

**Genome-centric metagenomic
investigation of 132 samples collected
from biogas reactors revealed
important functional roles for microbial
species belonging to underexplored
taxa**

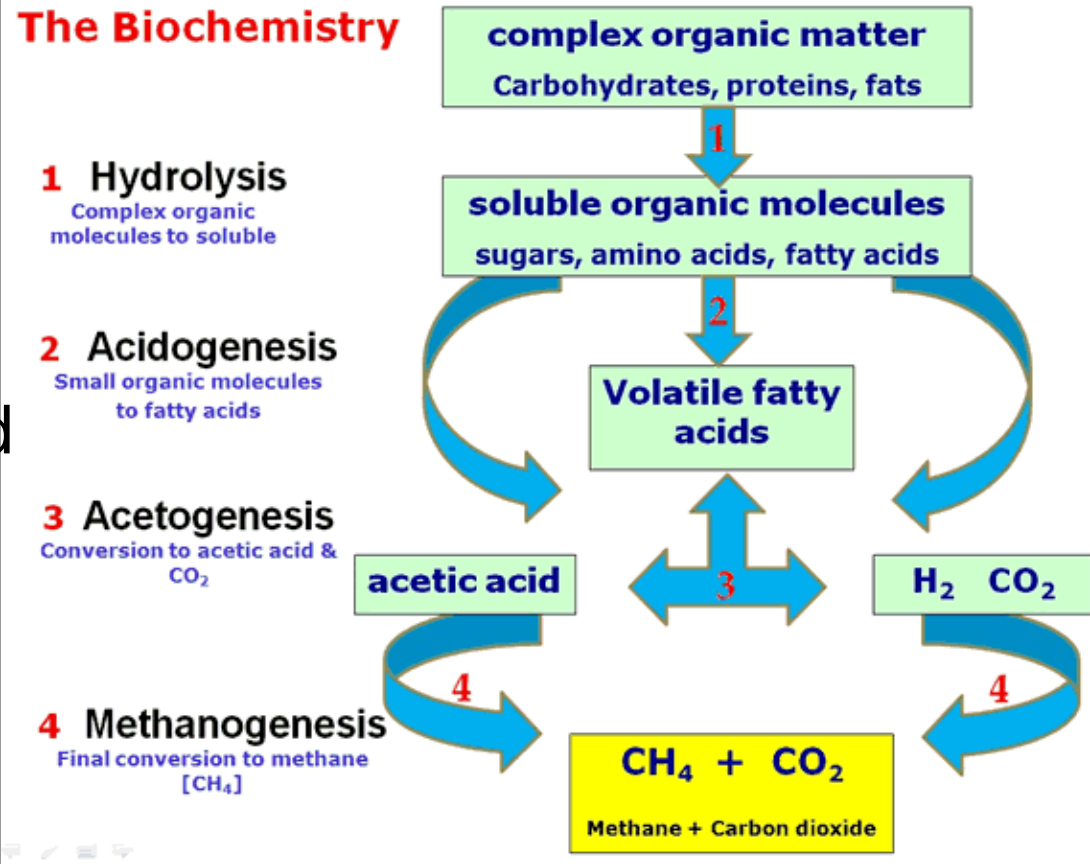
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HERAKLION 2019
7TH INTERNATIONAL
CONFERENCE ON SUSTAINABLE
SOLID WASTE MANAGEMENT

Anaerobic digestion

- Similarities in the biochemical process
- Complex microbiome and many uncultured
- Anaerobic digestion of organic matter is the largest biogenic source of methane on Earth



Anaerobic digestion

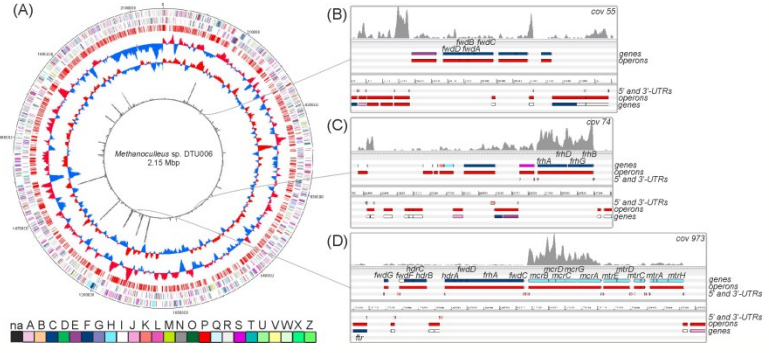
- Analyzing Anaerobic Digestion considering the microbial composition as a black box is like playing a chess match without knowing the pieces



Functional representation of the AD microbiome

Anaerobic digestion food chain

Metagenome Assembled Genome (MAG)

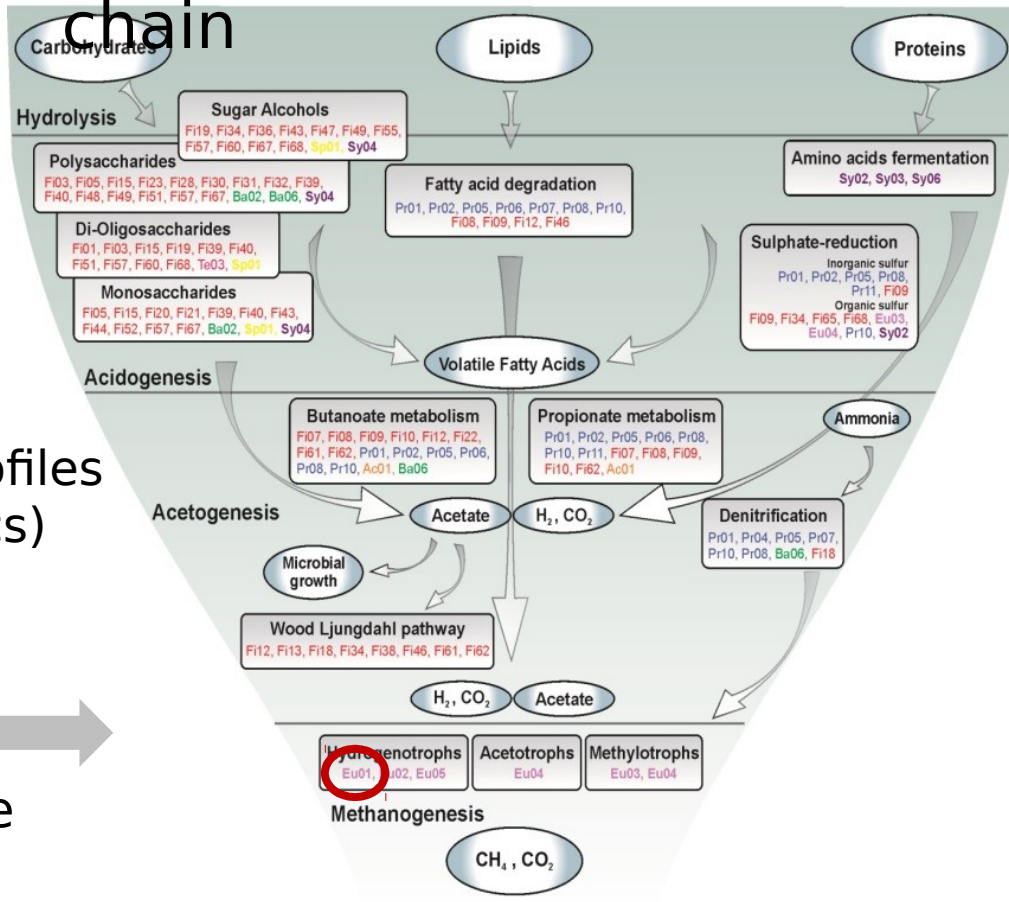


Functional analysis

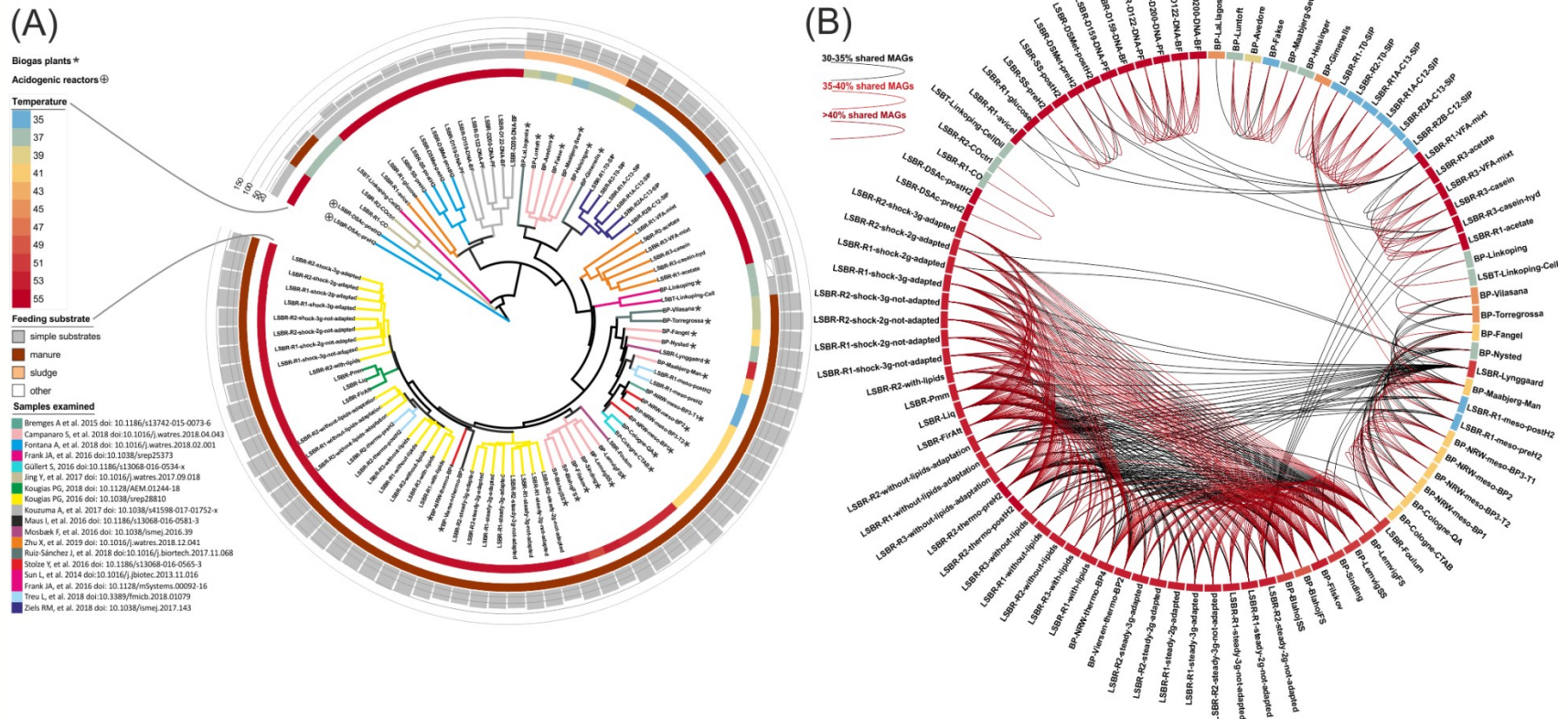
Gene expression profiles (metatranscriptomics)



PG assigned to the functional role in the AD food chain



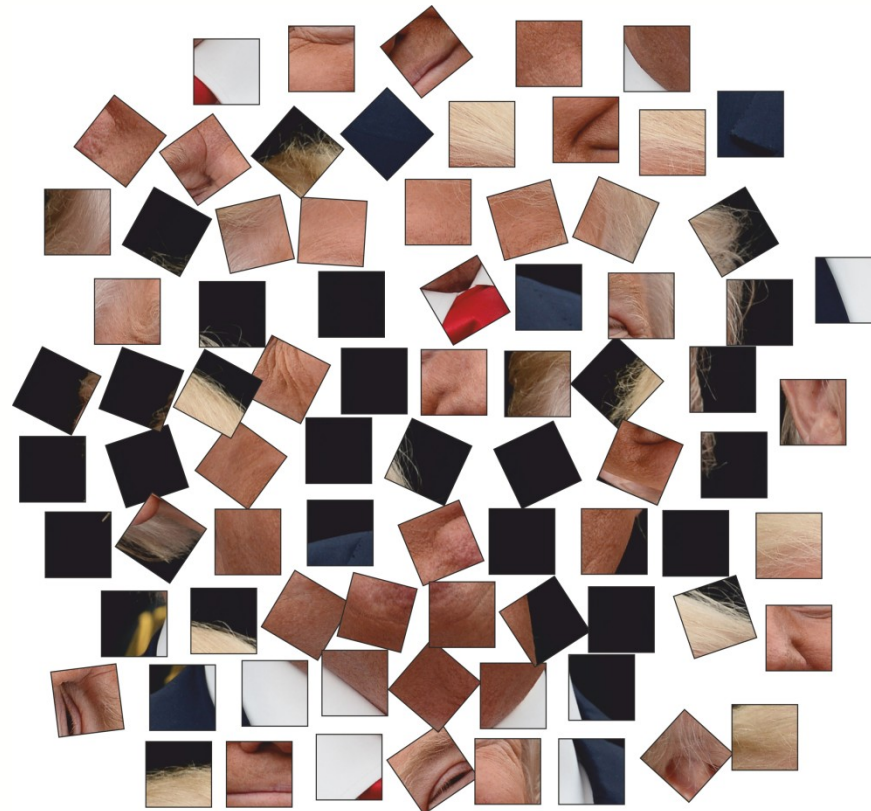
Summaries of the microbial communities identified in 132 samples



Beta diversity of 132 samples revealed temperature and feedstock as main drivers of the microbial community composition

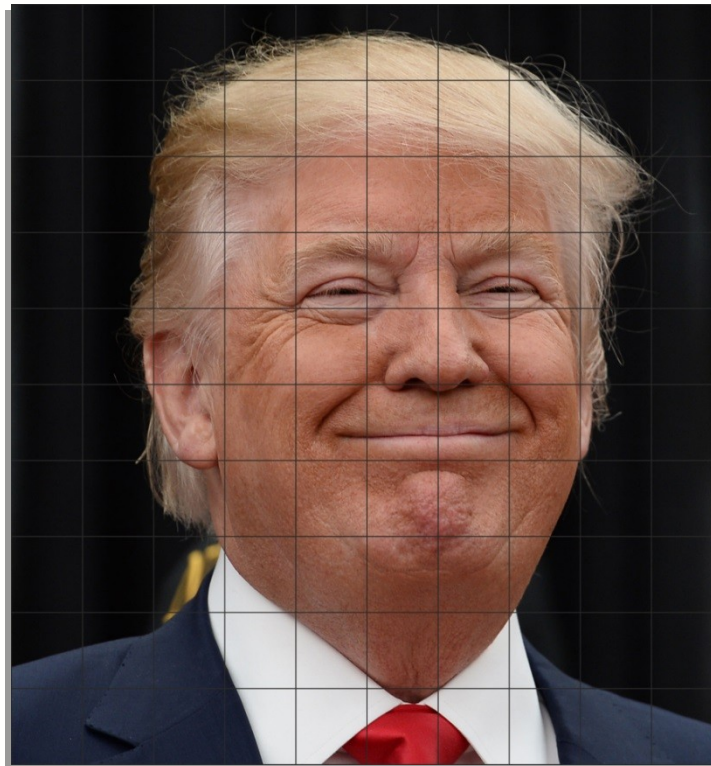
Genomes reconstruction

- Importance of reconstructing genomes...
- a puzzling view of the subject is making difficult to clarify his identity...



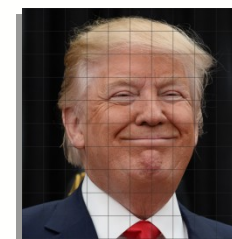
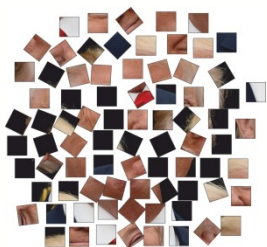
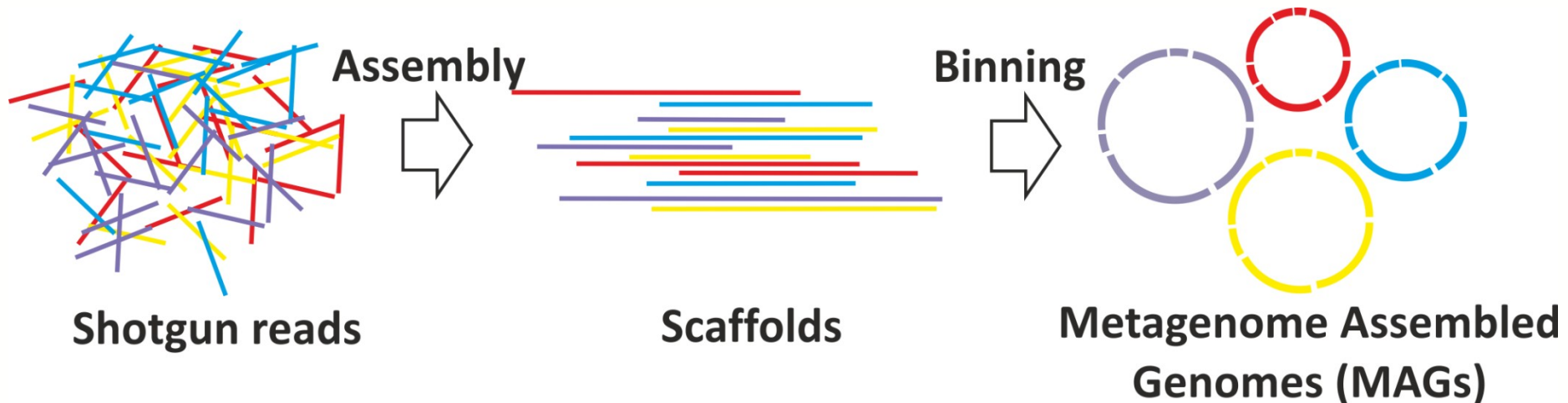
Genomes reconstruction

- Once reconstructed the full picture...
- you can have a more clear view of the subject...

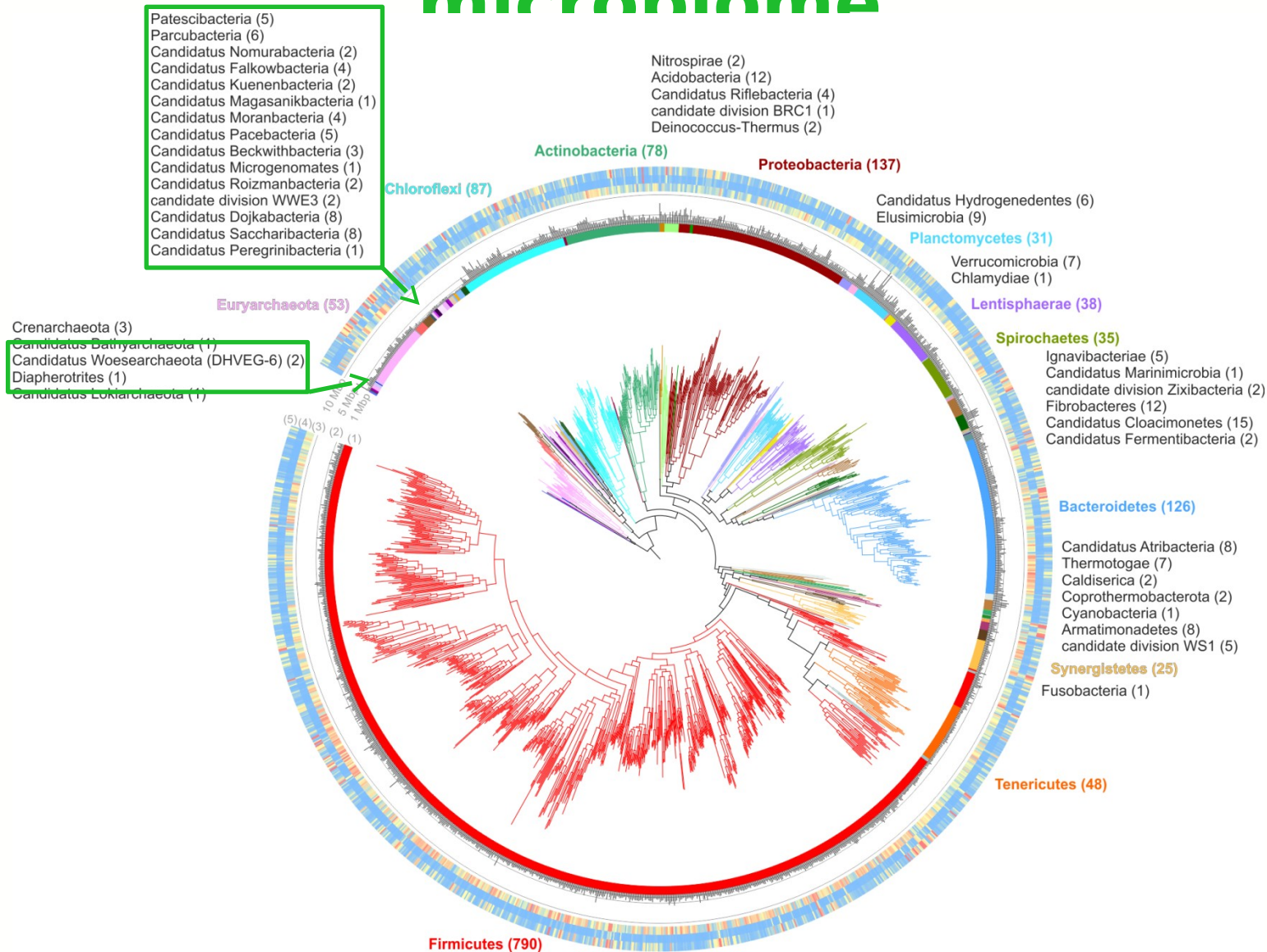


Genomic reconstruction

- This is why reconstruction of **Metagenome Assembled Genomes (MAGs)** can help to define microbiome composition and functional roles...



Taxonomy of 1,635 MAGs of the AD microbiome



MiGA database

- A dedicated MiGA database was developed for making all the information available to the scientific community
- were <http://microbial-genomes.org/projects/biogasmicrobiome>
- taxonomy and genomic characteristics of each species

The screenshot displays the MiGA database interface. The top navigation bar includes 'MiGA / Projects / biogasmicrob...', 'Home', 'Projects', and 'Log in'. The main content area is divided into two panels.

Left Panel (Search Results): Shows a search for 'methanoculleus'. The results list several datasets, each with a 'Taxonomy' field and an update date. The datasets listed are:

- METABAT AS10tIH2TH 381 (Taxonomy: d: Archaea » ... » g: Methanoculleus, Updated 2 months ago)
- METABAT AS19jrsBPTG 12 (Taxonomy: d: Archaea » ... » g: Methanoculleus, Updated 2 months ago)
- METABAT AS19jrsBPTG 18 (Taxonomy: d: Archaea » ... » g: Methanoculleus, Updated 2 months ago)
- METABAT AS19jrsBPTG 32 (Taxonomy: d: Archaea » ... » g: Methanoculleus, Updated 2 months ago)
- METABAT AS20ysBPTH 14 (Taxonomy: d: Archaea » ... » s: Methanoculleus, Updated 2 months ago)
- METABAT AS20ysBPTH 159 (Taxonomy: d: Archaea » ... » g: Methanoculleus, Updated 2 months ago)
- METABAT AS21ysBPME 11 (Taxonomy: d: Archaea » ... » g: Methanoculleus, Updated 2 months ago)
- METABAT AS22ysBPME 199 (Taxonomy: d: Archaea » ... » g: Methanoculleus, Updated 2 months ago)

Right Panel (Dataset Detail): Shows the details for 'METABAT AS20ysBPTH 14'. It includes a 'Taxonomy' section with the following classification:

```

d: Archaea
p: Euryarchaeota
c: Methanomicrobia
o: Methanomicrobiales
f: Methanomicrobiaceae
g: Methanoculleus
s: Methanoculleus thermophilus
  
```

Below the taxonomy is an 'AAI classification' section and an 'ANI clade' section. The 'Distances' section states: 'The closest relatives found by MiGA in the database were METABAT AS22ysBPME 199 (80.88% AAI) and METABAT AS23ysBPME 211 (80.14% AAI)'. The 'Genome relatedness' section shows an 'AAI table' and a 'Learn more' link. The 'MyTaxa Scan' section includes 'Ribosomal RNA (small subunit)' and 'Quality (essential genes)' metrics:

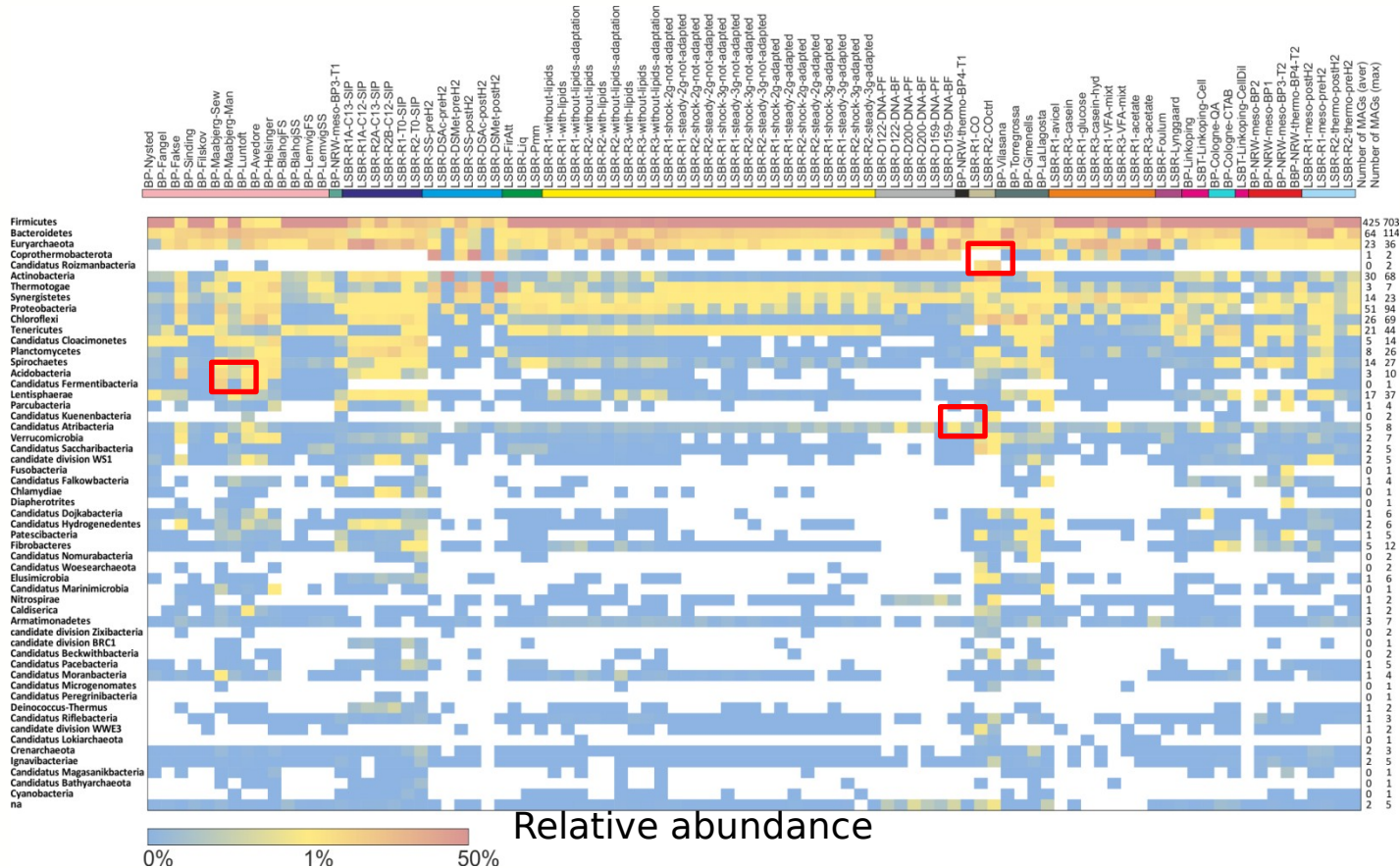
Metric	Value	Quality
Completeness	96.2%	very high
Contamination	3.8%	very low
Quality	77.2%	high

At the bottom, it notes: 'Genes commonly found in Bacteria and Archaea detected:'.

Relative abundance

- In most samples these taxa represent **2-3% of the AD** microbiome, but in specific samples they can represent **more than 10%**

CPR phyla



Metabolic reconstruction

Evaluation of their gene content suggests:

Ability to **synthesize nucleotides (not all the phyla)**;

Relevant number of glycoside hydrolases and glycoside transferases (particularly in *Candidatus* Dojkabacteria and *Candidatus* Magasanikbacteria) -> **degradation of some polymeric substances**

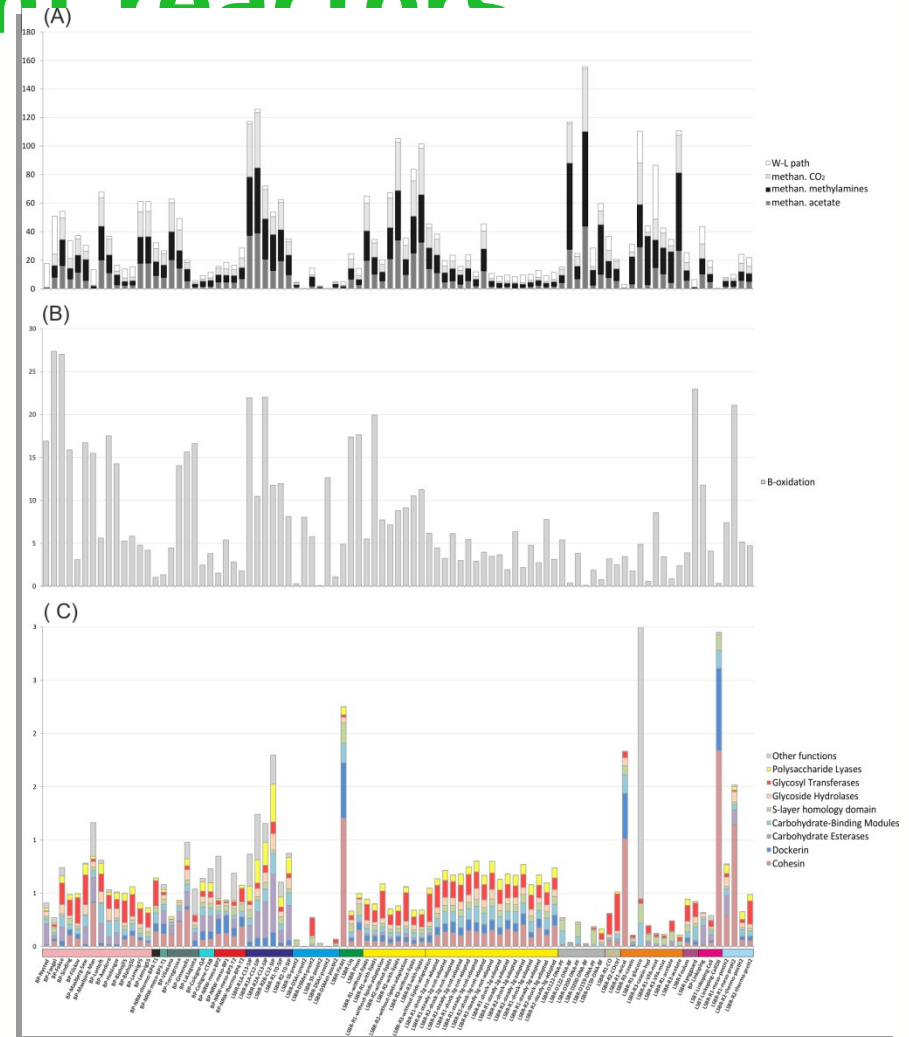
The presence in some species of the «phosphate acetyltransferase-acetate kinase pathway, involved in the conversion of acetyl-CoA to acetate» -> **production of acetate**

Absence of pathways involved in amino acids and lipids biosynthesis -> **proteins and lipids scavenging from the environment**

Presence of numerous proteases and transaminases -> **salvage amino acids from environmental sources**

Relative abundance of functional pathways in different reactors














- Relative abundance of MAGs associated to the encoded metabolic pathways allowed to estimate the relevance of different pathways in each sample:
- (A) methanogenesis
- (B) WL-pathway
- (C) polysaccharides degradation.





New Results

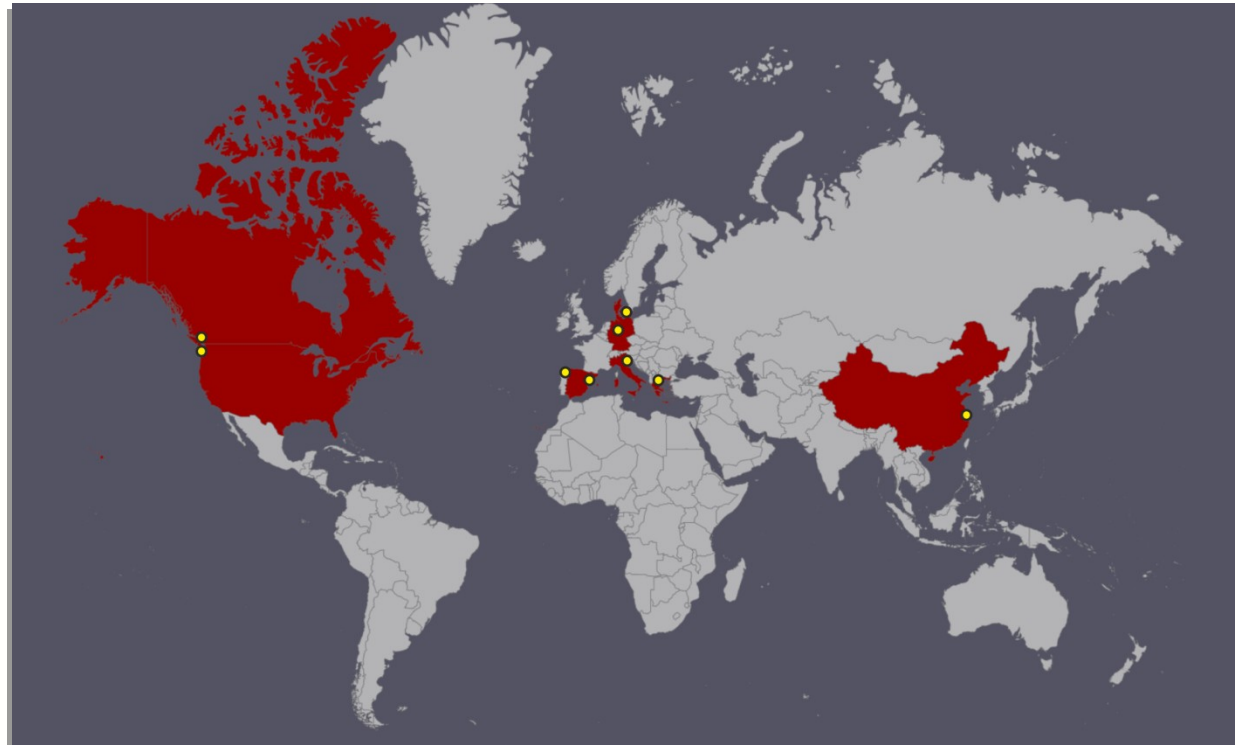
The anaerobic digestion microbiome: a collection of 1600 metagenome-assembled genomes shows high species diversity related to methane production

 Stefano Campanaro,  Laura Treu,  Luis M Rodríguez Rojas,  Adam Kovalovszki,  Ryan Ziels,  Irena Maus,  Xinyu Zhu,  Panagiotis G Kougias,  Arianna Basile,  Gang Luo,  Andreas Schlüter,  Konstantinos T Konstantinidis,  Irimi Angelidaki

doi: <https://doi.org/10.1101/680553>

A network of laboratories to study the AD system

To deal with the complexity of this metagenomic study we are forming a network of laboratories from Denmark, Greece, Spain, Germany, China, USA, Canada...
...and more collaborations are welcome!



Aknowledgments

- Bioenergy group, Technical University of Denmark: Xinyu Zhu, Adam Kovalovszki, Irini Angelidaki
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- Department of Civil Engineering, University of British Columbia: Ryan M Ziels
- Soil and Water Resources Institute Hellenic Agricultural Organization DEMETER: Panagiotis Kougias
- Center for Biotechnology (CeBiTec), Bielefeld University: Irena Maus, Andreas Schlüter
- Department of Environmental Science and Engineering, Fudan University: Gang



CRIBI Biotechnology Center

- **CRIBI is an interdepartmental Biotechnology Center located at Padova University**
- 22 different research groups of 14 different departments
- 4 facilities dedicated to research
 - - Next generation sequencing;
 - - Proteomics
 - - Microarrays, real-time PCR, digital PCR
 - - Peptide synthesis

<http://www.cribi.unipd.it/>



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Thank you!

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Genome size

MAGs genome size according to taxonomic assignment

The low completeness associated to MAGs belonging to some phyla suggests that essential gene sets has to be revisited

