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## ► To cite this version:

Thomas Paillet, Julien Lossouarn, Clarisse Figueroa, Inès Pedros, Cédric Midoux, et al.. Bacteriophages infecting surface bacteria from a smear-ripened cheese are persistent and contaminate the dairy plant. *Viruses of Microbes (VoM)* 2022, Jul 2022, Guimarães, Portugal. hal-04416527

**HAL Id: hal-04416527**

**<https://hal.inrae.fr/hal-04416527>**

Submitted on 25 Jan 2024

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# Bacteriophages infecting surface bacteria from a smear-ripened cheese are persistent and contaminate the dairy plant

Thomas Paillet<sup>1</sup>, Julien Lossouarn<sup>2</sup>, Clarisse Figueroa<sup>1</sup>, Inès Pedros<sup>1</sup>, Cédric Midoux<sup>3</sup>, Olivier Rué<sup>3</sup>, Marie-Agnès Petit<sup>2</sup>, Eric Dugat-Bony<sup>1</sup>

<sup>1</sup>Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91120, Palaiseau, France; <sup>2</sup>Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, F-78352, Jouy-en-Josas, France; <sup>3</sup>Université Paris Saclay, INRAE, MaIAGE, 78350 Jouy-en-Josas, France



## Context

Bacteriophages are known to be major drivers of microbial communities in several ecosystems. In cheese, phages infecting lactic acid bacteria (LAB) starter cultures are well described. However, very little is known about those infecting ripening bacteria and their impact on the microbial successions observed during cheese maturation [1]. In a recent study, 5 bacteriophages infecting non-LAB starter cultures were isolated and characterized, giving first clues of a potential ecological role within rind community [2]. Here, we sequenced the whole viral communities from smear-ripened cheeses sampled on 2017, 2019 and 2022. Then we tracked the 5 previously sequenced phage genomes within assembled contigs to simultaneously investigate their presence and persistence on cheese rinds. Finally, their origin within the dairy plant producing the sampled cheeses was questioned.

## Materials & Methods

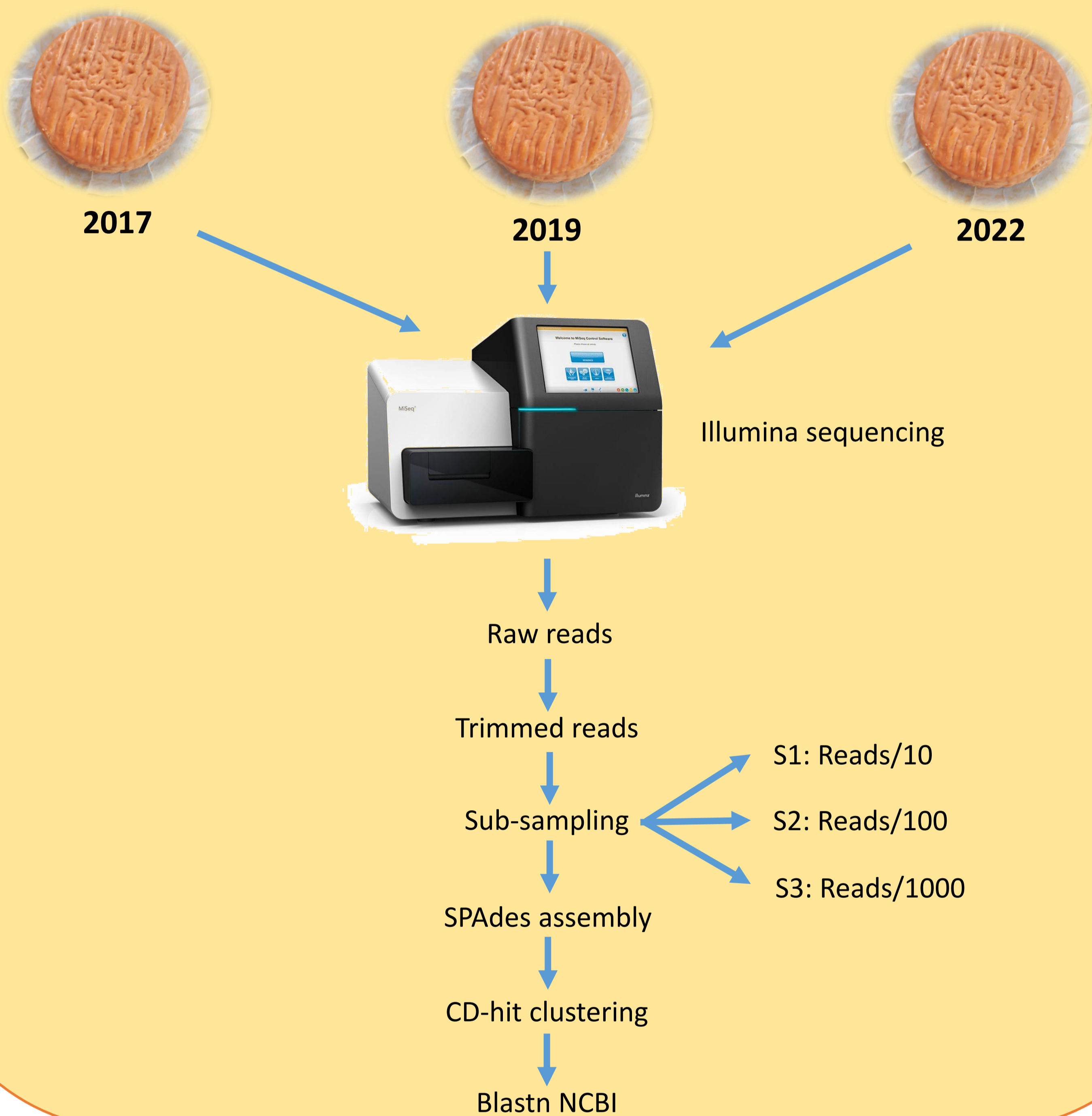
### Viral Metagenomics

Viral fractions were extracted from cheese rind at 3 different times (2017, 2019, 2022, each in triplicate) and viral DNA was sequenced by Illumina technology (5M of 2 x 150 bp reads). Then, trimmed reads were assembled in contigs with SPAdes [3]. Most abundant contigs were tested against the NCBI database through a Blastn [4].

### Phage Reservoirs

Viral fractions were extracted from the following cheese dairy samples, each in triplicate: milk after inoculation with acidification ferments, salting tables (after cleaning), cheese turning line (after cleaning), and two washing solutions. They were then tested on 5 indicator strains, on which the 5 phages were previously isolated, through a spot assay (10  $\mu$ l spots), with and without prior enrichment. Clear lysis plaques were picked with a sterile tip, and resuspended in 30  $\mu$ l sodium-magnesium buffer. Then, PCR amplification using diagnostic primers for each of our 5 characterized phages and Sanger sequencing was conducted to test whether the phage detected in the sample corresponded to the one previously isolated from cheese rind.

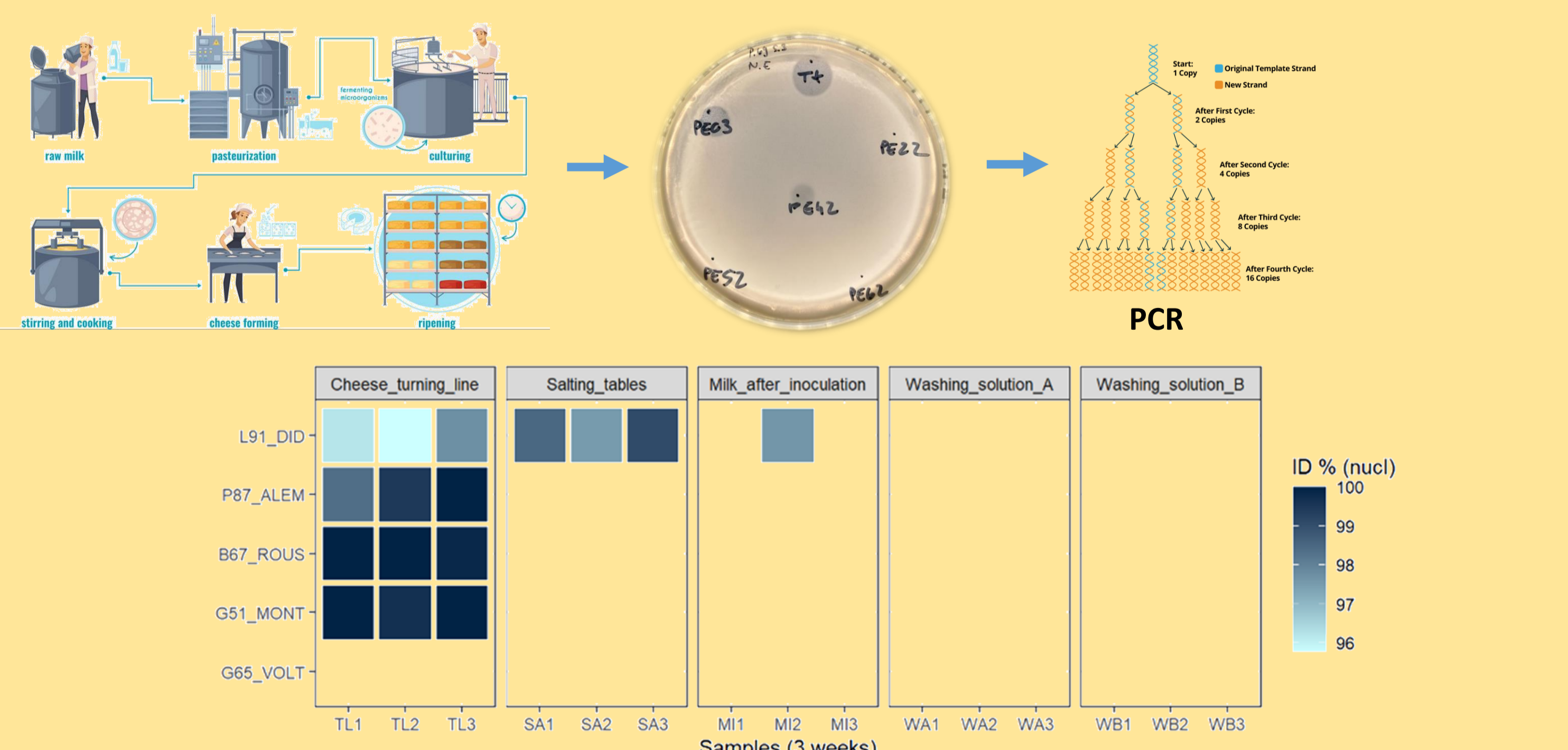
## Viral metagenomics



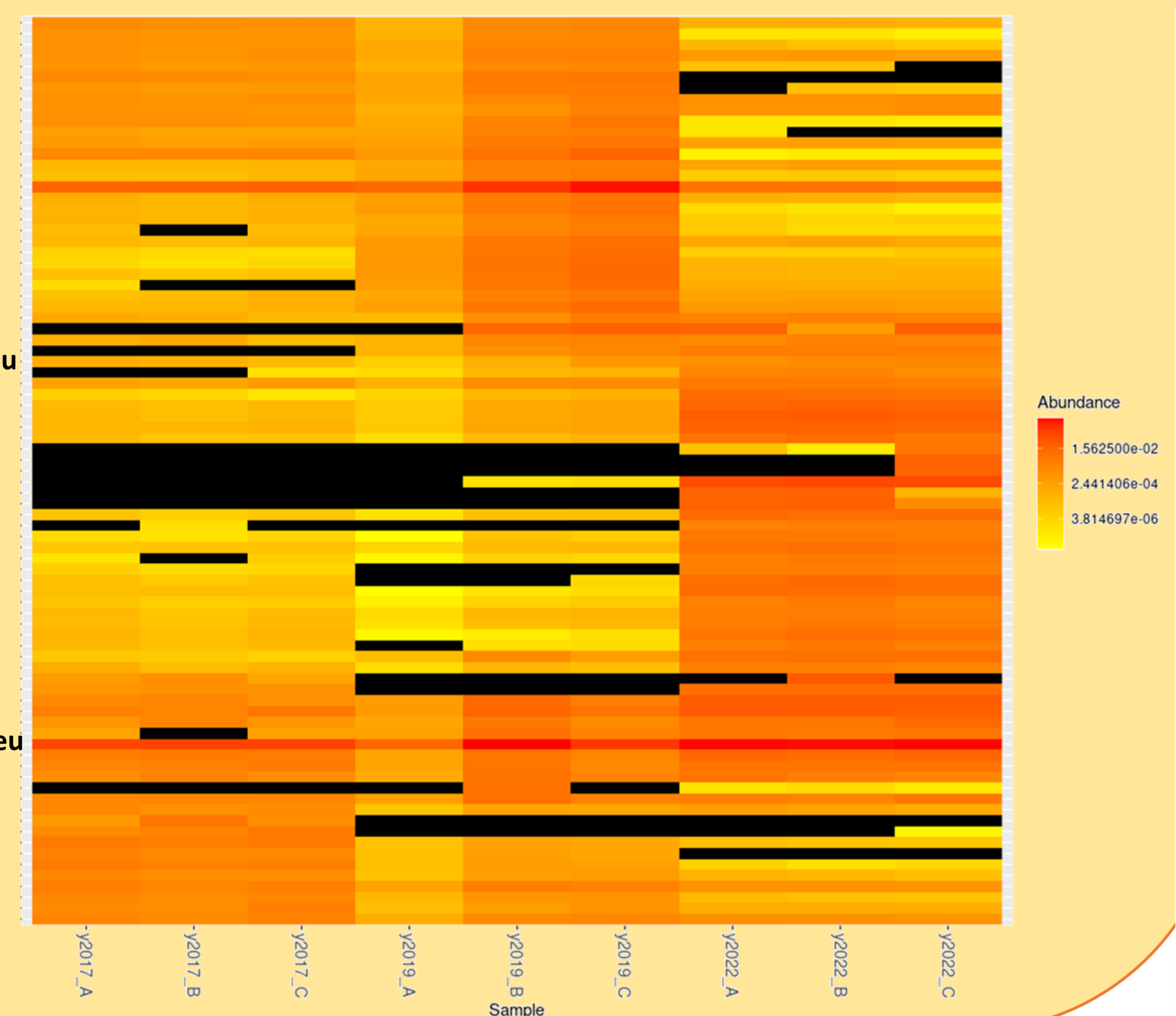
## Presence and persistence of phages



## Detection of phages within the dairy plant



All phages were detected within the dairy plant except Voltaire. The cheese turning line concentrates the most important diversity of dairy phages. The analysis of the PCR-amplified fragment used for the detection of Diderot revealed some sequence variations between Diderot-like phages present in the dairy plant, suggesting co-occurrence of multiple closely-related *Leuconostoc* phages within this environment.



## Conclusions

Phages may have an ecological role in cheese ripening, as they are able to infect ripening and NSLAB bacteria. They persist on the rind from year to year, sometimes at high abundancies. Then, it is not surprising to find them within the cheese plant, although their genomes continue to evolve. It would be of great interest to test the infect of these 5 isolated phages in model-cheeses to understand better their impact on bacteriophage community dynamics.

## Acknowledgments

T.P. is the recipient of a doctoral fellowship from the French Ministry of Higher Education, Research and Innovation (MESRI) and the MICA department of INRAE.

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