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Bacteriophage Significantly Reduces *Listeria monocytogenes* on Raw Salmon Fillet Tissue†

KAMLESH A. SONI AND RAMAKRISHNA NANNAPANENI*

Department of Food Science, Nutrition and Health Promotion, P.O. Box 9805, Mississippi State University, Mississippi State, Mississippi 39762, USA

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ABSTRACT

We have demonstrated the antilisterial activity of generally recognized as safe (GRAS) bacteriophage LISTEX P100 (phage P100) on the surface of raw salmon fillet tissue against *Listeria monocytogenes* serotypes 1/2a and 4b. In a broth model system, phage P100 completely inhibited *L. monocytogenes* growth at 4°C for 12 days, at 10°C for 8 days, and at 30°C for 4 days, at all three phage concentrations of 10^4, 10^6, and 10^8 PFU/ml. On raw salmon fillet tissue, a higher phage concentration of 10^8 PFU/g was required to yield 1.8-, 2.5-, and 3.5-log CFU/g reductions of *L. monocytogenes* from its initial loads of 2, 3, and 4.5 log CFU/g at 4 or 22°C. Over the 10 days of storage at 4°C, *L. monocytogenes* growth was inhibited by phage P100 on the raw salmon fillet tissue to as low as 0.3 log CFU/g versus normal growth of 2.6 log CFU/g in the absence of phage. Phage P100 remained stable on the raw salmon fillet tissue over a 10-day storage period, with only a marginal loss of 0.6 log PFU/g from an initial phage treatment of 8 log PFU/g. These findings illustrate that the GRAS bacteriophage LISTEX P100 is listericidal on raw salmon fillets and is useful in quantitatively reducing *L. monocytogenes*.

Listeria monocytogenes continues to be a problem in raw salmon and cold-smoked salmon products *(13, 14, 20, 25)*. The control or elimination of *L. monocytogenes* contamination in raw salmon is a first critical step for enhancing the safety of finished cold-smoked salmon that has not undergone adequate listericidal steps *(15)*. Several authors have reported the prevalence of *L. monocytogenes* in raw fish including salmon in the range of 0.1 to 25% *(9, 11, 19, 26, 38)*. The current intervention strategies for *L. monocytogenes* control on raw salmon include chemical treatments such as chlorine, chlorine dioxide, acidified sodium chlorite, ozone, and electrolyzed oxidizing water. Of these, dipping fish fillets in chlorine water is a widely used industrial practice *(7, 32)*. Eklund et al. *(12)* observed that the practice of dipping fish in a chlorine solution increases the chances for cross-contamination, as the chlorine solution quickly becomes ineffective in the absence of active management of its concentration. Antimicrobial treatment of salmon fillets with 50 ppm of acidified sodium chlorite or electrolyzed water produced only marginal 0.2- to 1.0-log reductions in *L. monocytogenes* populations, whereas 200 ppm of chlorine dioxide or 1.5 ppm of ozone decreased the quality attributes of the fillets *(10, 22, 27, 33)*. In addition to the chemical interventions, physical treatment such as steam treatment and UV exposure were also evaluated for *L. monocytogenes* control on raw salmon fillets. Bremer et al. *(6)* reported a ~4-log surface decontamination of *L. monocytogenes* from salmon skin surfaces by using an 8-s steam pasteurization treatment, without altering the quality of cold-smoked salmon. When *L. monocytogenes*–inoculated salmon skin and fillet samples were exposed to UV light for up to 60 s, a 0.7- to 1-log CFU/g reduction in *L. monocytogenes* counts were attained. However, this UV exposure increased the temperatures of fillets to 100°C, which resulted in visual color changes and decreased quality *(28)*.

One promising approach for *L. monocytogenes* control is the use of bacteriophages as an antilisterial agent *(15, 17, 20, 29)*. Bacteriophages (phages) infect bacterial cells; phages are specific for a target genus, serotype, or a strain. All phages are obligate parasites, and each relies on a specific host for propagation. In the absence of a host bacterium, a phage exists in a metabolically inert state. Phages are ubiquitous in nature, and as many as 10^8 phage particles can be isolated from 1 g of soil or water *(29)*. Phages are also isolated from several food products such as meat, dairy, vegetables, etc. *(2, 5, 18, 21, 39)*. The two broad categories of ''virulent'' or ''temperate'' phages differ in their respective modes of action. After entry into the host, virulent phages rapidly multiply inside the target bacterial cell without integrating with the host DNA, whereas temperate phages integrate into the host DNA and replicate along with it. For biocontrol strategies, phages with the ability to lyse bacterial cells rapidly without integration into the host bacterial DNA are recommended *(15, 20, 30, 31)*.

Recently, the U.S. Food and Drug Administration approved two bacteriophage preparations (LISTEX P100 and LMP-102) for use in certain foods to combat *L. monocytogenes* contamination *(34–36)*. Of these two phages, LISTEX P100 is approved for use for all raw and ready-to-eat foods, at levels not to exceed 10^9 PFU/g. As there is no effective method for the control of *L. monocytogenes* on raw salmon fillets, we evaluated the efficacy of phage LISTEX P100 on raw salmon fillet tissues for *L. monocytogenes*

* Author for correspondence. Tel: 662-325-7697; Fax: 662-325-8728; E-mail: nannapaneni@fsnhp.msstate.edu.

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biocontrol as a function of (i) phage dose, (ii) phage contact time, and (iii) storage temperature and storage time.

MATERIALS AND METHODS

***L. monocytogenes* strains.** Two *L. monocytogenes* serotypes, 1/2a (EGD strain) and 4b (Scott A strain), were used in this study. These strains were maintained in tryptic soy agar (TSA) slants and cultured at 37°C for 24 h in 10 ml of tryptic soy broth (TSB) to obtain cell concentrations of 10^9 CFU/ml (equivalent to an optical density at 600 nm [OD_{600}] of ~1.2). To prepare inoculum, broth cell suspensions were washed twice by obtaining the cell pellets via centrifugation at 10,000 × *g* for 10 min, and resuspension in physiological saline (0.9% NaCl). The serial dilutions of the *L. monocytogenes* cell suspensions were then prepared in saline to obtain the desired cell concentrations. The two-strain mixture of *L. monocytogenes* EGD and Scott A was prepared by mixing equal volumes of the washed cell suspension containing 10^9 CFU/ml, and then performing serial dilutions in saline.

Bacteriophage source and plaque-forming assay. The U.S. Food and Drug Administration– and the U.S. Department of Agriculture, Food Safety and Inspection Service–approved bacteriophage preparation LISTEX P100 (phage P100) *(34, 36)* was obtained from EBI Food Safety, Inc. (Wageningen, The Netherlands). Phage P100 is reported active against multiple serovars of *L. monocytogenes (8)*. Phage P100 stock solution in buffered saline had an approximate concentration of 10^{11} PFU/ml. This concentration was determined with the following assay: The bacteriophage suspension was serially diluted in sterile buffer (100 mM NaCl, 10 mM $MgSO_4$, and 50 mM Tris-HCl [pH 7.5]), and 100 μl of each phage suspension was mixed with a 150 μl of *L. monocytogenes* EGD or Scott A (OD_{600} ~ 1.2) cells in 4 ml of sterile soft agar (TSB containing 0.4% agar) at 42°C. The soft agar mixture was gently vortexed prior to pouring onto a TSA plate, and then distributed evenly by gentle rotation of the agar plate. The soft agar was allowed to solidify for 30 min at room temperature, and these plates were subsequently incubated in an inverted position for 18 to 24 h at 30°C. After the incubation period, the number of visible plaques was counted, and the resulting number multiplied by a dilution factor to obtain the counts, expressed as PFU per milliliter.

Effect of phage P100 on *L. monocytogenes* growth at different temperatures in broth. The effect of phage P100 on the inhibition of growth of *L. monocytogenes* EGD and Scott A was determined via a 24-well plate assay by measuring the optical density at 630 nm, and by viability testing. *L. monocytogenes* cell suspensions were serially diluted to achieve a concentration of 10^4 CFU/ml, and then they were distributed at 1.8 ml per well into the 24-well plates. These plates were incubated at 4 or 10°C for 1 h, or at 30°C for 30 min, to achieve temperature equilibration before being challenged with phage P100. Phage P100 was added at 0.2 ml per well to suspensions of 10^5, 10^7, and 10^9 PFU/ml in TSB to yield final phage concentrations of 10^4, 10^6, and 10^8 PFU/ml, respectively. The untreated control consisted of 0.2 ml per well of TSB instead of phage P100. The media-only controls were also placed in each 24-well plate. Four replicate wells were maintained for each treatment. The plates were immediately placed at 4, 10, and 30°C, and OD_{630} was recorded with a 24-well plate reader (ELx800NB, BioTek Instruments, Winooski, VT) at specific intervals. To minimize the temperature fluctuation, each plate was removed from the incubator for a maximum of 20 to 30 s for the optical density readings. At the end of the incubation period at different temperatures (2 days at 30°C, 8 days at 10°C, and 12 days at 4°C), 250 μl per well of each treatment was spread plated on polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol (PALCAM) agar to recover surviving *L. monocytogenes* cells that could be present.

Salmon fillet tissue samples. Fresh, whole raw salmon fillets were purchased from a local retail grocery store and kept at 4°C for use within 24 h. For each experiment, raw salmon fillet tissue samples (10 g) of approximately 2-cm^2 blocks were prepared by cutting a large fillet by using a sterile knife on a sterile cutting board. For the purpose of inoculation, packing, and storage, two such tissue samples per treatment were placed in a sterile, polystyrene dish measuring 10.2 cm in diameter (hexagonal polystyrene weighing dishes, Fisher Scientific Co., Pittsburgh, PA), with the flesh side facing up. Each such polystyrene dish containing samples was sealed in a Ziploc bag (16.5 by 14.9 cm).

Effect of different phage P100 concentrations on *L. monocytogenes* reduction on raw salmon fillet tissue. Each raw salmon fillet tissue sample was inoculated with 50 μl of a serially diluted, two-strain (serotype 1/2a and 4b) mixture of *L. monocytogenes* suspension by depositing 5- to 10-μl drops to the flesh side, to yield an inoculation level of approximately 4 log CFU/g. The samples were allowed to air dry for 15 min in a Biosafety Level 2 laminar flow hood for the attachment of *L. monocytogenes* cells. Each sample was then surface treated with serially diluted phage P100 on the flesh side by adding 100-μl suspensions of 10^{10}, 10^9, 10^8, 10^7, and 10^6 PFU/ml in physiological saline to yield final doses 10^8, 10^7, 10^6, 10^5 and 10^4 PFU/g, respectively. For the untreated control, each sample received 100 μl of saline solution in lieu of phage P100. The duplicate samples per treatment were placed in a polystyrene dish, sealed immediately in a Ziploc bag for incubation at 4°C for 2 h, and then enumerated for *L. monocytogenes*.

Effect of phage P100 against low and high *L. monocytogenes* inoculum levels on raw salmon fillet tissue. The serial dilution of the *L. monocytogenes* cell suspension that contained both serotypes 1/2a and 4b was spot inoculated at 50 μl to yield 2, 3, or 4 log CFU/g on the flesh side of the 10-g raw salmon tissue sample, and then allowed to air dry as described above. These tissue samples were then surface treated with phage P100 by adding 100 μl of phage suspension to the flesh side, for a phage application dose of 10^8 PFU/g per 10 g of tissue sample. Each treatment, which consisted of duplicate tissue samples in a polystyrene dish, was immediately packed in a Ziploc bag for incubation at 4°C or room temperature (22°C), and then enumerated for *L. monocytogenes* CFU after 30 min and 2 h.

Effect of phage P100 on *L. monocytogenes* growth during the shelf life of raw salmon fillet tissue. After the inoculation of 10-g samples of raw salmon fillet tissue with approximately 2 log CFU/g of *L. monocytogenes* serotype mixture (1/2a and 4b) to the flesh side, the samples were air dried as described above. For the phage P100 treatment, a 100-μl phage suspension was added to the flesh side to give a phage dose of 10^8 PFU/g per 10 g of tissue sample. After the phage treatment, the polystyrene dish containing duplicate tissue samples was immediately packed in a Ziploc bag for storage at 4°C, and then assayed for *L. monocytogenes* levels at 0, 1, 4, 7, and 10 days, at 4°C.

***L. monocytogenes* enumeration.** Each salmon fillet tissue sample was aseptically placed in a stomacher bag containing 25 ml of peptone water (0.1% peptone and 0.02% Tween 80), and then homogenized for 2 min in a stomacher (model 400C, Seward

Medical, London, UK) at 230 rpm. Ten milliliters of the homogenate was then concentrated by centrifugation at 12,000 $\times$ *g* for 5 min. This centrifugation step also significantly removed the phage P100 from the stomached rinses prior to direct plating for *L. monocytogenes* enumeration. After centrifugation, the top supernatant containing the phage P100 was removed, and the pellet containing *L. monocytogenes* cells was resuspended in 1 ml of peptone water. Subsamples of 100 or 250 μl (to yield a countable plate) from the resuspended pellet were then spread plated on PALCAM agar that contained the following *Listeria*-selective antibiotics: polymyxin B sulfate (10 mg/liter), acriflavin (5 mg/liter), and ceftazidime (6 mg/liter). When required, a serial dilution step was performed after resuspending the pellet to yield a countable plate for *L. monocytogenes*. While enumerating the low levels of *L. monocytogenes,* the entire pellet in 1 ml was spread plated by using 250 μl per plate on four PALCAM plates.

Determination of phage P100 stability on salmon fillet tissue. The stability of phage P100 on salmon fillet tissue was determined at 4°C during the 10-day storage period. Each 10-g fillet tissue sample was surface treated with phage P100 by adding 100 μl of phage suspension to the flesh side to yield a final application of 10^8 PFU/g. After phage treatment, polystyrene dishes containing the duplicates tissue samples were packed immediately in a Ziploc bag for storage at 4°C for up to 10 days. Phage P100 was enumerated at 0, 1, 4, 7, and 10 days by using a plaque-forming assay. After each incubation period, the fillet sample was homogenized in 25 ml of peptone water by stomaching (as described above), and 1 ml of homogenate was filter sterilized with a 0.22-μm filter syringe. The filtrate was tested for PFU counts, as described earlier.

Statistical analysis. All experiments were repeated three times. *L. monocytogenes* counts were converted into log CFU per gram, and then analyzed with the SPPS statistical analyses software package (version 12.0, SPPS, Inc., Chicago, IL). Analysis of variance (ANOVA) was used for determining mean significant differences between controls and within phage treatments.

RESULTS

Phage P100 inhibits *L. monocytogenes* growth at different temperatures in broth. Figure 1 shows the inhibition in growth of *L. monocytogenes* in TSB at 4, 10, and 30°C in the presence of 10^4, 10^6, and 10^8 PFU/ml of phage P100. As expected, the *L. monocytogenes* growth was temperature dependent ($P < 0.05$). At 4°C, the OD_{600} measurements for both *L. monocytogenes* EGD and Scott A strains remained below the threshold levels up to 8 days, and growth was only detected at 12 days. At 10°C, *L. monocytogenes* growth was observed after approximately 2 days, while at 30°C, growth occurred immediately after incubation. All phage concentrations of 10^4, 10^6, and 10^8 PFU/ml were equally effective at all three temperatures in inhibiting *L. monocytogenes* growth. In the presence of phage P100, OD_{630} measurements remained at initial threshold levels at all three temperatures throughout the test periods. No surviving *L. monocytogenes* cells (minimum detection limit of 10 CFU/ml) were detected at all three temperatures in phage-treated cell suspensions, which was determined by spread plating samples on PALCAM plates at the end of each incubation period. In Figure 1, the threshold levels of OD_{630} $\sim$ 0.05 may be due to cell debris.

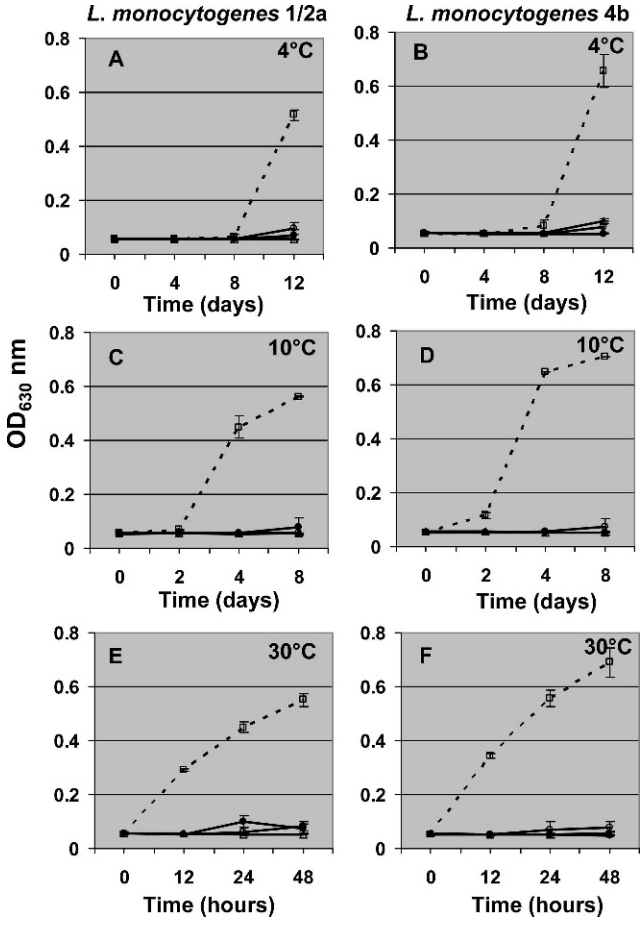

FIGURE 1. *The inhibition of the growth of* Listeria monocytogenes *serotypes 1/2a (A, C, E) and 4b (B, D, F) in the presence of different phage P100 concentrations at three temperatures.* □, *No phage;* △, *phage P100 at* 10^4 *PFU/ml;* ○, *phage P100 at* 10^6 *PFU/ml;* ●, *phage P100 at* 10^8 *PFU/ml.*

Reduction of *L. monocytogenes* counts on raw salmon tissue as a function of phage P100 density. Figure 2 shows the effect of varying phage concentrations (10^4 to 10^8 PFU/g) on the reduction of *L. monocytogenes* counts from raw salmon fillet tissue samples that were inoculated with 4 log CFU/g of *L. monocytogenes*. The reductions in *L. monocytogenes* counts were proportional to the phage density, i.e., with an increase in phage concentration, there was a greater decrease in *L. monocytogenes* counts. There was no significant ($P > 0.05$) reduction in *L. monocytogenes* counts on raw salmon fillet tissue when treated with a lower dose of phage P100 at 10^4 PFU/g, compared with the untreated control. Phage P100 doses of 10^5 and 10^6 PFU/g, though statistically significant ($P < 0.05$), resulted in marginal reductions of 0.5 and 1.2 log CFU/g of *L. monocytogenes,* respectively. The phage treatment of 10^7 PFU/g resulted in 2-log CFU/g reductions in *L. monocytogenes* counts, while the higher phage treatment of 10^8 PFU/g was the most effective and yielded a ~3.5-log CFU/g reduction in *L. monocytogenes* counts within 2 h. With the exception of the 10^4 PFU/g treatment, all other phage treatments had reduced ($P < 0.05$) *L. monocytogenes* counts when compared with the untreated control. In addition, there was a significantly ($P < 0.05$)

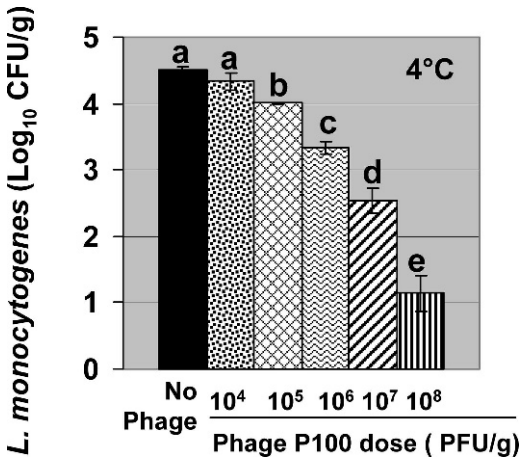

FIGURE 2. *Reductions of* Listeria monocytogenes *within 2 h at 4°C on raw salmon fillet tissue with different concentrations of phage P100. Bars with different letters represent significant* ($P \leq 0.05$) *differences based on the least-squares difference, one-way ANOVA test.*

higher reduction in *L. monocytogenes* counts with an increase in phage density.

Reduction in *L. monocytogenes* counts as a function of inoculum load and temperature. Figure 3 shows the reduction in *L. monocytogenes* counts by the phage P100 treatment within 30 min or 2 h, at 4°C or room temperature (22°C). The *L. monocytogenes* serotype mixture of 1/2a and 4b was surface inoculated at 2-, 3-, and 4-log CFU/g levels on the raw salmon fillet tissue, and then surface treated with phage P100 at 10^8 PFU/g. Over all, there was a significant ($P < 0.05$) reduction in *L. monocytogenes* counts at both temperatures (4 or 22°C), due to the phage P100 treatment. In addition, similar levels of reduction in *L. monocytogenes* counts were observed between the 30-min and 2-h phage treatments at both temperatures. The 4-log CFU/g inoculum levels of *L. monocytogenes* decreased to approximately 1 log CFU/g (a 3-log reduction when treated with phage P100 treatment at both temperatures). At the 3-log CFU/g inoculum level, the decrease in *L. monocytogenes* counts were 2.5 to 2.9 log, and at the 2-log CFU/g inoculum level, the decrease in *L. monocytogenes* counts was approximately 1.9 log at both temperatures.

Reduction of *L. monocytogenes* growth by phage P100 during the refrigerated shelf life of raw salmon fillets. Figure 4 shows the effect of phage P100 on *L. monocytogenes* during the 10-day storage period of raw salmon fillets at 4°C. During this storage period, the *L. monocytogenes* population in the untreated control grew by 1 log (from the initial load of 1.6 to 2.6 log CFU/g by day 10). Considering the initial load of *L. monocytogenes* at day 0, the phage P100 treatment resulted in an approximately 1.4-log reduction in *L. monocytogenes* count at day 1. During the subsequent storage period, *L. monocytogenes* counts in phage-treated samples remained at or below 0.3 log CFU/g, with no further decrease or increase. At the end of 10 days, the reductions in *L. monocytogenes* counts in phage treatment were about 2.3 log lower, compared with

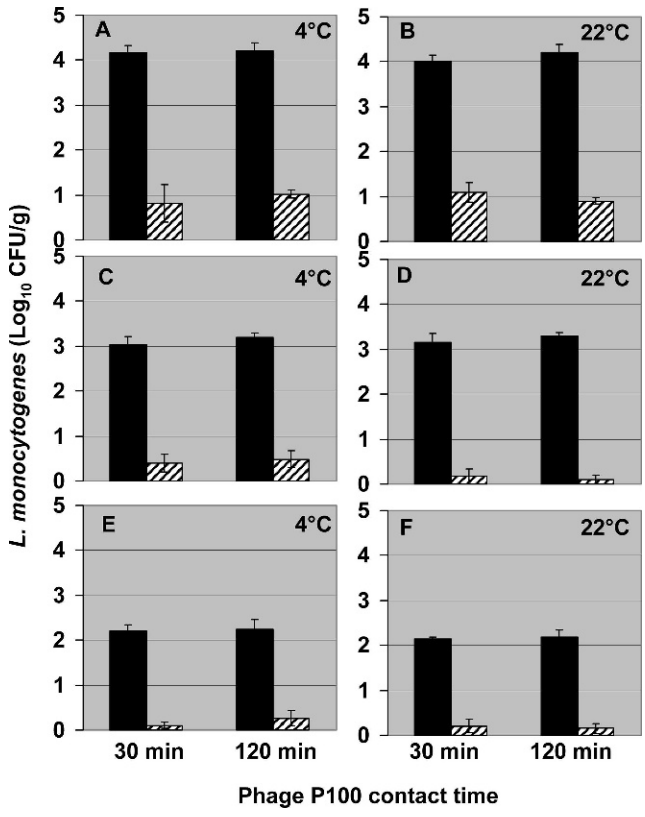

FIGURE 3. *Reductions of* Listeria monocytogenes *on raw salmon fillet tissue within 30 min or 2 h at 4°C or room temperature (22°C) by phage P100. An* L. monocytogenes *serotype 1/2a (EGD) and 4b (Scott A) mixture was inoculated at roughly 4 log CFU/g (A and B), 3 log CFU/g (C and D), and 2 log CFU/g (E and F) and treated with a phage concentration of 10^8 PFU/g. ■, No phage; ▨, phage P100 treatments.*

the no-phage control. Throughout the test period, *L. monocytogenes* counts were statistically ($P < 0.05$) lower in phage-treated samples at 1, 4, 7, and 10 days, compared with the no-phage control.

Stability of phage P100 on salmon fillet tissue stored at 4°C. Figure 5 shows the stability of phage P100 at 4 and 10°C during the 10-day storage period of raw salmon fillets.

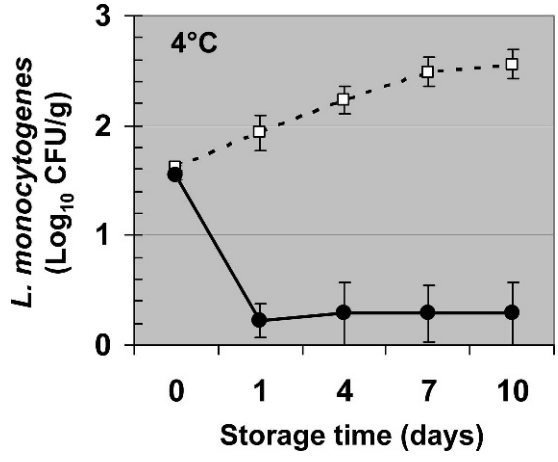

FIGURE 4. *Reduction of* Listeria monocytogenes *growth during the 10-day shelf life of raw salmon fillets at 4°C by phage P100. □, No phage; ●, phage P100 at 10^8 PFU/g.*

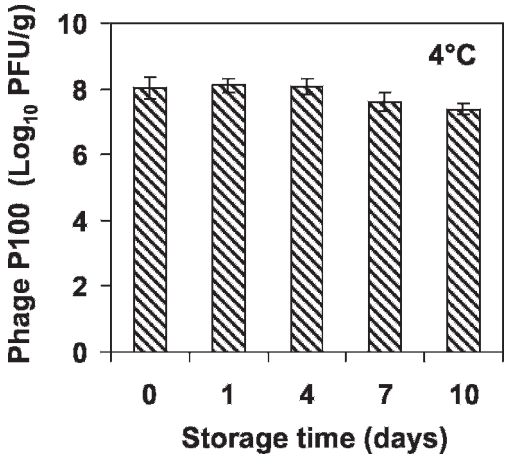

FIGURE 5. *Stability of phage P100 during the 10-day shelf life of raw salmon fillet tissue at 4°C.*

The phage P100 titer was relatively stable on the raw salmon fillet tissue during storage. Of the initial 8 log PFU/g, there was only a marginal decrease in the phage P100 titer (by 0.6 log PFU/g) at the end of the 10-day storage period on raw salmon fillet tissue samples that were stored at 4°C.

DISCUSSION

In this study, we have demonstrated that bacteriophage P100 was able to reduce *L. monocytogenes* counts in a model broth system and on raw salmon fillet tissue, as a function of phage dose, *L. monocytogenes* inoculum level, and phage contact time and storage temperature. In broth medium, phage P100 was effective at all tested phage concentrations of 10^4, 10^6, and 10^8 PFU/ml. On salmon fillets, phage efficacy was highly dependent on the phage concentration, i.e., at a higher phage concentration, there was a proportionately higher reduction of *L. monocytogenes*. At a higher phage concentration of 10^8 PFU/g, *L. monocytogenes* counts on fillet tissue were decreased by three orders of magnitude. Guenther et al. *(16)* reported similar results, and noticed that there were higher magnitudes of *L. monocytogenes* reductions in broth conditions when compared with solid matrices of ready-to-eat food products, possibly due to better diffusion of phage particles in liquid. In addition, in other phage-challenge studies with *Salmonella* and *Campylobacter,* phage densities in the range of 10^6 to 10^8 PFU/g (or cm^2) were required for appreciable reductions in host bacterial counts *(1, 4, 23, 24)*.

From a food safety application perspective, the ability of the selected phage to target host cells under refrigerated storage is important *(20)*. The activity of a selected phage at a lower temperature depends on its successful adsorption to the host bacterium's surfaces and active function *(20, 37)*. *L. monocytogenes* cells are able to grow under refrigerated temperature conditions, and are metabolically active at such low temperatures. By using a model broth system, we observed that phage P100 effectively inhibited *L. monocytogenes* growth at 4, 10, and 30°C (Fig. 1). Moreover, no surviving *L. monocytogenes* cells were found in phage-treated broth samples at the end of these experiments. Similar to broth assays, phage P100 was also found equally effective on fillet surfaces at both 4°C and room temperature (22°C). This is of practical importance because this phage technology can be successfully used in fillet processing: fillets could be stored immediately under refrigeration conditions after listericidal treatment.

While raw salmon may be frequently contaminated with *L. monocytogenes,* levels of contamination are usually low and limited to 0.1 to 10 CFU/g at the initial stage of contamination, with only sporadic possibilities of this pathogen being isolated in high numbers *(14)*. To yield a measurable count of *L. monocytogenes,* the efficacy of antimicrobial compounds are normally tested at higher inoculum levels (~4 log CFU/g). On salmon fillet tissue, we tested the effect of phage P100 at *L. monocytogenes* levels as low as 2 log CFU/g or as high as 4 log CFU/g. Using a centrifugation step, 10 ml of stomached subsample rinses was concentrated to accurately recover the low numbers of surviving *L. monocytogenes* cells after phage treatment. This was also confirmed by the extraction of inoculated samples with known concentrations of *L. monocytogenes* by this assay method. This centrifugation step was also useful in significantly reducing phage P100 numbers from the stomached rinses prior to direct plating for *L. monocytogenes* enumeration. This is evident in Figure 5; the phage application dose of 8 log PFU/g was almost entirely recovered in centrifuged supernatant. Our experiments with different *L. monocytogenes* inoculum loads revealed strong listericidal activity by phage P100 treatment. At inoculum levels of 2, 3, and 4 log CFU/g, the decreases in *L. monocytogenes* populations by phage treatments were approximately 1.8, 2.5, and 3 log CFU/g, respectively. In terms of percentages, the levels of *L. monocytogenes* reduction at different inoculum levels were approximately 99.9%. At a phage concentration of 10^8 PFU/g, the proportion of number of phage particles available for each host bacterium were 10^6:1, 10^5:1, and 10^4:1 (phage P100:*L. monocytogenes*) for 2-, 3-, and 4-log CFU/g levels of *L. monocytogenes* inoculum, respectively. Although the phage particles available for each host bacterium were higher in tissue samples containing less *L. monocytogenes* inoculum, this did not result in the complete elimination of target *L. monocytogenes* cells. As explained by Hagens and Offerhaus *(17),* phage particles at high concentrations may not be near all target host bacterial cells. There were measurable reductions in *L. monocytogenes* counts at all temperatures within the first 30 min of phage contact time. We did not observe any meaningful differences in *L. monocytogenes* reductions between 30-min and 2-h contacts with phage P100. Recently, Bigwood et al. *(3)* used a predictive modeling that suggested that phage particles could decrease the host population significantly (~2 log) within 1 h of phage exposure.

The usefulness of a phage in preventing the proliferation of a host bacterium during longer product storage time depends on the stability of phage particles in any particular food matrices and its surface water content for phage mobilization *(16, 17)*. Guenther et al. *(16)* tested the viability of *L. monocytogenes*–specific phage A511 on different food matrices, and noticed that phage particles were more stable on ready-to-eat meat products (approxi-

mately 0.6-log reductions) compared with cabbage and lettuce (approximately 2-log reductions) during a 6-day storage period. In our study, phage P100 was found stable on salmon fillet tissue surfaces, where phage counts showed a marginal decrease of only 0.6 log after 10 days of storage (Fig. 5). Consequently, on salmon fillets challenged with 2 log CFU/g of *L. monocytogenes* and treated with phage P100, the *L. monocytogenes* population decreased and remained at approximately 0.2 to 0.3 log CFU/g during the 10-day storage period.

In conclusion, this study demonstrates the strong listericidal activity of the generally recognized as safe bacteriophage preparation LISTEX P100 on raw salmon fillet tissue. Since phage preparation is applied in a saline solution, there may not be the quality or appearance defects that are frequently noticeable with the use of other antimicrobial treatments such as chlorine dioxide, ozone, or irradiation *(10, 22, 28)*. However, since phage P100 is highly specific to only *Listeria* spp., there are obstacles to overcome; additional testing and measures are necessary for the suppression and eradication of other harmful pathogens and spoilage microflora. In addition, experiments with whole fillets (to mimic the commercial fillet processing operation) are needed to test the efficacy of phage P100 against the wide range of *L. monocytogenes* isolates that frequently originate in salmon fillet processing facilities.

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REFERENCES

1. Atterbury, R. J., P. L. Connerton, C. E. Dodd, C. E. Rees, and I. F. Connerton. 2003. Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 69:6302–6306.
2. Atterbury, R. J., P. L. Connerton, C. E. Dodd, C. E. Rees, and I. F. Connerton. 2003. Isolation and characterization of *Campylobacter* bacteriophages from retail poultry. *Appl. Environ. Microbiol.* 69: 4511–4518.
3. Bigwood, T., J. A. Hudson, and C. Billington. 2009. Influence of host and bacteriophage concentrations on the inactivation of food-borne pathogenic bacteria by two phages. *FEMS Microb. Lett.* 291:59–64.
4. Bigwood, T., J. A. Hudson, C. Billington, G. V. Carey-Smith, and J. A. Heinemann. 2008. Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiol.* 25:400–406.
5. Binetti, A. G., and J. A. Reinheimer. 2000. Thermal and chemical inactivation of indigenous *Streptococcus thermophilus* bacteriophages isolated from Argentinean dairy plants. *J. Food Prot.* 63:509–515.
6. Bremer, P. J., I. Monk, C. M. Osborne, S. Hills, and R. Butler. 2002. Development of a steam treatment to eliminate *Listeria monocytogenes* from king salmon (*Oncorhynchus tshawytscha*). *J. Food Sci.* 67:2282–2287.
7. Bremer, P. J., and C. M. Osborne. 1998. Reducing total aerobic counts and *Listeria monocytogenes* on the surface of king salmon (*Oncorhynchus tshawytscha*). *J. Food Prot.* 61:849–854.
8. Carlton, R. M., W. H. Noordman, B. Biswas, E. D. de Meester, and M. J. Loessner. 2005. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regul. Toxicol. Pharmacol.* 43: 301–312.
9. Chou, C. H., J. L. Silva, and C. Wang. 2006. Prevalence and typing of *Listeria monocytogenes* in raw catfish fillets. *J. Food Prot.* 69:815–819.
10. Crapo, C., B. Himelbloom, S. Vitt, and L. Pedersen. 2004. Ozone efficacy as a bactericide in seafood processing. *J. Aquat. Food Prod. Technol.* 13:111–123.
11. Duffes, F. 1999. Improving the control of *Listeria monocytogenes* in cold smoked salmon. *Trends Food Sci. Technol.* 10:211–216.
12. Eklund, M. W., M. E. Peterson, F. T. Poysky, R. N. Paranjpye, and G. A. Pelroy. 2004. Control of bacterial pathogens during processing of cold-smoked and dried salmon strips. *J. Food Prot.* 67:347–351.
13. Gombas, D. E., Y. Chen, R. S. Clavero, and V. N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. *J. Food Prot.* 66:559–569.
14. Gram, L. 2001. Potential hazards in cold-smoked fish: *Listeria monocytogenes*. *J. Food Sci.* 66:1072–1081.
15. Greer, G. G. 2005. Bacteriophage control of foodborne bacteria. *J. Food Prot.* 68:1102–1111.
16. Guenther, S., D. Huwyler, S. Richard, and M. J. Loessner. 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. *Appl. Environ. Microbiol.* 75:93–100.
17. Hagens, S., and M. L. Offerhaus. 2008. Bacteriophages: new weapons for food safety. *Food Tech.* 4:46–54.
18. Hsu, F. C., Y. S. Shieh, and M. D. Sobsey. 2002. Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat. *J. Food Prot.* 65:93–99.
19. Hu, Y., K. Gall, A. Ho, R. Ivanek, Y. T. Grohn, and M. Wiedmann. 2006. Daily variability of *Listeria* contamination patterns in a cold-smoked salmon processing operation. *J. Food Prot.* 69:2123–2133.
20. Hudson, J. A., C. Billington, G. Carey-Smith, and G. Greening. 2005. Bacteriophages as biocontrol agents in food. *J. Food Prot.* 68:426–437.
21. Kennedy, J. E., Jr., C. I. Wei, and J. L. Oblinger. 1986. Methodology for enumeration of coliphages in foods. *Appl. Environ. Microbiol.* 51: 956–962.
22. Kim, J. M., T. S. Huang, M. R. Marshall, and C. I. Wei. 1999. Chlorine dioxide treatment of seafoods to reduce bacterial loads. *J. Food Sci.* 64:1089–1093.
23. Leverentz, B., W. S. Conway, Z. Alavidze, W. J. Janisiewicz, Y. Fuchs, M. J. Camp, E. Chighladze, and A. Sulakvelidze. 2001. Examination of bacteriophage as a biocontrol method for salmonella on fresh-cut fruit: a model study. *J. Food Prot.* 64:1116–1121.
24. Leverentz, B., W. S. Conway, W. Janisiewicz, and M. J. Camp. 2004. Optimizing concentration and timing of a phage spray application to reduce *Listeria monocytogenes* on honeydew melon tissue. *J. Food Prot.* 67:1682–1686.
25. Neetoo, H., M. Ye, and H. Chen. 2008. Potential antimicrobials to control *Listeria monocytogenes* in vacuum-packaged cold-smoked salmon pate and fillets. *Int. J. Food Microbiol.* 123:220–227.
26. Norton, D. M., M. A. McCamey, K. L. Gall, J. M. Scarlett, K. J. Boor, and M. Wiedmann. 2001. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. *Appl. Environ. Microbiol.* 67:198–205.
27. Ozer, N. P., and A. Demirci. 2006. Electrolyzed oxidizing water treatment for decontamination of raw salmon inoculated with *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A and response surface modeling. *J. Food Eng.* 72:234–241.
28. Ozer, N. P., and A. Demirci. 2006. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. *Int. J. Food Sci. Technol.* 41:354–360.
29. Petty, N. K., T. J. Evans, P. C. Fineran, and G. P. Salmond. 2007. Biotechnological exploitation of bacteriophage research. *Trends Biotechnol.* 25:7–15.
30. Plunkett, G., III, D. J. Rose, T. J. Durfee, and F. R. Blattner. 1999. Sequence of Shiga toxin 2 phage 933W from *Escherichia coli* O157:H7:Shiga toxin as a phage late-gene product. *J. Bacteriol.* 181: 1767–1778.
31. Sakaguchi, Y., T. Hayashi, K. Kurokawa, K. Nakayama, K. Oshima, Y. Fujinaga, M. Ohnishi, E. Ohtsubo, M. Hattori, and K. Oguma. 2005. The genome sequence of *Clostridium botulinum* type C neurotoxin–converting phage and the molecular mechanisms of unstable lysogeny. *Proc. Natl. Acad. Sci. USA* 102:17472–17477.

32. Silva, J. L., G. R. Ammerman, and S. Dean. 2001. Processing channel catfish. Southern Regional Aquaculture Center Publications, Stoneville, MS.
33. Su, Y. C., and M. T. Morrissey. 2003. Reducing levels of *Listeria monocytogenes* contamination on raw salmon with acidified sodium chlorite. *J. Food Prot.* 66:812–818.
34. U.S. Food and Drug Administration. 2006. Agency response letter GRAS notice no. GRN 000198. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm154675.htm. Accessed 13 August 2009.
35. U.S. Food and Drug Administration. 2006. Food additives permitted for direct addition to food for human consumption: bacteriophage preparation, p. 47729–47732. 21 CFR, part 172. Available at: http://edocket.access.gpo.gov/2006/E6-13621.htm. Accessed 13 August 2009.
36. U.S. Food and Drug Administration. 2007. Agency response letter GRAS notice no. GRN 000218. Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153865.htm. Accessed 13 August 2009.
37. Verthe, K., and W. Verstraete. 2006. Use of flow cytometry for analysis of phage-mediated killing of *Enterobacter aerogenes*. *Res. Microbiol.* 157:613–618.
38. Vogel, B. F., L. V. Jorgensen, B. Ojeniyi, H. H. Huss, and L. Gram. 2001. Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smokehouses as assessed by random amplified polymorphic DNA analyses. *Int. J. Food Microbiol.* 65:83–92.
39. Whitman, P. A., and R. T. Marshall. 1971. Isolation of psychrophilic bacteriophage–host systems from refrigerated food products. *Appl. Microbiol.* 22:220–223.