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

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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.

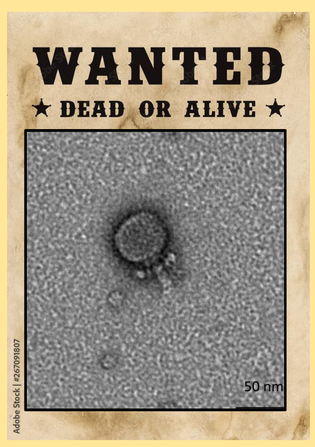
Materials & Methods

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 µl spots), with and without prior enrichment. Clear lysis plaques were picked with a sterile tip, and resuspended in 30 µl 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

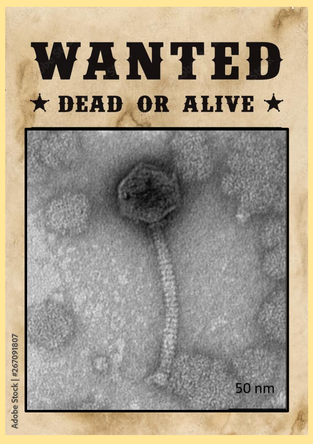


Glutamicibacter ϕ **Voltaire**

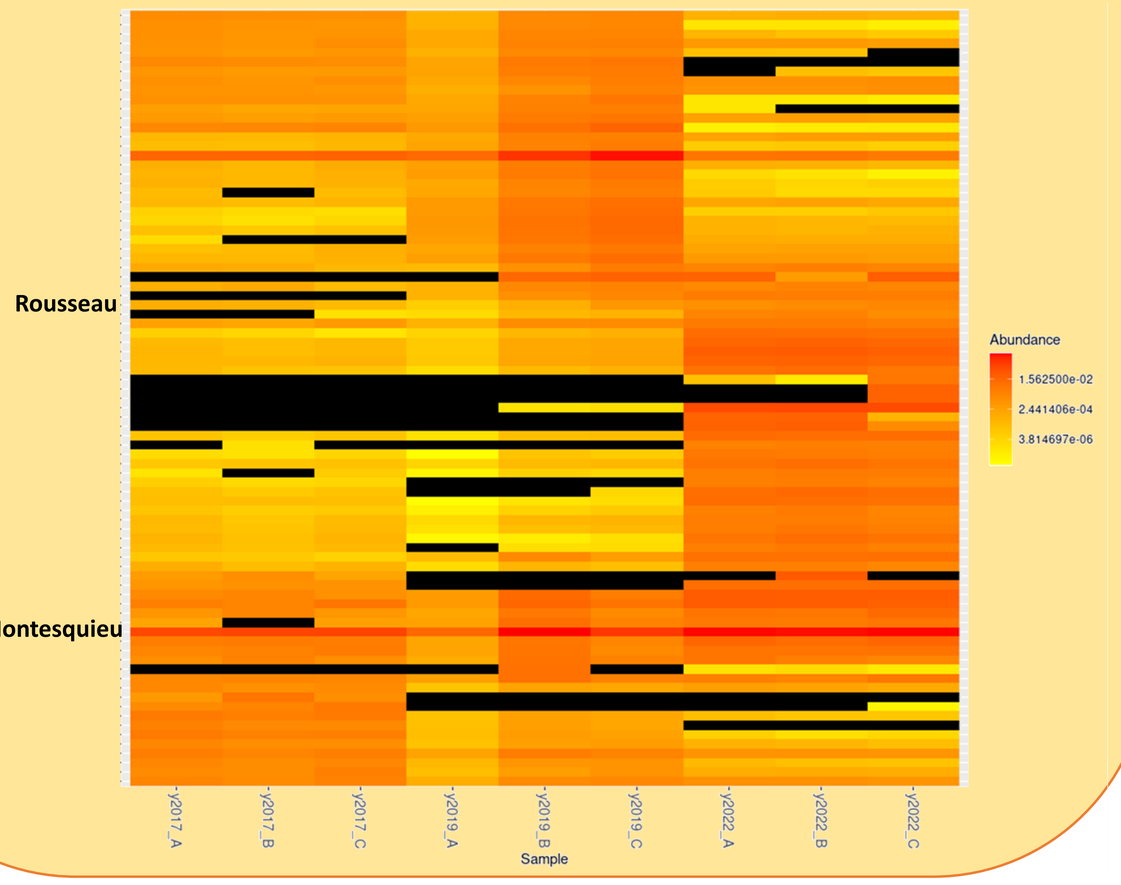
Glutamicibacter ϕ **Montesquieu**

Brevibacterium ϕ **Rousseau**

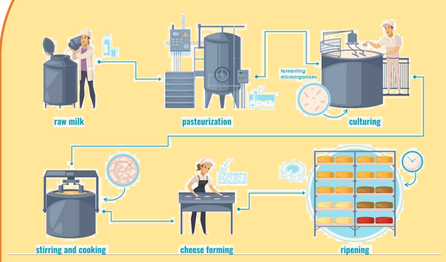
Psychrobacter ϕ **D'Alembert**

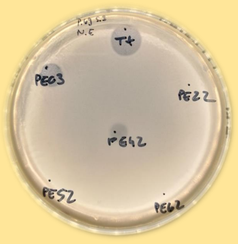


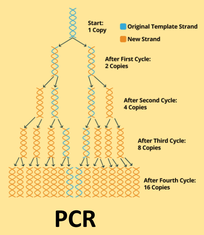
Leuconostoc ϕ **Diderot**



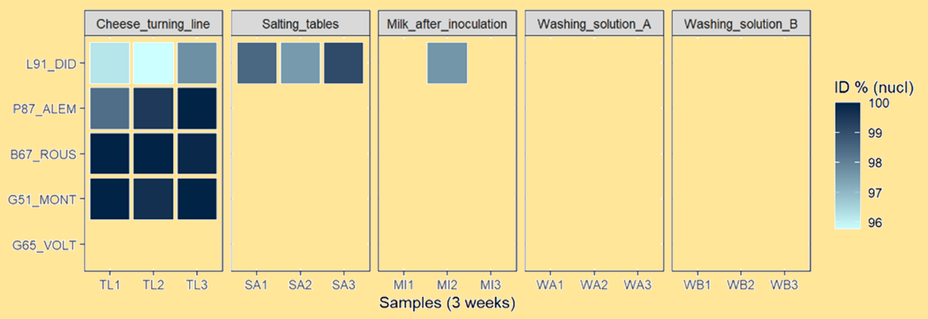
Detection of phages within the dairy plant







PCR



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 bacteirla community dynamics.

Acknowledgments

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