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# Diversity of novel archaeal viruses infecting methanogens NRAU discovered through coupling of stable isotope probing and metagenomics

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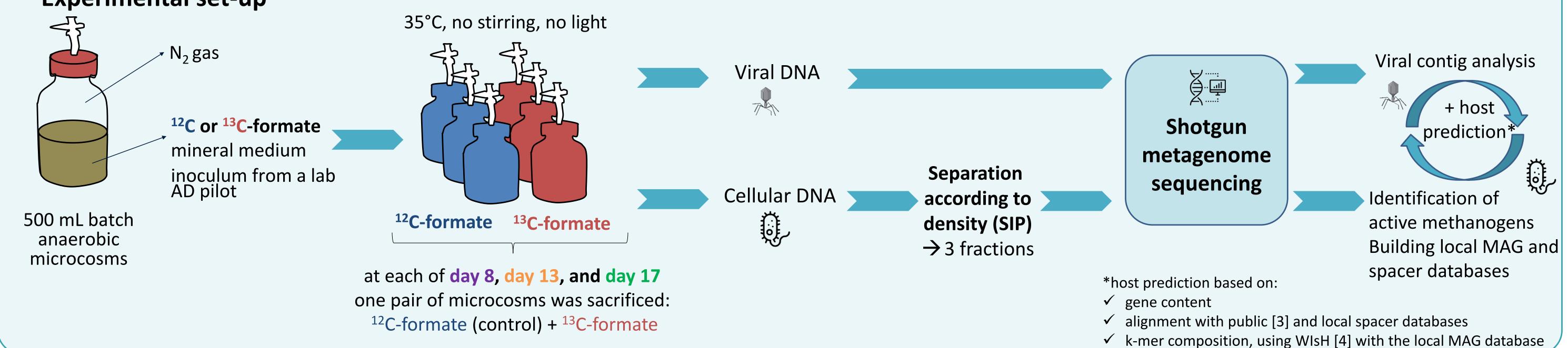
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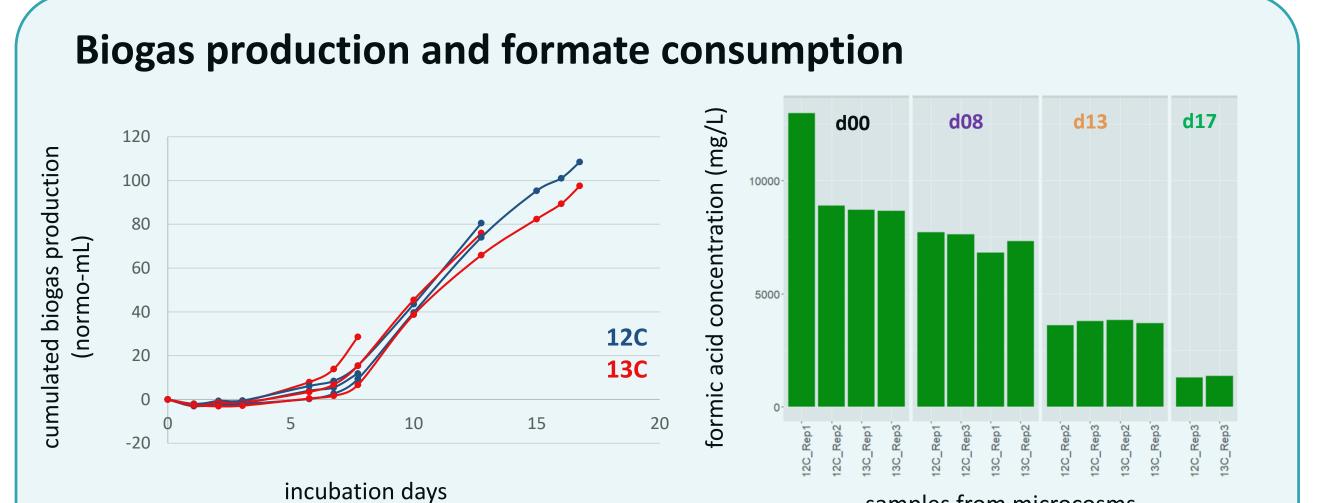
4. Université Paris-Saclay, INRAE, BioinfOmics, MIGALE bioinformatics facility, 78350, Jouy-en-Josas, France 5. Institut Pasteur, Université de Paris, CNRS UMR6047, Archaeal Virology Unit, F-75015 Paris, France

## Context

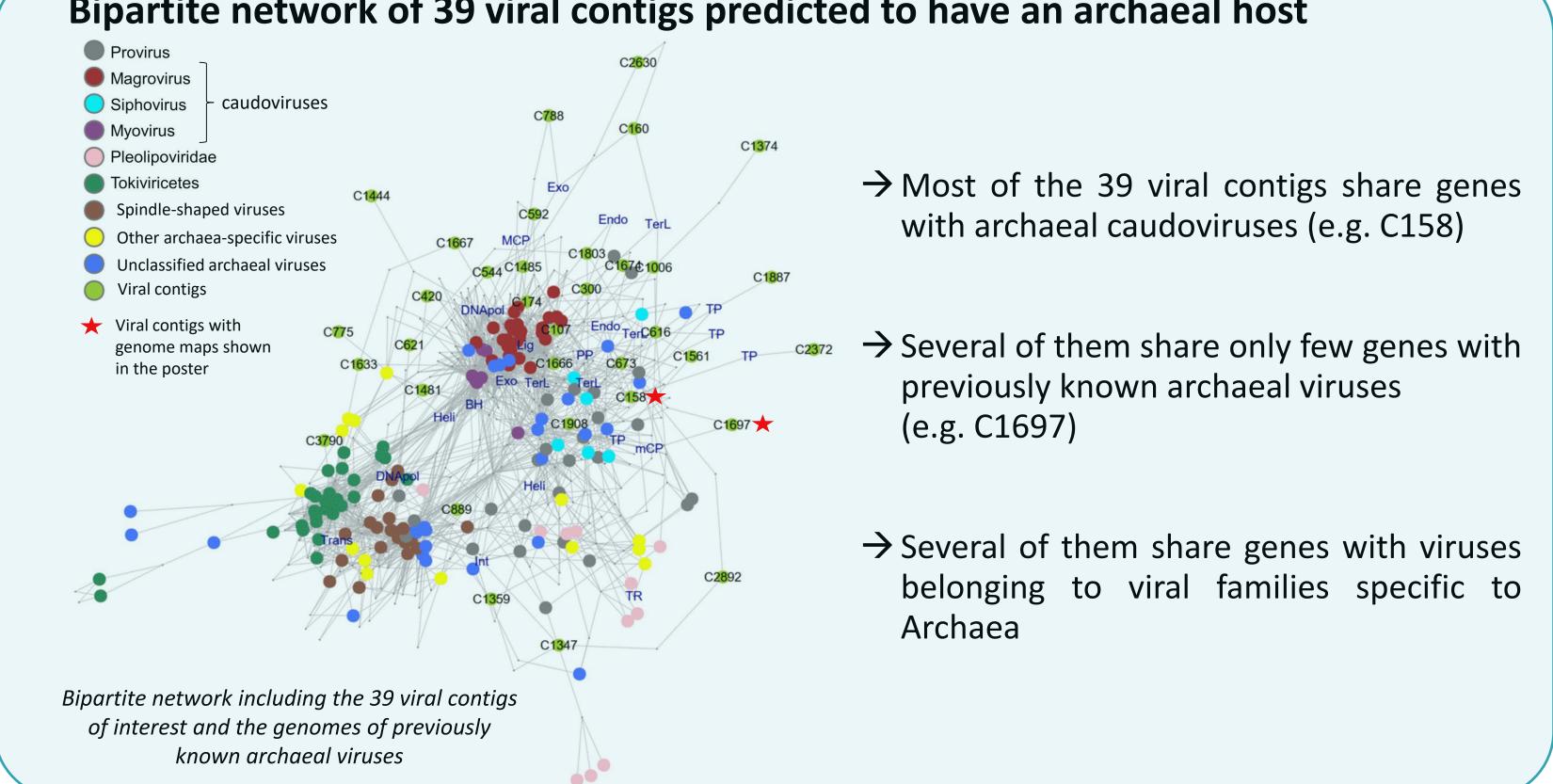
Viruses of microbes are major players in various ecosystems, typically affecting the structuration and dynamics of microbial communities [1]. They nevertheless remain poorly explored in anaerobic digestion (AD) plants [2], where biowaste is valorised into methane-rich biogas. Here, we explored the diversity of viruses infecting methanogens involved in AD processes. We developed an original coupling of metagenomics and stable isotope probing (SIP), to identify viruses infecting formate-assimilating methanogens during AD. Only a dozen of viruses of methanogens have been isolated so far, mostly head-tailed.

## **Experimental set-up**





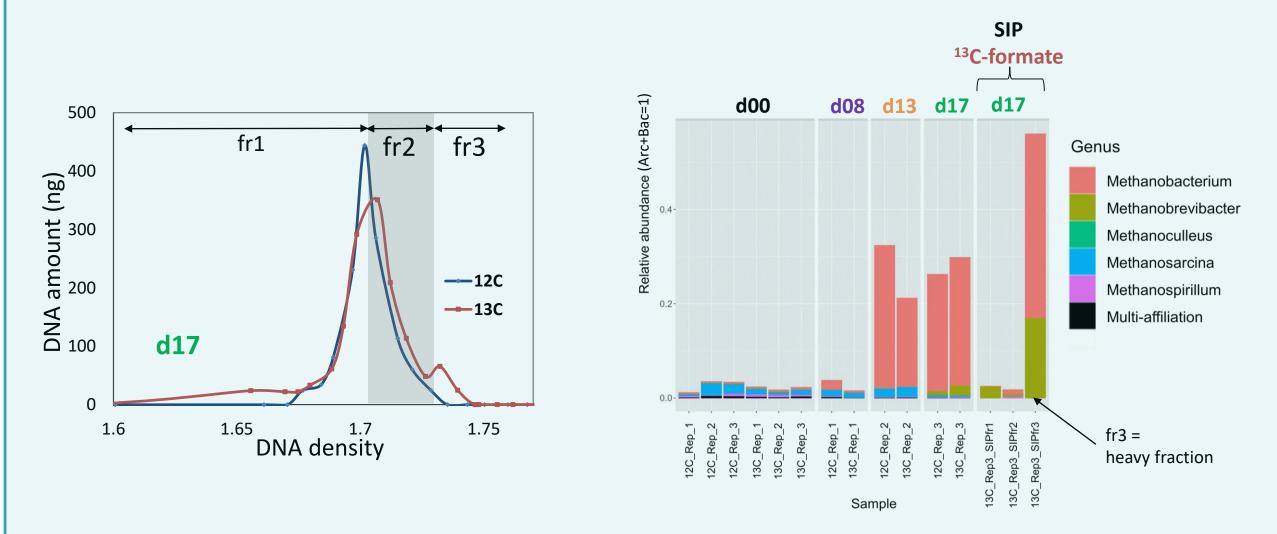
### Bipartite network of 39 viral contigs predicted to have an archaeal host



samples from microcosms

 $\rightarrow$  As expected, biogas was produced and formate was consumed, with a good reproducibility among replicates

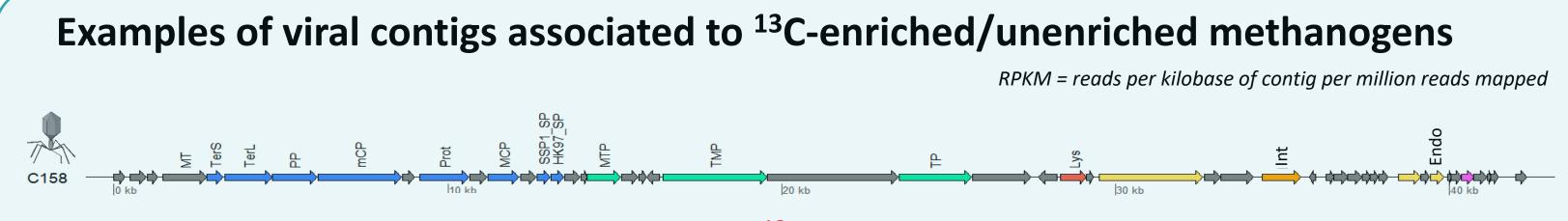




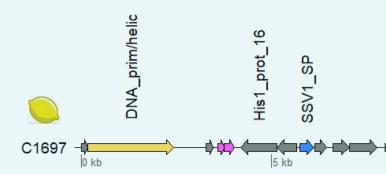
 $\rightarrow$  Methanogenic archaea of the genera *Methanobacterium*, and to a lesser extent *Methanobrevibacter*, assimilated <sup>13</sup>C-formate and reached high proportions at d17, including in the heavy fraction (fr3)

Rod-shaped VLPs

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Caudovirus, complete genome, predicted host = <sup>13</sup>C-enriched *Methanobacterium*, based on gene content and k-mer composition - new family proposed: *Speroviridae* (abundance: RPKM  $\approx$  263 at d17)

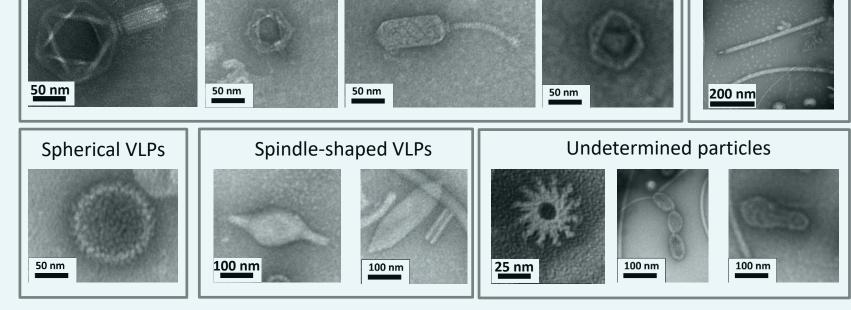


Spindle-shaped virus, partial genome, host = unenriched *Methanosarcina*, based on gene content and k-mer composition - likely represents a new family (abundance: RPKM  $\approx$  9 at d17)

SSV1\_SP: similar to the main structural protein of SSV1, a spindle-shaped virus (Fuselloviridae) indecting Sulfolobus solfataricus, an acidothemophilic archaeon His1\_prot\_16: similar to prot\_16 of His1, a spindle-shaped virus (Halspiviridae) infecting Haloarcula hispanica, a halophilic archaeon

## Conclusions

- ✓ Significantly expands the knowledge on the diversity of viruses of methanogens



Virus-like particle (VLP) diversity

Icosaedral VLPs (dominant morphotype)

Transmission Electron Microscopy images

morphotypes was observed in the microcosms, including head-tailed, rodshaped, spherical and spindle-shaped ones.

Spindle-shaped viruses

are specific to Archaea.

 $\rightarrow$  A great diversity of

(one new caudovirus family, two putative new families of spindle-shaped viruses) ✓ Reinforces the notion of wide environmental and phylogenetic distribution of

spindle-shaped archaeal viruses

✓ Work recently published: Ngo *et al, Environmental microbiology*, 2022, 1–16

## Perspectives

Original approach that can be applied to virtually any type of microbial community and coupled with other experimental methods for host identification (e.g. PhageFISH, epicPCR, ...).

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### References

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