A molecular ecology approach using Stable Isotope Probing and metagenomics to study viruses of methanogens' diversity Context

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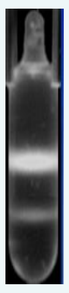
Context

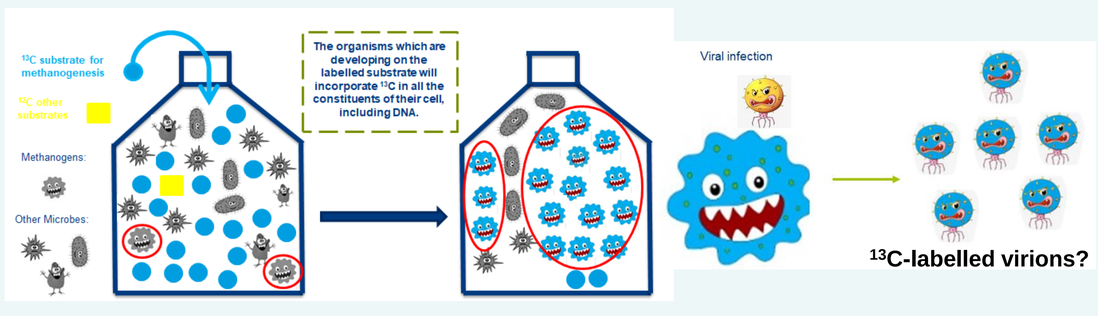
Identifying hosts from viruses in complex microbial communities is still non-trivial

- Very promising approaches have recently emerged to identify hosts from viruses in complex ecosystems (1, 2)
- However, most of them require either host cultivability or prior knowledge of host and virus partial genome sequence
- Moreover, **none of them establish a direct link with the host metabolism**

Stable Isotope Probing (SIP) should enable to identify viruses infecting hosts which assimilate a specific substrate, within complex ecosystems



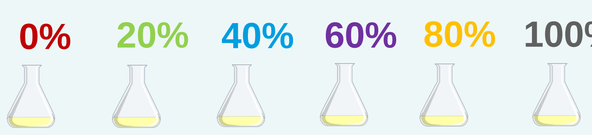
The use of various ^{13}C-labeled methanogenesis substrates enables us to activate different populations of methanogenic archaea and enrich them with ^{13}C (3). Logically, viruses that infected them should also be labelled. The viral DNA will be separated by ultracentrifugation on a CsCl gradient (4), collected and further studied (sequencing, …).



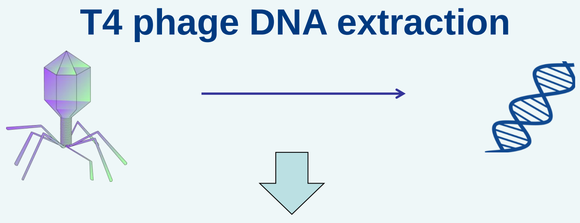
Proof of concept with a simple biological model

T4 bacteriophage (DSM 613) production on *Escherichia coli* B cells (DSM 4505) grown in M9 minimal medium

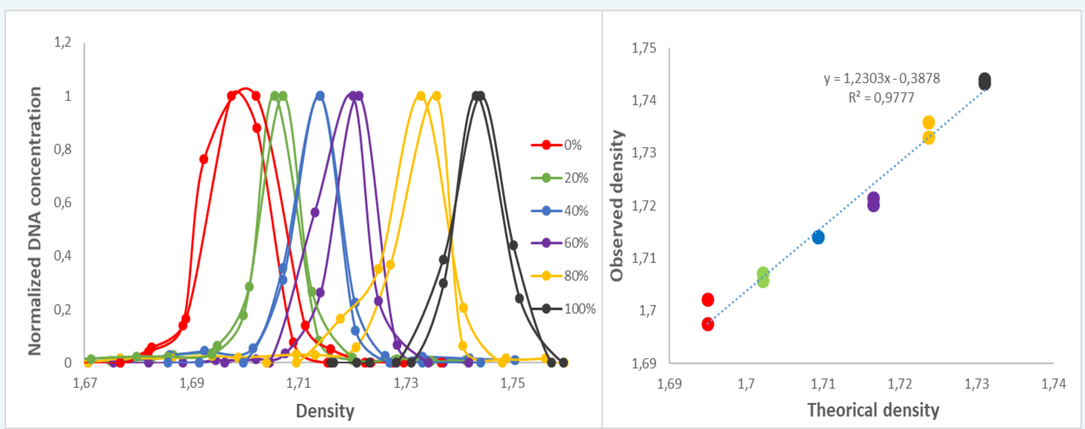
% of ^{13}C-glucose in the growth medium



T4 phage DNA extraction



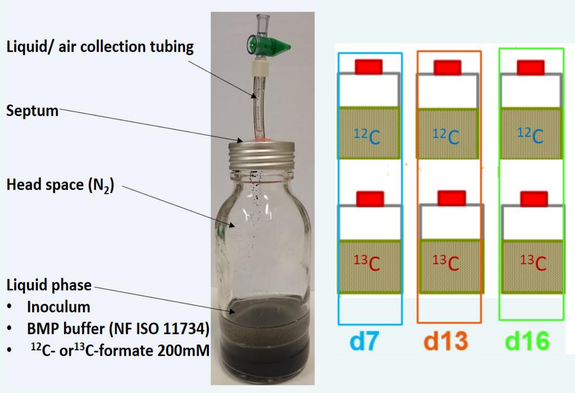
SIP profiles

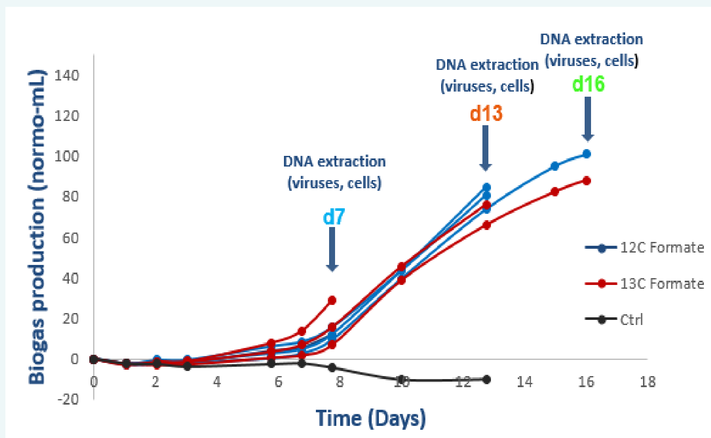


- Significant differences in positions of density peaks
- Good reproducibility between the expected and observed peak positions, according to the ^{13}C-glucose percentages

Test of SIP applied to organic waste anaerobic digestion microbial communities

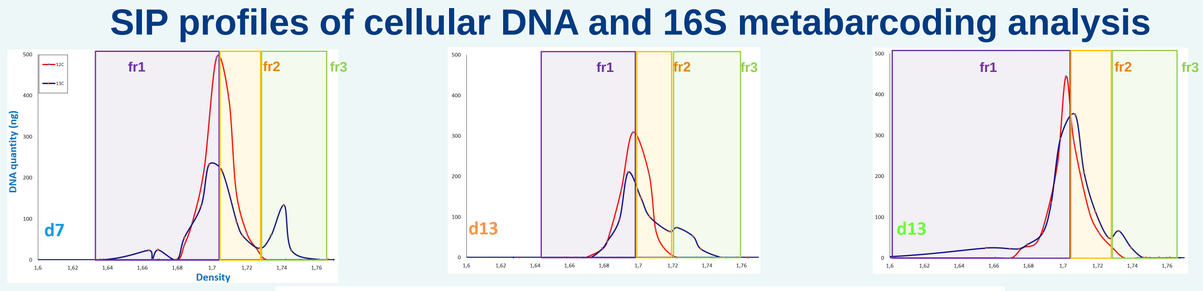
Incubation with ^{12}C- or ^{13}C-formate as substrate

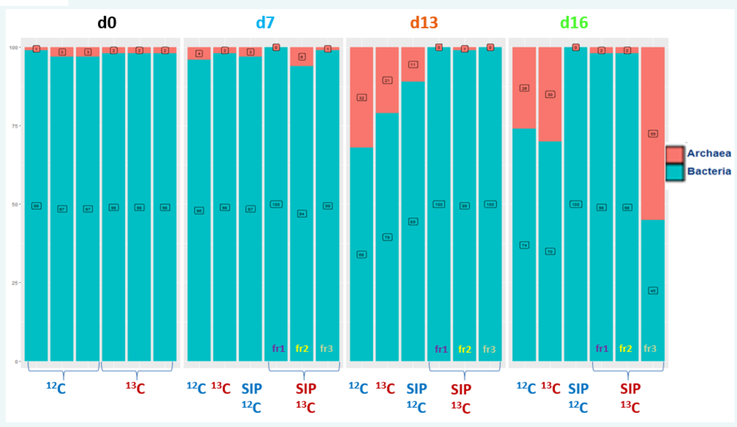




- Good reproducibility of biogas production
- DNA extraction at 3 different time points to select for the samples with the optimal amounts of enriched DNA from archaeal cells or viruses

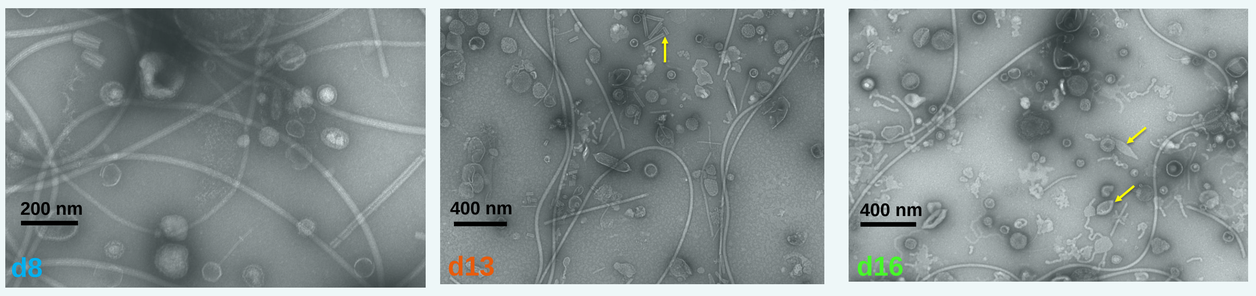
SIP profiles of cellular DNA and 16S metabarcoding analysis





- Archaea were mostly methanogens and their proportion increased over time
- The highest proportion of archaea was reached in the fr3 ^{13}C fraction at d16

Virions observation (TEM)



- At d13-d16, virus-like particles with morphotypes similar to archaea-specific viral families were observed
- No significant differences in viral DNA quantities collected at d7, d13 or d16

Perspectives

- Perform the SIP analysis of viral DNA and sequence the fractions of interest
- Identify contigs of archaeal viruses thanks to bioinformatics analysis of the metavirome sequences (pipeline development).

References

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