

Phages in Trentingrana cheeses' natural whey starters: new insights from characterization and genomic comparison

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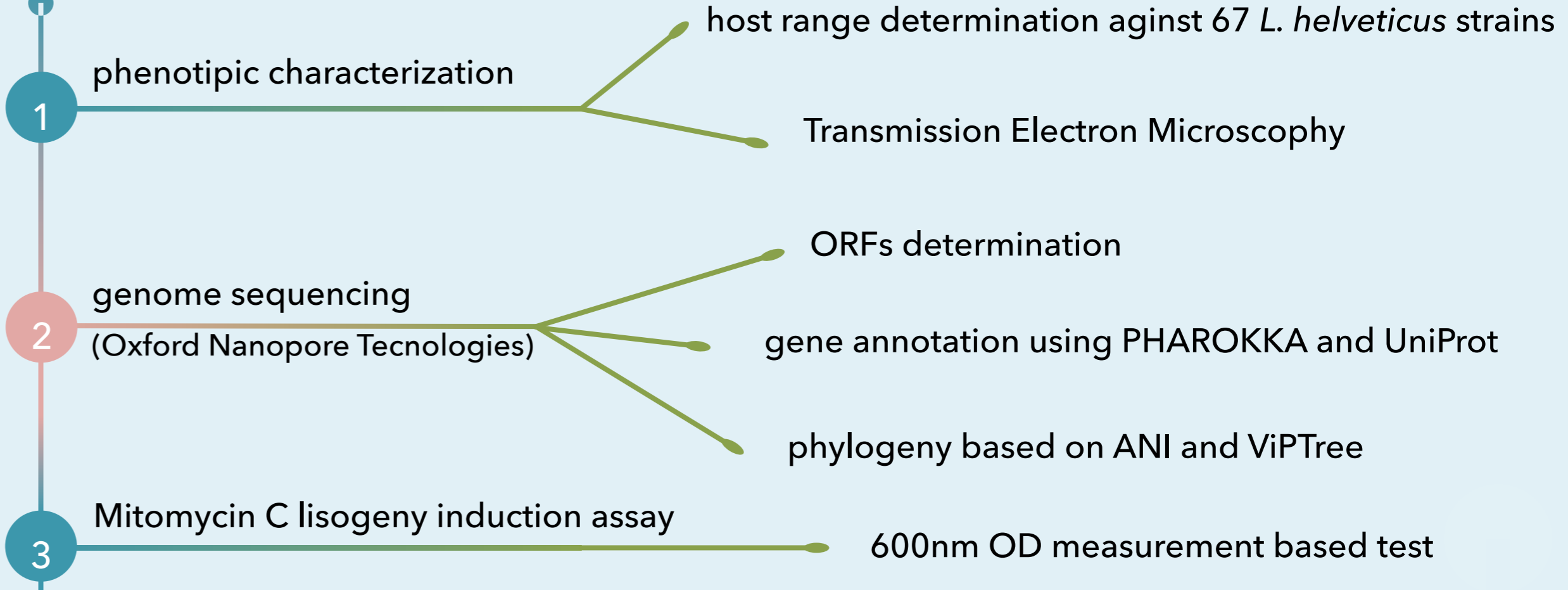
Introduction

Trentingrana, a Protected Designation of Origin (PDO) hard Grana-like cheese produced in the north of Italy, is characterized by the use of a natural whey starter culture (NWS) in the cheese making process. These NWSs greatly influence the peculiarity and organoleptic characteristics of this cheese and are mainly characterized by thermophilic lactic acid bacteria (LABs) as *Lactobacillus helveticus*. The presence of phage in NWSs could determine a loss of acidification activity inducing as a consequence 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.

Only few studies have characterized phages derived from Grana-like cheeses. Herein, seven *Lactobacillus helveticus* phages, isolated from Trentingrana NWS over one year of cheese production, were deeply characterized by focusing on their host range and morphology, as well as their genomic comparison.

Methods

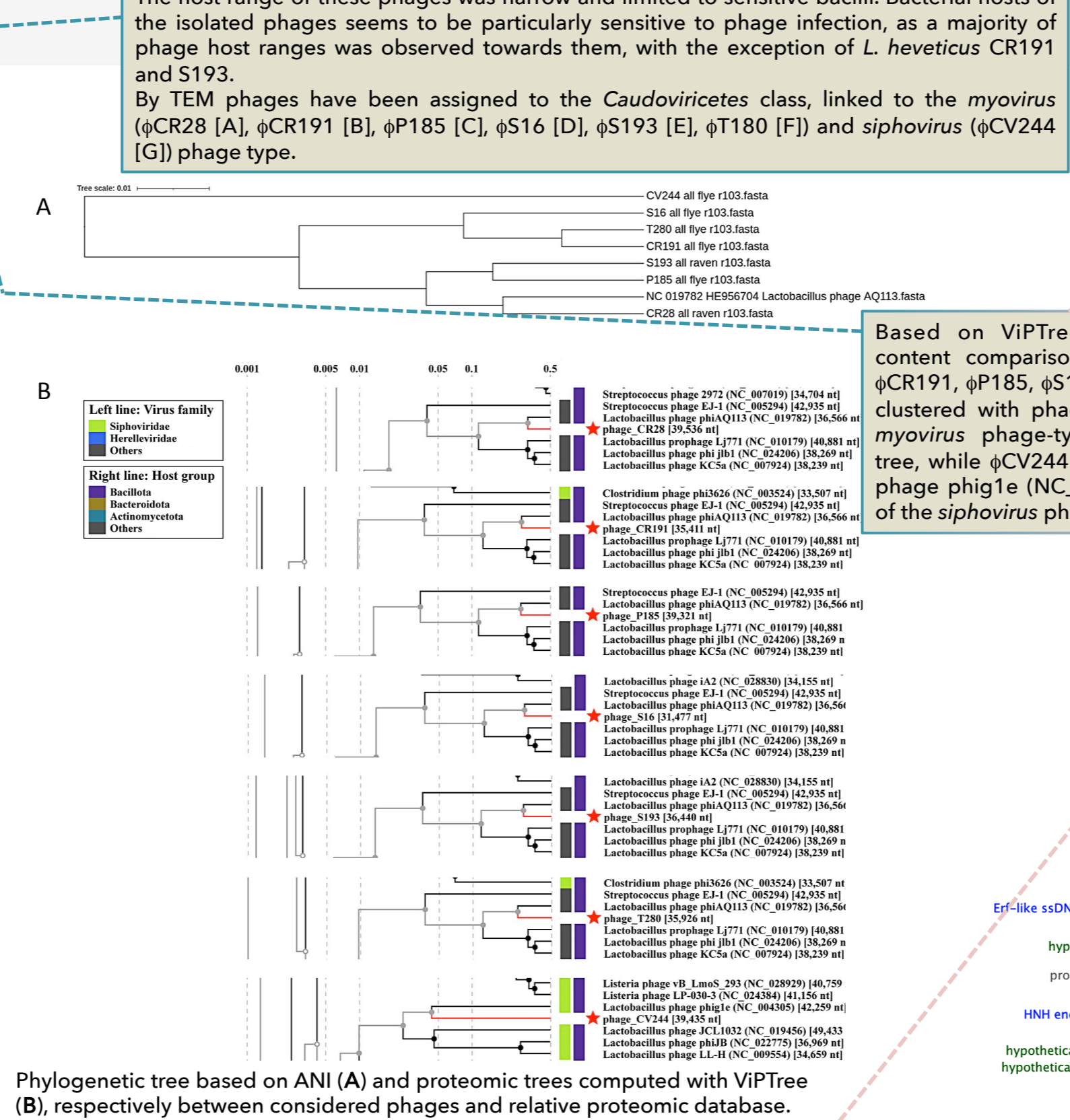
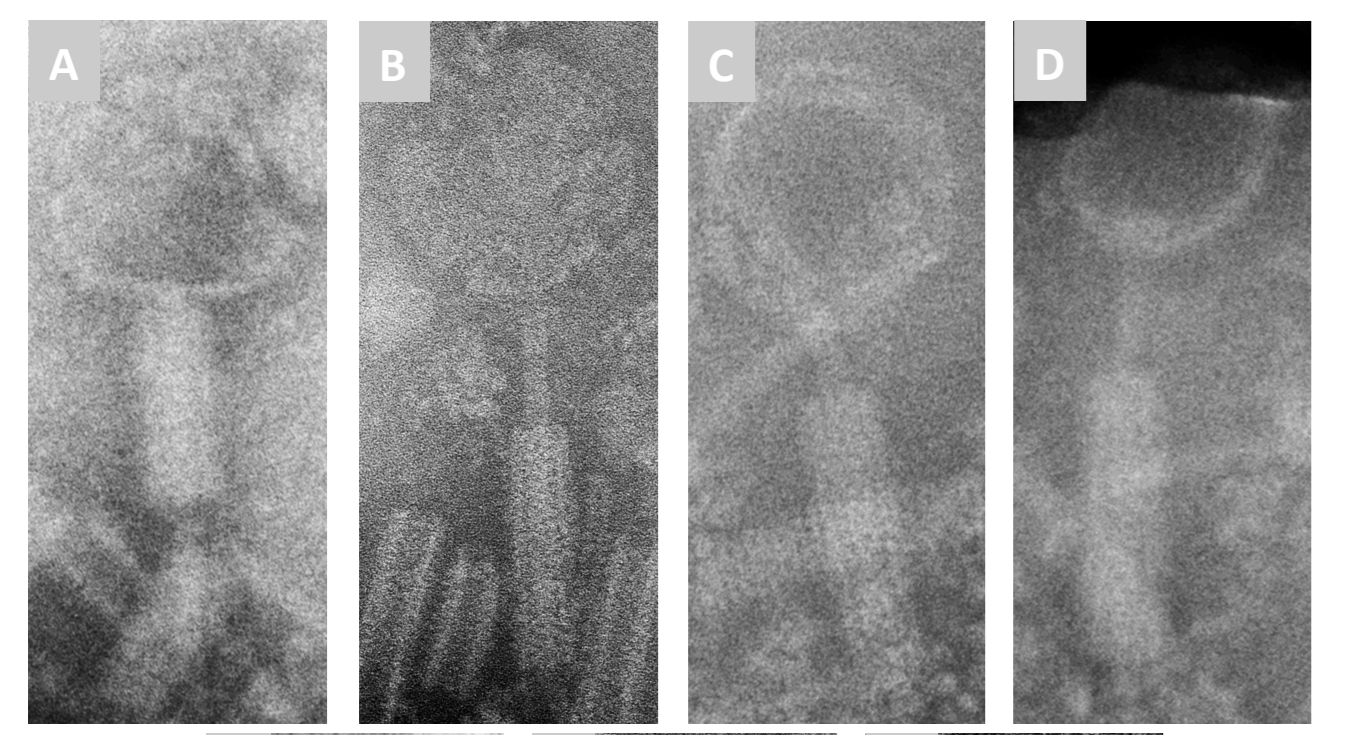
Considered phage ϕ CR28, ϕ CR191, ϕ P185, ϕ CV244, ϕ S16, ϕ S193 and ϕ T280, were isolated from five dairy implants in the Trentino province



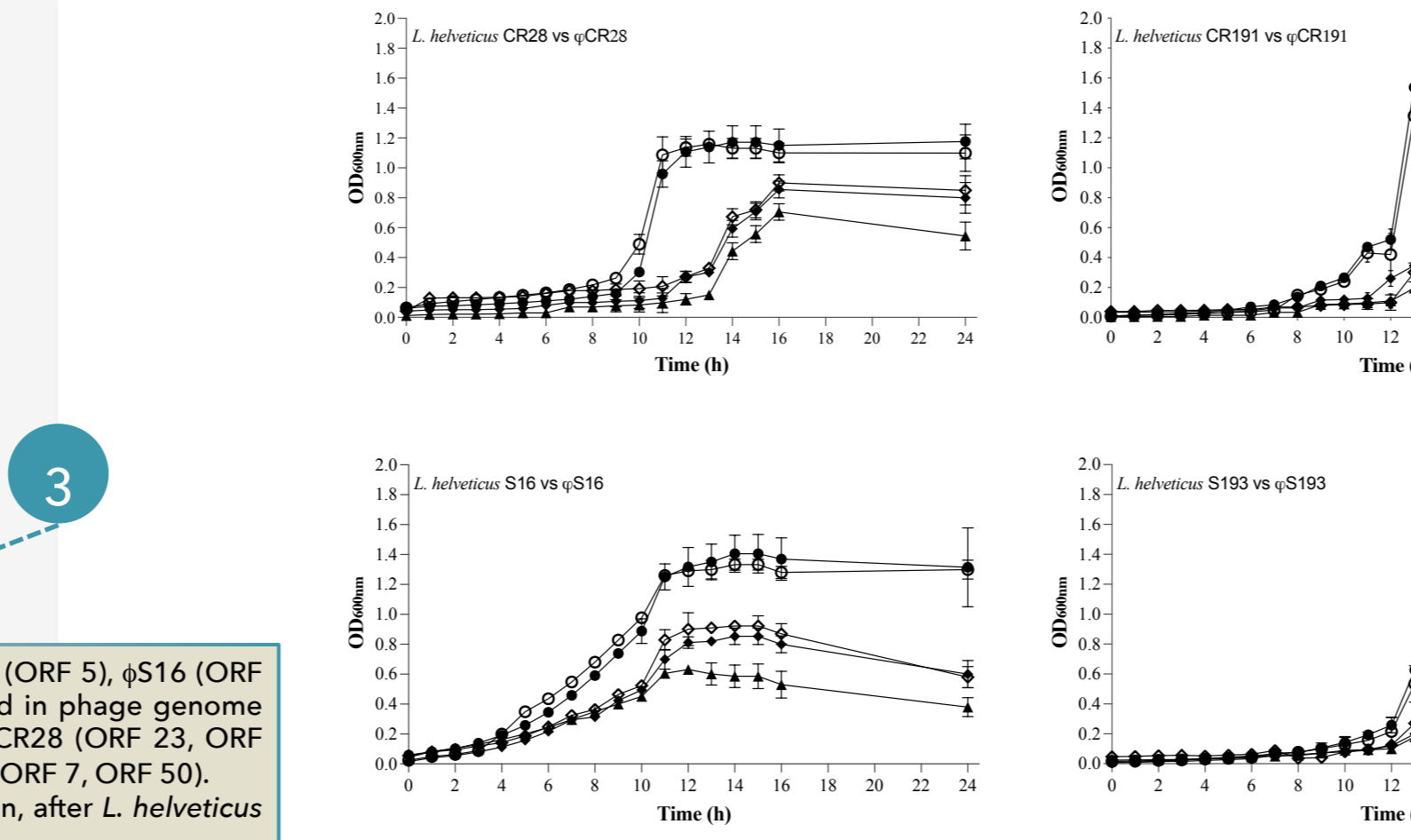
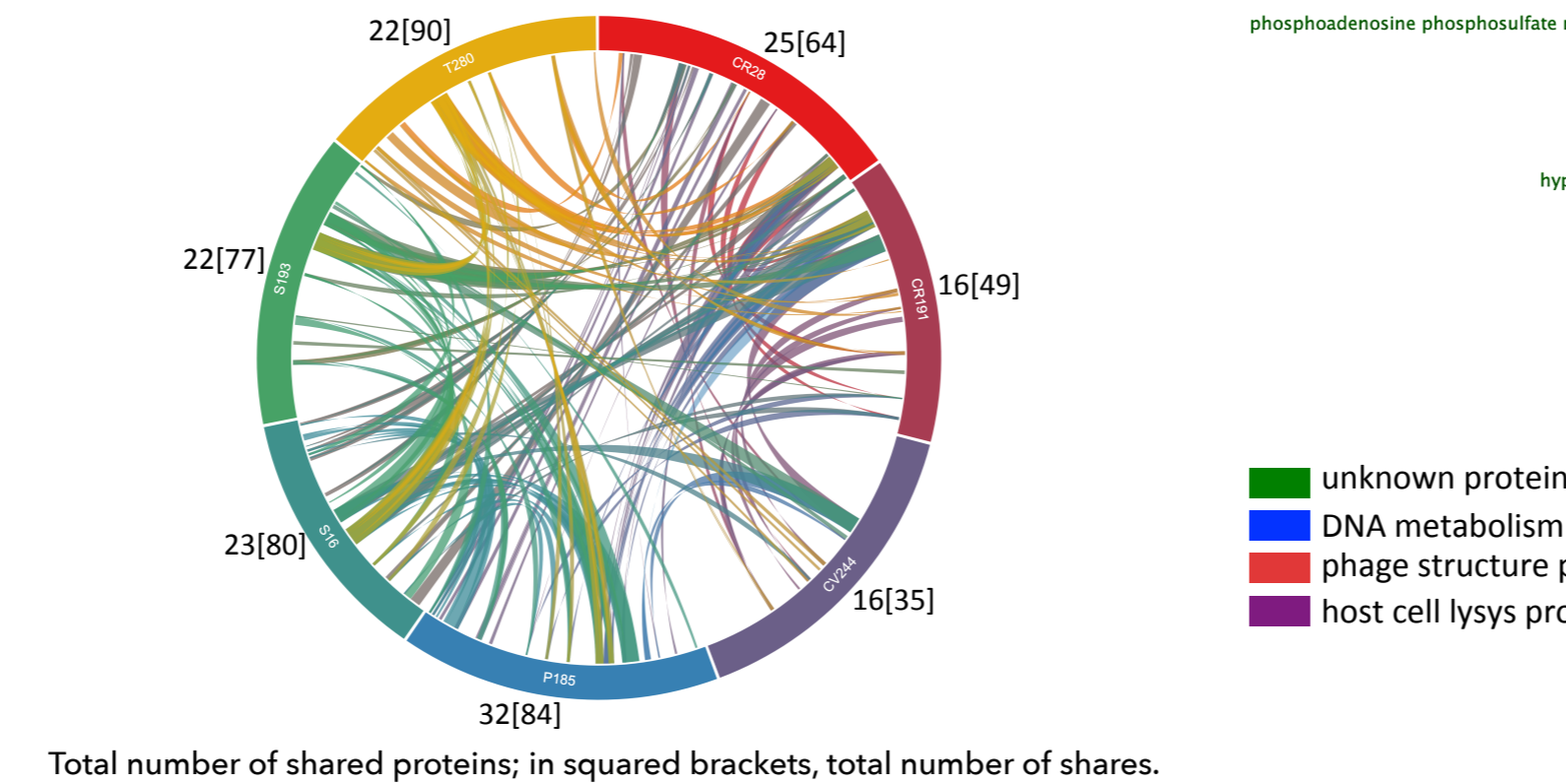
Results

Strain	Dairy implant	Period of isolation	Phage						
			ϕ CR28	ϕ CR191	ϕ CV244	ϕ P185	ϕ S16	ϕ S193	ϕ T280
<i>L. helveticus</i> _CR62	CR	February							
<i>L. helveticus</i> _CR67	CR	February							
<i>L. helveticus</i> _CR72	CR	February							
<i>L. helveticus</i> _CR80	CR	February							
<i>L. helveticus</i> _CR81	CR	February							
<i>L. helveticus</i> _CR93	CR	May							
<i>L. helveticus</i> _CR100	CR	May							
<i>L. helveticus</i> _CR102	CR	May							
<i>L. helveticus</i> _CR104	CR	May							
<i>L. helveticus</i> _CR106	CR	September							
<i>L. helveticus</i> _CR108	CR	September							
<i>L. helveticus</i> _CR119	CR	December	++						
<i>L. helveticus</i> _CR113	CR	December							
<i>L. helveticus</i> _S348	S	December							
<i>L. helveticus</i> _T173	T	September							
<i>L. helveticus</i> _T195	T	September							
<i>L. helveticus</i> _T221	T	September	+	+	+	+	+	+	++
<i>L. helveticus</i> _T260	T	December							
<i>L. helveticus</i> _CR28	CR	September	+++						
<i>L. helveticus</i> _CR191	CR	September	+	+++					
<i>L. helveticus</i> _CV244	CV	September			+++	++			+++
<i>L. helveticus</i> _P185	P	September	+++						+++
<i>L. helveticus</i> _S16	S	February	++					+++	
<i>L. helveticus</i> _S193	S	May	+					+++	
<i>L. helveticus</i> _T280	T	December							+++

Note. Strains susceptibility to phage infection are indicated as degree of "-" to "++++", "++++" indicates a completely clear zone; "++" indicates a center clear zone with a blurred ring; "+" indicates a generally clear zone; "-" indicates no lysis with light grey cells; black cells refers to complete lysis in respective phages host cells.



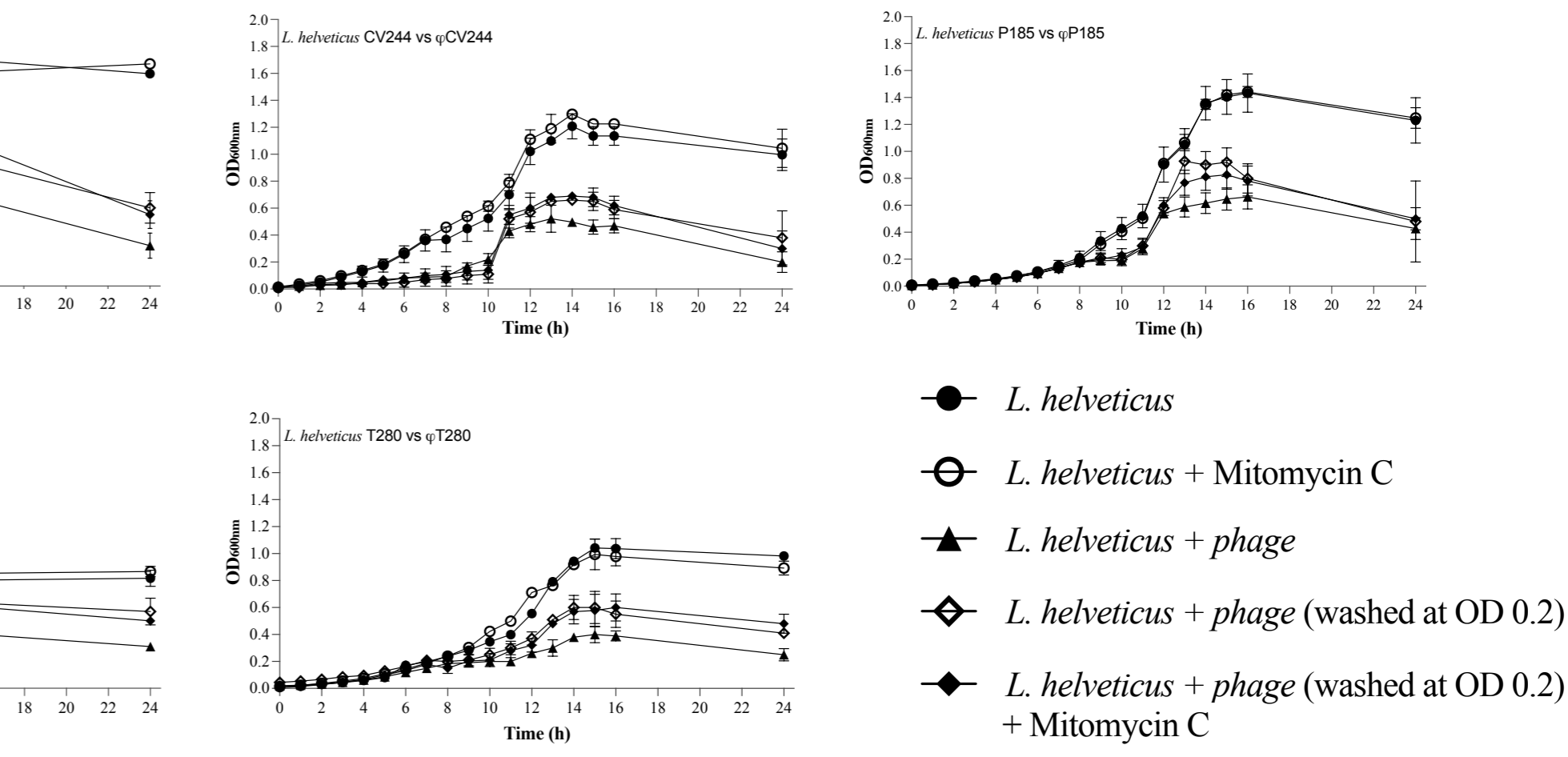
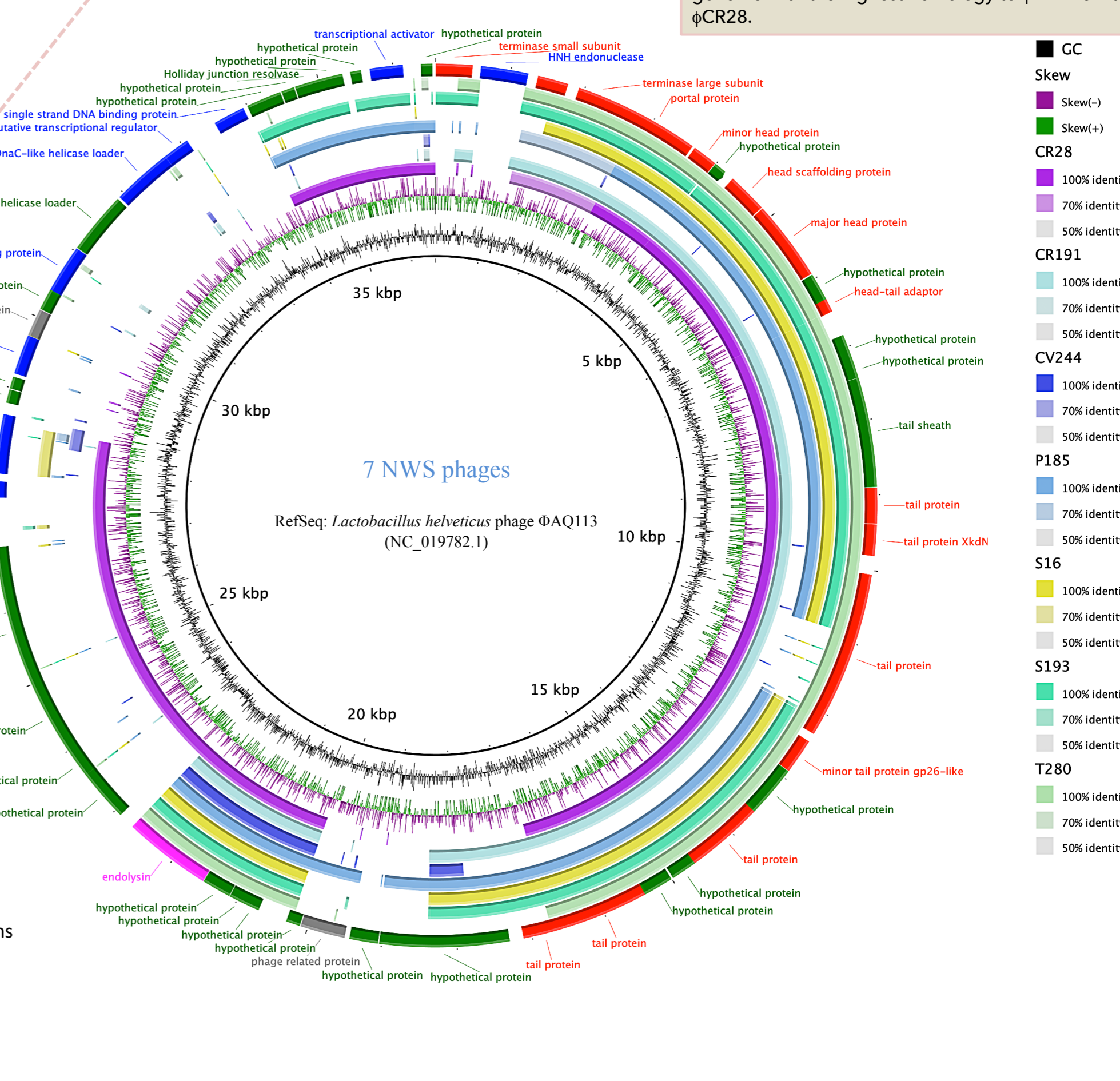
Hypothetical/unknown proteins present singularly or in multiple copies between genomes. In particular, we counted 40 ORFs for ϕ CR28, 34 ORFs for ϕ CR191, 40 ORFs for ϕ CV244, 42 ORFs for ϕ P185, 35 ORFs for ϕ S16, 36 ORFs for ϕ S193 and 39 ORFs for ϕ T280. Half of these hypothetical/unknown proteins (n=44) were conserved and shared between genomes.



Representative growth curves (OD_{600nm}) of the phage induction test on the seven *Lactobacillus helveticus* strains and phages considered in this work. Mitomycin C [1 μ g/mL].

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, with the exception of *L. helveticus* CR191 and S193. By TEM phages have been assigned to the *Caudoviricetes* class, linked to the *myovirus* (ϕ CR28 [A], ϕ CR191 [B], ϕ P185 [C], ϕ S16 [D], ϕ S193 [E], ϕ T180 [F]) and *siphovirus* (ϕ CV244 [G]) phage type.

Based on ViPTree global protein content comparison, phages ϕ CR28, ϕ CR191, ϕ P185, ϕ S16 and ϕ S193 were clustered with phage ϕ AQ113 in the *myovirus* phage-type group of the tree, while ϕ CV244 was grouped with phage ϕ 1g1e (NC_004305.1), as part of the *siphovirus* phage-type group.



Discussion

With a constant income of new viruses carried by the raw milk, NWSs represent an ideal niche of interaction between bacteria and phages that may impact on the technological parameters for cheese production. Here we characterized seven phages isolated from Trentingrana NWSs and able to infect *Lactobacillus helveticus*. Classified as part of the tailed-phages groups of myovirus and siphovirus, they harbor most of the needed genes for a complete lytic life in *Lactobacillus* spp, while the founded lysogeny-associated genes could represent remnants of a temperate origin. Interestingly 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 times.

Conclusion

The presence of these phages, without affecting the starter acidification activity, is opening new interesting insights into the beneficial functions for the host. A deep characterization of phage genomic information would allow a better understanding of their role in NWSs, to maintain the excellence in Grana cheese production, e.g. for Trentingrana

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