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Ecological modelling of phage, bacterial and yeast populations structure in Epoisses cheese under changing ripening conditions using factorial experimental design and metavirome analysis

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Introduction

Bacteriophages, i.e. bacterial viruses, are always present in microbial ecosystems and contribute to the ecosystem functioning by regulating the population structure through predation and cell lysis. How these phages are involved in regulating food ecosystems remains an unresolved question so far, in particular when the food is a solid and spatially structured matrix. It should also be noted that phages genomes are difficult to assemble from metagenomics data due to their high genomic plasticity, adding another challenge to the task of correlating phages populations with bacterial populations.

Our work aimed at proposing an analytical strategy to improve our capacity in characterizing phages genomes in metagenomics data and to correlate them with cognate bacterial host using Epoisses, a French washed-rind cheese, as a model.

Materials and Methods

A central composite design was carried out to allow variations of two abiotic parameters, namely temperature and oxygen level, during the ripening of Epoisses cheese. This design had nine different ripening conditions, chosen specifically with the purpose to induce changes in the bacterial and yeast populations and hence in the co-evolving viral populations. Community structure analysis was determined using metagenetic approaches for bacterial and yeasts populations (targeting *gyrB* and ITS2, respectively), and shotgun metagenomic for phages after viral particles extraction from the cheese matrix. The variance in phage populations was used as a criterion to better assemble phages genomes from the metavirome dataset. Then, we tried to correlate the characterized phage clusters in terms of virulence/lysogeny behavior. In a second step, we established significant correlations (positive or negative) between the abundances of viral clusters with those of bacterial species.

Results

Our results showed that the two abiotic parameters induced important changes both in the bacterial, yeast and phage communities' structure. Based on these correlations, we were able to characterize bacterial/phages co-abundance (in case of lysogenic phages) or bacterial/phages co-exclusion (in case of virulent phages) and to model the response of these relationships according to the temperature and oxygen levels during ripening. We could identify known host-phage relationship such as *Lactococcus lactis* and a virulent phage belonging to the 936 group but also to assign host

relationship between unknown phages genomes to bacterial species from the rind such as *Halomonas sp.* or *Pseudalteromonas sp.*

Conclusions

Our strategy is thus very promising for better characterizing metavirome populations in complex ecosystems. This work also shed light onto the potential ecological role of viruses in food microbial ecosystems.