

# THE ABILITY OF BACTERIOPHAGES TO ELIMINATE LISTERIA MONOCYTOGENES ON RED-SMEAR SOFT CHEESE

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## BACKGROUND

Many food products are susceptible to contamination with Listeria monocytogenes (“Lm”) which can lead to severe food poisoning and death. Currently available methods do not achieve full eradication, thus there is a need for better methods to prevent such contamination.

## GOAL

To demonstrate that phages can significantly reduce the counts of L monocytogenes, (1) in liquid culture, and (2) on surface-ripened soft cheeses contaminated with Listeria - in both cases and, to the maximum extent possible, without phage-resistant substrains emerging.

## MATERIALS & METHODS

(1) EBI’s proprietary phage strain, denoted “P100”, is a strictly virulent phage with an extremely broad host range, unable to integrate its genome into the host bacterium’s DNA. (2) The strain of Lm used in these experiments was isolated as a predominant, long-term contaminant in a cheese manufacturing plant. (3) Initial pilot experiments were conducted at NIZO food research, using an established model cheese surface ripening process. Experimental modifications included (a) spiking the bare cheese surface with Lm (at concentrations of 1 CFU or 10 CFU per gram of cheese), and (b) addition of phage P100 at various intervals to the salt brine wash, at concentrations of approximately  $5 \times 10^8$  plaque-forming units (“PFU”) per  $\text{cm}^2$ . (4) The results shown in Figures 1 and 2 were obtained by the cheese-making client, in its own laboratory, using its own proprietary “technical” cheese ripening flora, and its own parameters of inoculation, ripening time and temperature, packaging, etc. (5) The limit of detection in the standard enumeration assay was  $< 10$  CFU per gram sample of cheese. If no Lm were found in a given sample, enrichment procedures were used to detect the possible presence of very low concentrations of Lm.

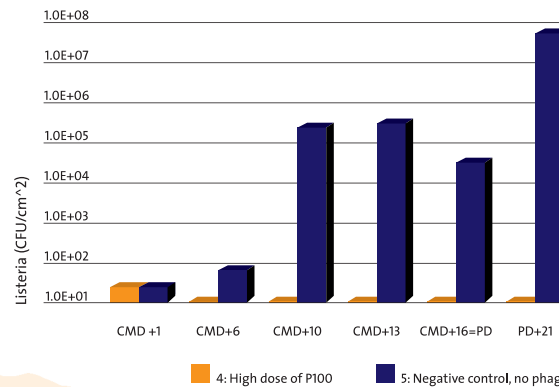


Fig. 1. Applying Phage P100 to surface of surface-ripened cheeses completely prevents outgrowth of Lm, as confirmed by enrichment as well as direct enumeration. (CMD= cheese-making day, PD = packaging day)

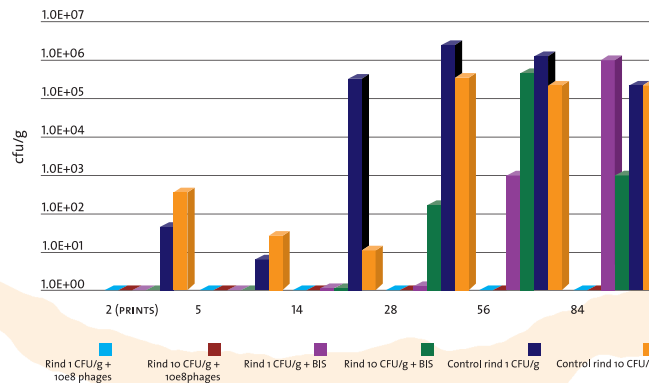


Fig. 2. Comparing phages to a strain of bacteria producing a bacteriocin known to inhibit Lm:  
 1 Lm grows quickly to significant levels on control cheese  
 2 Total eradication with Phage P100 (Limit of detection is  $< 10$  CFU/g . None found in 25g of cheese)  
 3 The bacteriocin appeared at first to eradicate Lm, but after Day 28 the bacteria overcame the inhibition, re-emerged, and eventually reached high counts on the cheese surface.

Conclusion: Phages were far more effective than the bacteria inhibiting strain, in this set of experimental conditions.

## RESULTS

- In liquid cultures with Listeria monocytogenes cells at relevant counts of approximately  $10^4$  CFU/ml, the titer of phage had to be  $5 \times 10^8$  PFU/mL in order to infect and kill all viable cells present, and prevent emergence of phage resistant cells.
- Phage treatment eradicated all Lm beyond the initial spiking done on Day 1, whereas on untreated cheeses, Listeria grew up to high titers of approximately  $10^8$  CFU per gram of cheese (Fig. 1).
- Phage treatment and bacteriocin treatment appeared to be equally effective up until Day 28, after which in the bacteriocin-treated samples (purple and green bars) the bacteria overcame the inhibitory effects and started to regrow to high titers, to levels seen in the controls (Fig. 2).
- Phage P100 did not affect the functioning of the “technical” flora, i.e. the microorganisms involved in cheese ripening. There were no obvious, immediately visible changes to the final product.
- The phage-resistant forms of Listeria which emerged when using low phage concentrations did not result in any phenotypic changes with respect to their susceptibility to several clinically used antibiotics (compared to the background strain).

## CONCLUSIONS

- In the proper ratios and conditions, Phage P100 appears to be effective for eradication and control of Listeria monocytogenes from surface-ripened cheeses. Experiments were confirmed in the factory laboratory of a client company.
- The data strongly suggest that a phage-based approach could be very useful for controlling Listeria monocytogenes in a wide variety of food products. Such approach may also be helpful in decontaminating food processing plants.

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