

18th International Symposium on Microbial Ecology (ISME), 14-19 August 2022

'Cold-adapted lipases and lipolytic bacteria found by metaproteogenomics in low-temperature anaerobic membrane bioreactors treating domestic wastewater'
 & **'Monitoring the anaerobic digestion microbial community as a foaming risk prediction method'**
 Authors: Reihaneh Bashiri *et al.*

I am the first author of two accepted abstracts (both as poster presentation) in the ISME 18th conference. One of the abstracts is from my PhD (*Cold-adapted lipases and lipolytic bacteria found by metaproteogenomics in low-temperature anaerobic membrane bioreactors treating domestic wastewater*) and the other is from the EBNet Proof of Concept (PoC) funding (*Monitoring the anaerobic digestion microbial community as a foaming risk prediction method*).

Both of these abstracts have value for the environmental or industrial biotechnology sector, and use combined omics tools: metagenomics to identify microbial individuals and metaproteomics to determine what are they doing within the communities of different environmental samples.

In my PhD research, I looked for the cold-adapted bacterial lipases and their producers in anaerobic treatment of domestic wastewater at low temperatures. Cold-adapted bacterial lipases have value for many industries like detergent manufacturers.

In the EBNet PoC, I looked for foam causing/stabilising bacteria and their biomarker molecules which correlates to foaming incidents in full-scale anaerobic digesters with a wide range of mixed solid and liquid wastes. This project can both offer a prediction tool for foaming incidents in anaerobic digestion systems and produce value for industries which are interested in biosurfactant (foam-causing substances with microbial origin) production by identifying novel biosurfactant producers.

Monitoring the anaerobic digestion microbial community as a foaming risk prediction method
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Foaming in anaerobic digestion (AD)

- Causes serious process instability
- Most foam-forming/stabilising bacteria in full-scale AD plant with mixed solid and liquid wastes are unknown

Methods

Hartlepool, Non-foaming plant
 Imperial Park, Foaming plant

Metagenomics
 MinION & Illumina
 Metaproteomics

Who are the putative foam-causing bacteria?
What are the putative foam-causing proteins?

Results

Who are the putative foam-forming bacteria and what are they doing? → Metaproteomics

Cold-adapted lipases and lipolytic bacteria found by metaproteogenomics
 in low-temperature anaerobic membrane bioreactors treating domestic wastewater
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Introduction

Cold-adapted bacteria can treat domestic wastewater at low temperatures. But poor lipid degradation is still a barrier.

Methods

We used metagenomics and metaproteomics [2].

Metagenomics → Who are the lipase producing bacteria?
 Metaproteomics → Are the lipases produced?
 Bioinformatics → KBase

Feed: Domestic wastewater (primary influent) from a full-scale activated sludge plant
Inoculum: Soils & sediments from Lake Geneva, Switzerland and Svalbard, Norway

Figure 1: Concentration of unhydrolysed organic matters in cold-adapted *Acetivibrio* [2].

Figure 2: Cold-adapted lab-scale *Acetivibrio* speciation and our approach.

Results

Who are the lipase producing bacteria?

40 lipolytic MAGs with 78 lipases
 Genome completeness ≥ 90%
 Contamination ≤ 10%
 At least one lipase (EC 3.1.1.3) gene

Out of 1519 total MAGs and 903 lipases (metagenome-assembled genomes)

How abundant are the lipolytic genera in reactors?

Non-stable 15°C, Stable 15°C, Non-stable 4°C, Stable 4°C

Figure 3: Taxonomic classification of lipolytic MAGs at phylum and genus level.

Who are the lipolytic genera from the metaproteomics side?

Metaproteomic found no lipase!
 Fatty (long-chain fatty acid transporter) was found as a lipolytic biomarker protein in 4 genera not recovered in lipolytic MAGs by metagenomics!

Figure 4: Relative abundance of the lipolytic genera recovered in MAGs in the reactors.

Figure 5: Relative abundance of the putative lipolytic genera with an expressed *lipA* in reactors.

Conclusions

- Extracellular lipases and lipolytic bacteria were not easily identifiable by metagenomics and metaproteomics.
- Metaproteomics did not provide sufficient proteome coverage for lower abundant proteins such as lipases.
- Better protein extraction methods for metaproteomics of enzymes should be developed.

References

1. Pothof-Groen, E., et al. 2018. Environmental Science: Water Research & Technology, 4, 1002-1013.
 2. Bashiri, R. et al. 2021. Science Advances, 7(12), 1-15.