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Advances in understanding the ecology of bacteriophages in cheese

Thomas Paillet,¹ Julien Lossouarn,² Quentin Lamy-Besnier,² Marie-Agnès Petit,² **Eric Dugat-Bony**¹

¹Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91120 Palaiseau, France

²Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78352 Jouy-en-Josas, France

The production of smear-ripened cheese relies on the successive development of various microorganisms including LAB, yeasts and ripening bacteria. Bacteria play a major role in milk acidification, allowing coagulation, and in the ripening process, where they contribute to sensory properties such as colour, odour and taste. Recent studies indicate that a viral community, mainly composed of bacteriophages, coexists with cellular microorganisms in this ecosystem, but its ecological significance remains to be elucidated. Here, we used both a cultivation-based approach and viral metagenomics to get novel insights into the bacteriophage ecology of a French smear-ripened cheese.

First, five virulent phages infecting four non-starter culture bacterial species, namely *Glutamicibacter arilaitensis*, *Brevibacterium aurantiacum*, *Psychrobacter aquimaris* and *Leuconostoc falkenbergense*, were isolated from cheese and characterized through genome sequencing, transmission electron microscopy and host-range determination. PCR detection was also used for evaluating their distribution within potential reservoirs of the cheese factory. Second, we applied metagenomics tools to assess both the dynamics of phages and bacterial groups on the cheese surface during maturation, and their persistence in ready-to-eat cheeses throughout the production years. We observed a clear shift in the phage community composition during maturation, with a reduced proportion of viral taxa associated with *Lactococcus* phages, subsequently replaced by phages targeting ripening bacteria belonging to *Brevibacterium*, *Glutamicibacter*, *Pseudoalteromonas*, and *Vibrio* genera. This dynamics was strongly associated with bacterial successions observed on the cheese surface. Additionally, we determined that a large core-virome composed of the most dominant phages was consistently detected in matured cheese across production years, indicating long-term persistence of the main phages in the cheese production environment.

Together, these results provide new insights into the microbial ecology of cheese, highlighting the importance of phages during the ripening process. It also offers new avenues of research to better control this key step of cheese production.