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 ($\geq$95% CH$_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)
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.

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
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% H₂CO₂
  - 100% H₂CO₂
  - 50% acetate + 50% H₂CO₂
- 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(unpublished)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 H₂ addition
Experimental design

- CSTR reactor in triplicate
- Acetate as initial substrate
- \( \text{H}_2 \) addition at constant flow
- Three sampling points
  - 18 hours before
  - 18 hours after
  - 36 days after

Zhu et al. 2020
A strain replacement was highlighted after the H₂ 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
Map SNVs to genes

- 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
Thank you

You can follow our progress on ...

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