



Short communication

A note on stability in food matrices of *Salmonella enterica* serovar Enteritidis-controlling bacteriophages

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ABSTRACT

Background: Lytic bacteriophages are bacterial viruses that upon infection kill their host cells and therefore have re-emerged as biological control agents of bacterial pathogens, particularly in the field of food related infections. Here, we investigated the stability in different food matrices of five phage isolates capable of controlling the foodborne pathogen *Salmonella enterica* serovar Enteritidis (SE).

Results: We found that two phages, originally isolated from food sources, were up to 5 logs more stable than three phages isolated from sewage, in ten food matrices (fresh and processed) at both 4°C and 18°C.

Conclusion: Lytic phages isolated from contaminated food sources seem to be a better choice when structuring phage cocktails to be used in the control of SE in food management protocols.

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1. Introduction

The use of bacteriophage, or phage, as controlling agents of spoilage bacteria and bacterial pathogens is increasingly being considered as a valid biocontrol strategy in the food industry [1,2]. However, a basic condition to be met by such strategy is that controlling phage can be stable in food matrices in which they will be employed. In this context, reports on the use of phage to control bacterial pathogens in food products have included data on phage stability therein. For example, Abuladze et al. [3] examined the phage-mediated reduction of *Escherichia coli* O157:H7 contamination of hard surfaces, food matrices of vegetable origin and ground beef. In the course of their study they evaluated the stability of a 3-phage cocktail at storage temperature (10°C) in the different matrices for 168 h, without distinguishing between individual phages. The phage cocktail remained stable in most matrices. Similarly, Guenther et al. [4] studied the control of *Listeria monocytogenes* in several food matrices using two lytic phages individually and determined the stability of one of them (A511) in the

different ready-to-eat foods employed in their investigation, following the A511 phage titer for 6 d at 6°C. In a related vein, Wagenaar et al. [5] determined the maintenance of a *Campylobacter jejuni* phage 71 in the caecal content of broilers while conducting phage therapy experiments. Phage follow-up was for 37 d.

Studies dealing with *Salmonella*-phage stability are also scarce and conducted as an addition to bacterial biocontrol experiments. Such is the case of the report by Guenther et al. [6] who followed the titer of the *Salmonella typhimurium* phage FO1-E2 in various ready-to-eat foods for 6 d, in the presence of the host bacterium at a low count of 1×10^3 cfu/g. In a previous investigation by Leverentz et al. [7] on biocontrol of serovar Enteritidis in fresh-cut fruit, they reported on the persistence of a 4-phage commercial mixture in the foods used as substrates, without distinguishing the behavior of each phage taken individually.

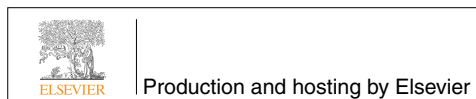
However, it is generally assumed that all phage in a cocktail are equally or similarly stable in the food matrix to which they are applied independent of their origin. In fact, Ryan et al. [8] point out the lack of phage stability studies in papers dealing with bacteriophage therapy. In this study we chose to evaluate the stability in different food matrices of five previously isolated phages in our collection. This is to test the idea that phages originally isolated from food matrices would show greater stability in a variety of foodstuffs of animal origin in contrast to phages coming from a heterologous source, namely sewage.

Our results showed that phage coming from food matrices tend to be more stable in the foodstuffs assayed in this study.

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2. Experimental

2.1. Phages and host

Bacteriophages are listed in the [Results and discussion](#) section. Methods for phage and bacteria propagation were as previously described [9]. High titer lysates were prepared using as host bacterial strain a nalidixic acid (NaI, 100 µg mL⁻¹) and rifampicin (Rif, 100 µg mL⁻¹) resistant mutant, derivative (VAL 222) of *Salmonella enterica* serovar Enteritidis PT4 (SE), provided by Dr. Roy Curtiss III, The Biodesign Institute, Arizona State University. The bacterium was routinely grown in LB liquid or solid (1.5% agar) media at 37°C.

2.2. Food matrices

All foodstuffs were obtained from a commercial source subject to routine inspection by the Chilean Public Health authority. Samples were homogenized in sterile Whirl Pak plastic bags in a stomacher and were examined for the presence of SE by enrichment in Rappaport–Vassiliadis broth followed by plating in XLD agar [10]. Then they were stored frozen at -20°C until used.

2.3. Phage stability determinations

Samples (5 g) of each food matrix were mixed with an equivalent volume of buffer SM [9] and vortexed for 1 min at top speed. Phage was then added as an inoculum of 6×10^8 plaque forming units (pfu) per sample. For each food matrix, three samples were incubated at 4°C and another three at 18°C for 10 d. After incubation, 1 mL of the different food matrix-phage mixes was centrifuged for 5 min at room temperature and 10,000 rpm in a fixed-angle rotor Eppendorf 5415D benchtop centrifuge. Phage in supernatants was titered using the soft-agar (0.7%) overlay technique in LB agar plates [9].

3. Results and discussion

In this study we sought to investigate the stability of five different phages in two groups of food matrices: fresh meat products and processed foods of animal origin. In turn, the phage isolates came from sewage samples (fSE7, fSE8 and fSE12) and from food matrices (fSE1C and fSE4S). The idea we tested was whether phage isolated from food sources would show greater stability in food matrices than those isolated from sewage. This, however, is within the limits of our experimental conditions: the use of frozen food samples and of buffer SM both of which might influence phage stability.

Results shown in [Table 1](#) indicate that phages fSE1C and fSE4S, isolated from pickle sauce and ground beef respectively, consistently showed higher stability in all the food matrices examined both at 4°C and 18°C. In contrast, phages coming from sewage samples are generally about 3 to 5 logs less stable. In fact, only phage fSE12 approximates the stability of fSE1C and fSE4S in most matrices, except for turkey meat, sausage and cheese either at 4°C or 18°C.

Within the context of the use of phage to curtail infections by *Salmonella* in foods, our results partially agree with those of Leverentz et al. [7] who found that a 4-lytic phage mixture was inactivated in apple slices at 5°C, 10°C and 20°C during an incubation period of 168 h. In contrast, the SCPLX-1 phage mix only diminished about 4 logs in honeydew melon slices under the same conditions of temperature and time of incubation. However, no distinctions between individual phages in the cocktail mix were made nor their origin indicated.

In a related study, Guenther et al. [6] measured the stability of phage FO1-E2, a *S. typhimurium* lytic phage, in wieners, turkey breast, mixed seafood, chocolate milk and egg yolk. They found that FO1-E2 remained stable for up to 6 d in all food matrices studied. These authors did not give any indications about the origin of phage FO1-E2 but it certainly

Table 1
Phage stability in food matrices.

Matrices ^a		Stability ^b of phages ^c						
		fSE7	fSE8	fSE12	fSE1C	fSE4S		
Fresh	Beef	18°C	0.772	0.688	0.874	0.960	0.979	
		4°C	0.455	0.683	0.763	0.967	0.977	
	Chicken	18°C	0.598	0.652	0.831	0.979	0.981	
		4°C	0.720	0.496	0.814	0.979	0.974	
	Pork	18°C	0.623	0.603	0.802	0.981	0.981	
		4°C	0.779	0.460	0.885	0.973	0.979	
	Salmon	18°C	0.752	0.541	0.834	0.979	0.974	
		4°C	0.826	0.520	0.720	0.971	0.982	
	Turkey	18°C	0.603	0.625	0.625	0.971	0.974	
		4°C	0.630	0.511	0.851	0.974	0.979	
	Processed	Cheese	18°C	0.455	0.562	0.683	0.977	0.974
			4°C	0.625	0.574	0.891	0.977	0.977
Salame		18°C	0.492	0.455	0.854	0.954	0.968	
		4°C	0.468	0.595	0.883	0.982	0.984	
Sausage		18°C	0.606	0.489	0.871	0.974	0.982	
		4°C	0.683	0.598	0.683	0.977	0.979	
Turkey Ham		18°C	0.786	0.739	0.810	0.979	0.962	
		4°C	0.730	0.726	0.856	0.979	0.977	
Wiener		18°C	0.722	0.455	0.806	0.973	0.960	
		4°C	0.589	0.548	0.786	0.977	0.981	

^a None showed presence of SE.

^b Stability expressed as log phage count at day 10/initial phage inoculum.

^c All phages listed formed clear, lytic plaques on SE, correspond to *Caudoviridae* and contain double stranded DNA. Phages fSE7, fSE8 and fSE12 were isolated from sewage, fSE1 from pickle sauce and fSE4 from ground beef.

behaves similar to our fSE1C and fSE4S isolates which remain stable for at least 10 d at the temperatures and food matrices we tested.

Overall, the stability of *Salmonella* phages in food matrices depends on the nature of the matrix being used as substrate. For example, it has been shown that phage M13 replication is inhibited by sterilized milk proteins [11]. However, our results indicate that phages fSE1C and fSE4S are not significantly affected by potential inhibitors in the matrices examined in contrast to the other three phages studied. To us this indicates that it would be advisable to isolate controlling phages from sources or using conditions akin to the substrate in which they will be employed, in what could be called “habitat-oriented phage isolation”.

Conflict of interest

The authors declare there is no conflict of interest.

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Author contributions

Proposed the theoretical frame: JR, CB; Conceived and designed the experiments: JR, CB, GT; Contributed reagents/materials/analysis tools: JR, CB; Wrote the paper: JR; Performed the experiments: GT, KH; Analyzed the data: JR, CB, GT.

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