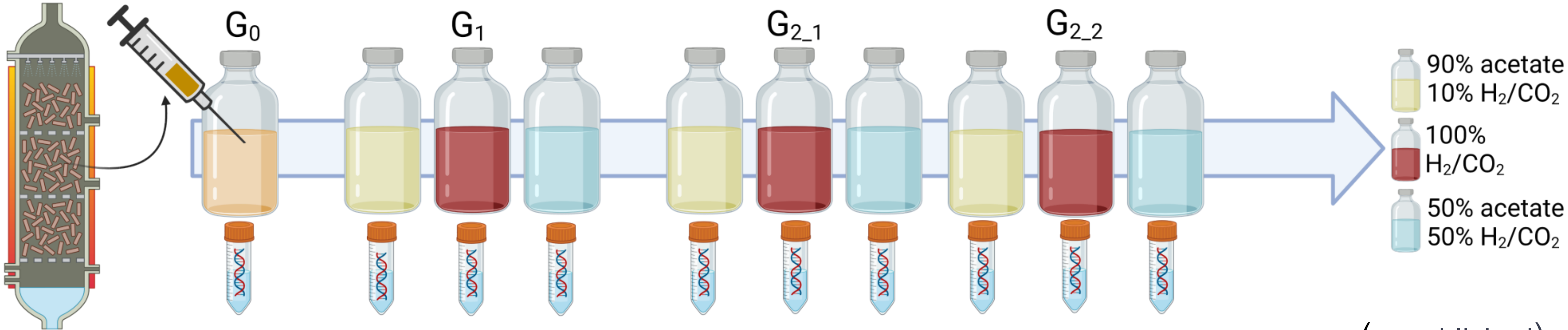


Case study 1

Carbon substrates

Experimental design

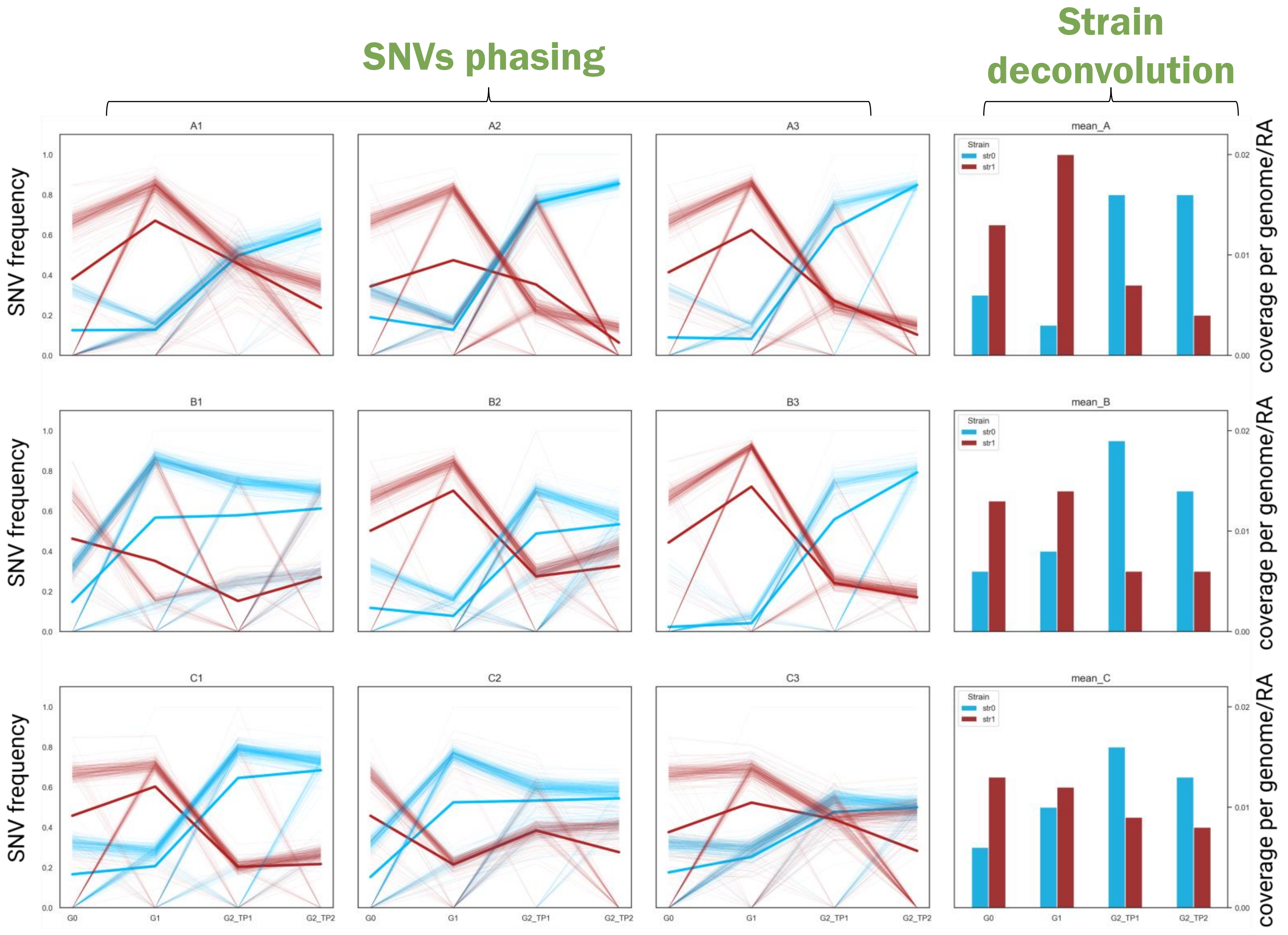
- Inoculum coming from TBR reactor
- Batch framework
- Three carbon sources:
  - **90% acetate + 10% H_2CO_2**
  - **100% H_2CO_2**
  - **50% acetate + 50% H_2CO_2**
- Sequential reinocula (G0, G1, G2)



(unpublished)

Variant selection determined by the shift in carbon substrates availability

- Reactors:
    - A 90% Ac + 10% H2CO2
    - B 100% H2CO2
    - C 50% Ac + 50% H2CO2
- Strain replacement between G1 and G2_TP1 for ***M. wolfeii***
- No change between substrates
- ~6% of these were affecting genes involved in **hydrogenotrophic methanogenesis**

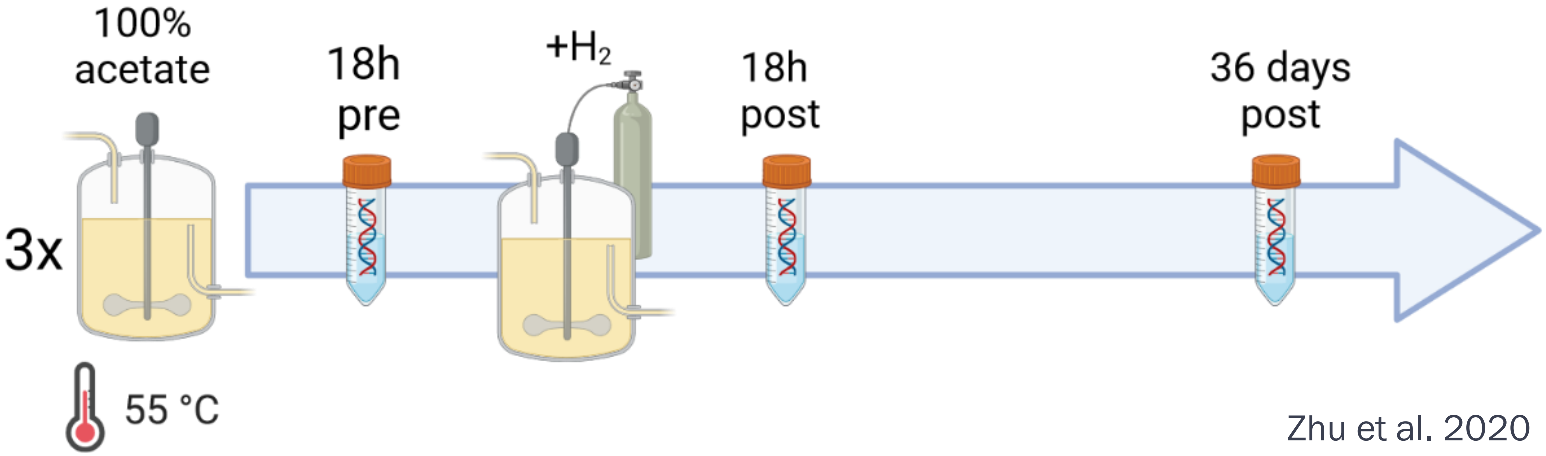


Case study 2

Exogenous H_2 addition

Experimental design

- CSTR reactor in triplicate
- Acetate as initial substrate
- **H_2 addition at constant flow**
- Three sampling point
  - 18 hours before
  - 18 hours after
  - 36 days after

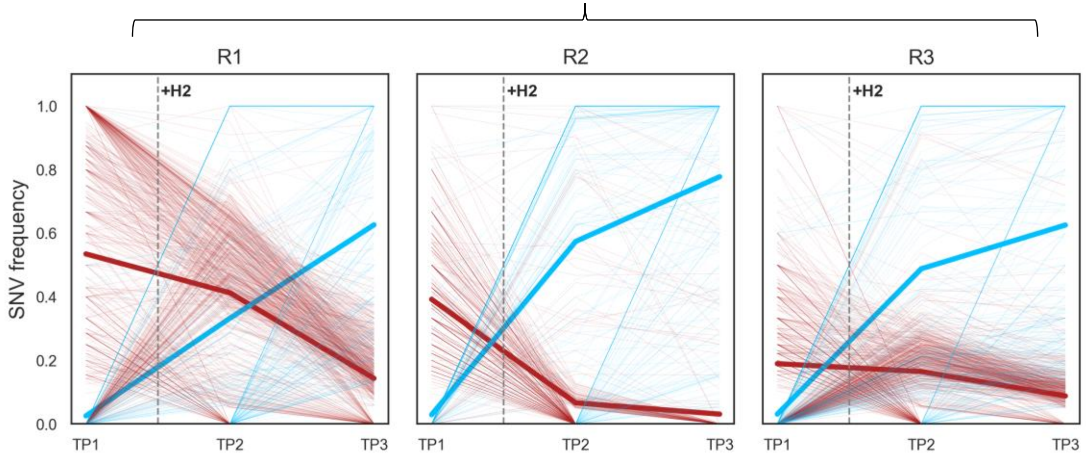


Zhu et al. 2020

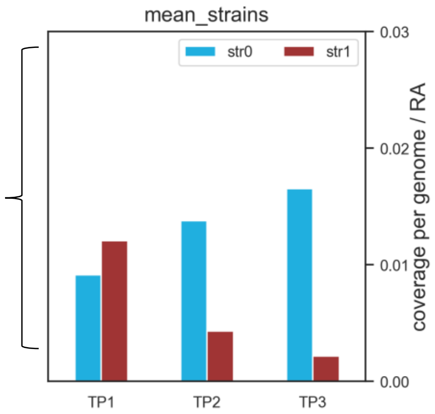
Variants selection in CSTRs upon exogenous H_2 addition

- A **strain replacement** was highlighted after the H_2 addition in ***M. thermophilus***
- On average 139 SNVs were positively selected in the new strain (blue)
- ~12% of these were affecting genes involved in **hydrogenotrophic methanogenesis**

SNVs phasing



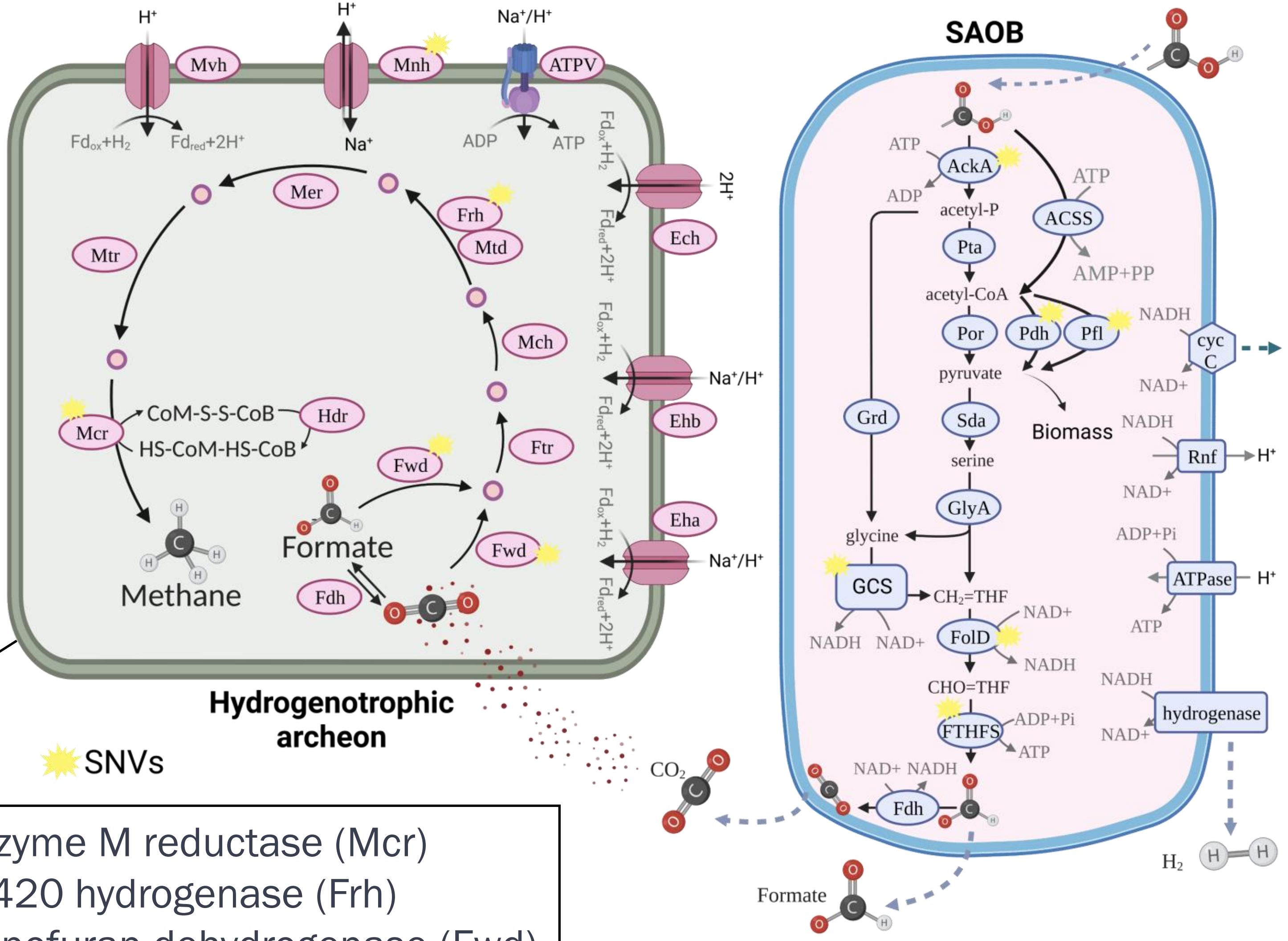
Strain deconvolution



Map SNVs to genes

Legend
- ● **Case study #1**
- ● **Case study #2**

- Genes involved in methanogenesis and WL pathway accumulated SNVs
- Methanogens:
  - ***M. wolfeii***
  - ***M. thermophilus***
- Putative SAOB:
  - **Firmicutes sp. mA_31**
  - ***Coprothermobacter proteoliticus***
  - **Synergistaceae sp. 24abBP_148**



methyl-coenzyme M reductase (Mcr)
coenzyme F420 hydrogenase (Frh)
formylmethanofuran dehydrogenase (Fwd)

Summary

Circular economy concept

Reduce carbon footprint through BU optimization

Novel approach for strain level analysis

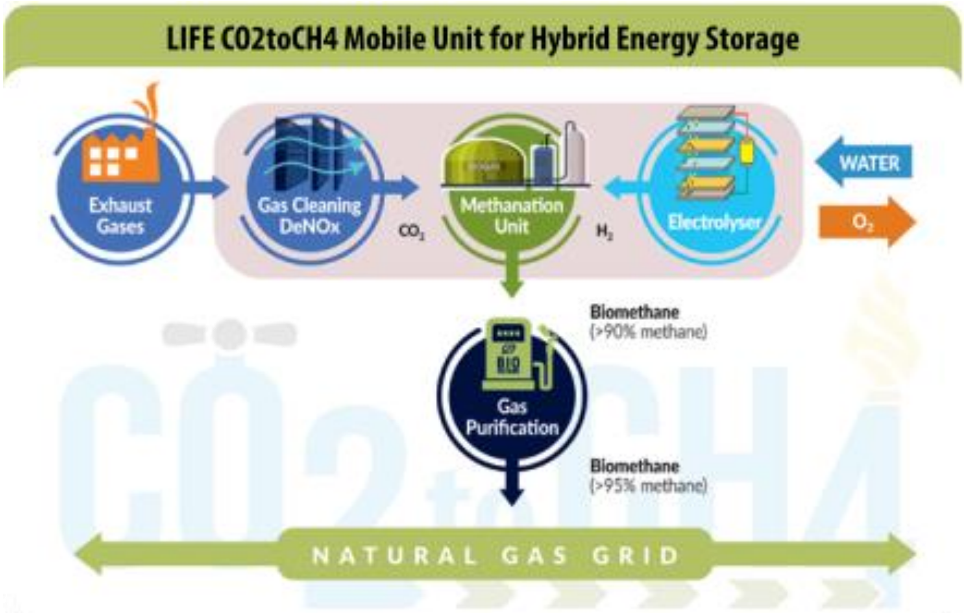
Combine SNVs and strain deconvolution

Selective pressures trigger strain-level dynamics

Track strains through time and follow their evolution

Insight in the role of SNVs

SNVs promote adaptation of microbes to environmental changes



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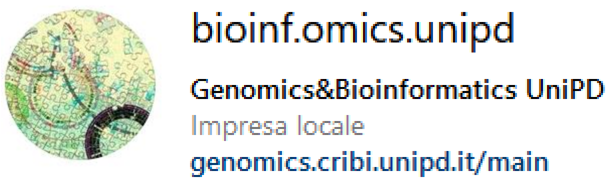
Thank you

You can follow our progress on …



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