

# Exploring inducible *Caudoviricetes* prophages in grana cheese production: characterization and implications

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FONDAZIONE EDMUND MACH dal 1874



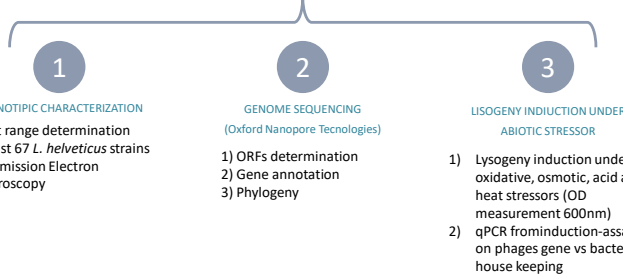
## 1 INTRODUCTION

Trentingrana, is a Protected Designation of Origin (PDO) hard Grana-like cheese produced in the north of Italy, where natural whey starter culture (NWS) is used in the cheese making process. NWSs are mainly characterized by thermophilic lactic acid bacteria (LABs) as *Lactobacillus helveticus* and strongly impact on the organoleptic characteristics of the final product. Presence of free or temperate phages within the NWSs could represent an important aspect potentially inducing a loss of acidification activity with a consequent slow and/or incomplete fermentation, incomplete whey purge from the curd, growth of pathogenic or spoilage bacteria, all factors that negatively affect the quality or the yield of the final product.

Considering the peculiarity of these cheese production and the economic importance within the market, only few studies have considered phages derived from Grana-like cheeses. In this work, seven *Lactobacillus helveticus* phages, isolated from Trentingrana NWS over one year of cheese production, were phenotypically and genetically characterized, focusing on their induction under abiotic stressor, as potential conditions they may hit in the dairy implant.

## 2 METHODS

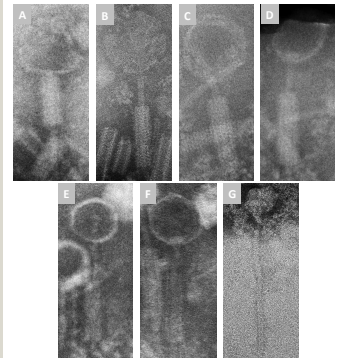
Seven phage,  $\phi$ CR28,  $\phi$ CR191,  $\phi$ P185,  $\phi$ CV244,  $\phi$ S16,  $\phi$ S193 and  $\phi$ T280, were isolated from five dairy implants in the Trentino province (north of Italy)



## 3 RESULTS

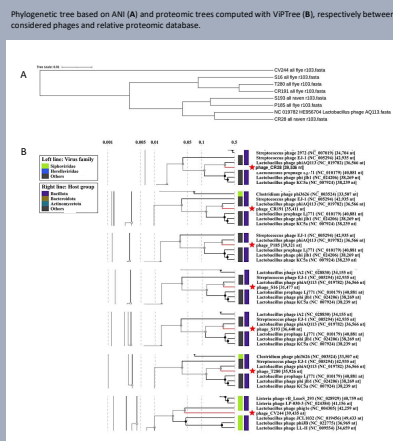
The host range of these phages was narrow and limited to sensitive bacilli. Bacterial hosts of the isolated phages seems to be particularly sensitive to phage infection, as a majority of phage host ranges was observed towards them.

### MORPHOLOGY



TEM images showed phages morphologies associated to the *Caudoviricetes* class, particularly linked to the myovirus ( $\phi$ CR28 [A],  $\phi$ CR191 [B],  $\phi$ P185 [C],  $\phi$ S16 [D],  $\phi$ S193 [E],  $\phi$ T180 [F]) and siphovirus ( $\phi$ CV244 [G]) phage type.

### PHYLOGENY



Based on ViPTree global protein content comparison, phages  $\phi$ CR28,  $\phi$ CR191,  $\phi$ P185,  $\phi$ S16 and  $\phi$ S193 were clustered with phage  $\phi$ AQ113 in the myovirus phage-type group of the tree, while  $\phi$ CV244 was grouped with phage phig1e (NC\_004305.1), as part of the siphovirus phage-type group.

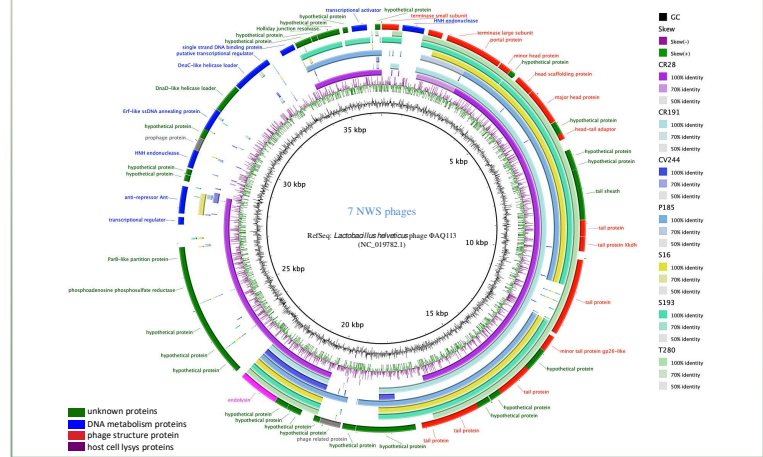
### GENOME INFORMATIONS

Genomic sequence information classified these phages as part of the *Caudoviricetes* class (myovirus phage type), with an average genome size of 36.792 bp and an overall average G+C content of 36.98%. For each of our phages, the first hit of genome similarity through BLASTn concerned *L. helveticus* phage  $\phi$ AQ113 (NC\_019782.1), taken as reference genome in this study.

Genomes contained ~60 putative ORFs (with ~19 functional CDSs and ~38 hypothetical proteins) that were divided based on the protein function into: host lysis (~3 ORFs), structural (~11 ORFs), DNA packaging and replication (~7 ORFs), unknown function (~38 ORFs).

Genome comparison showed high homology (over 70%) for all the studied phages, particularly for ORF encoding phage structural proteins (~14ORFs) and the type involved in host lysis (~2ORFs). Besides, a lesser sequence homology was observed for ORFs related to DNA metabolism (~9 ORFs) and hypothetical proteins (~14 ORFs). The phage genome with the highest homology to  $\phi$ AQ113 was  $\phi$ CR28.

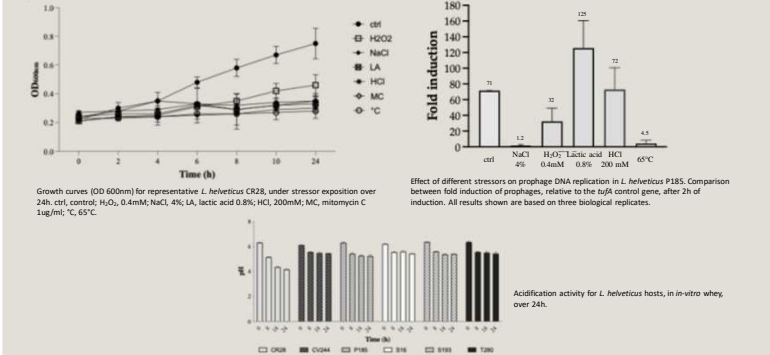
GenBank Accession Numbers
$\phi$ CR28 - OQ627802.1
$\phi$ CR191 - OQ627803.1
$\phi$ CV244 - OQ627804.1
$\phi$ P185 - OQ627805.1
$\phi$ S16 - OQ627806.1
$\phi$ S193 - OQ627807.1
$\phi$ T280 - OQ627808.1



### TEMPERATE PHAGES

Integrase genes were present in  $\phi$ CR191 (ORF 21),  $\phi$ P185 (ORF 5),  $\phi$ S16 (ORF 41) and  $\phi$ S193 (ORF 53). Other genes potentially involved in phage genome integration/prophage induction, were found in phages  $\phi$ CR28 (ORF 23, ORF 15, ORF 31),  $\phi$ CV244 (ORF 7, ORF 18, ORF 40) and  $\phi$ T280 (ORF 7, ORF 50).

Lysogenic behavior was observed over mitomycin C induction in *L. helveticus* hosts. Induction was tested also over oxidative (0.1, 0.2, 0.4mM H<sub>2</sub>O<sub>2</sub>), osmotic (1, 2, 4% NaCl), organic acid (0.1, 0.5, 0.8% lactic acid), acid (50, 200mM HCl) and heat (65°C) exposition for 2h.



## 4 DISCUSSION

NWSs represent an ideal niche of interaction between bacteria and phages. Here we characterized seven temperate phages isolated from *Lactobacillus helveticus* in Trentingrana NWSs. These tailed-phages harbor most of the needed genes for a complete lytic/lysogenic life; at the same time 57% of gene could not be annotated with specific functions, so was possible to speculate the potential presence here of a set of new phage-genes well conserved between these genomes and shared a total of 479 genes.

The phage induction seems to be related specifically to some abiotic stressor, such as lactic acid. Considering the sensitivity of the best acidifying strain to (pro)phages co-infection, this may represent a detrimental aspect able to compromise NWS acidification.

## 5 CONCLUSION

The presence of these phages within NWSs, is opening new interesting insights into the beneficial functions for the host and the role in the technological performance of NWS. A deep characterization of their induction susceptibility would allow a better understanding of critical conditions that may affect the cheese making process, to maintain the excellence in Grana cheese production, e.g. for Trentingrana.