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Original Article

Reduction of *Listeria monocytogenes* on the Surface of Fresh Channel Catfish Fillets by Bacteriophage Listex P100

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Abstract

Bacteriophage Listex P100 (phage P100) was approved by the U.S. Food and Drug Administration and U.S. Department of Agriculture's Food Safety and Inspection Service for *Listeria monocytogenes* control on both raw and ready-to-eat food products. In this article, we present the proof of concept on the influence of phage dose, phage contact time, and storage temperature on the listericidal activity of phage P100 in reducing the *L. monocytogenes* loads on the surface of fresh channel catfish fillet. The fresh catfish fillet samples were surface inoculated with $\sim$4.3 $\log_{10}$ colony forming units (CFU)/g of a two serotype mix (1/2a and 4b) of *L. monocytogenes* cells and then surface treated with phage P100. *L. monocytogenes* reduction was influenced by phage contact time and phage dose regardless of higher or lower temperature regimes tested on catfish fillet. The reduction in *L. monocytogenes* loads ($p < 0.05$) with the phage P100 dose of 2×10^7 plaque forming units (PFU)/g (7.3 $\log_{10}$ PFU/g) was 1.4–2.0 $\log_{10}$ CFU/g at 4°C, 1.7–2.1 $\log_{10}$ CFU/g at 10°C, and 1.6–2.3 $\log_{10}$ CFU/g at room temperature (22°C) on raw catfish fillet. The phage contact time of 30 min was adequate to yield greater than 1 $\log_{10}$ CFU/g reduction in *L. monocytogenes*, whereas 15 min contact time with phage yielded less than 1 $\log_{10}$ CFU/g reduction in *L. monocytogenes* loads on catfish fillet. Phage P100 titer was stable on catfish fillet samples, and overall reductions in *L. monocytogenes* counts were still maintained over a 10-day shelf life at 4°C or 10°C by phage P100 treatment. These findings illustrate the effectiveness of an alternative generally recognized as safe antimicrobial such as bacteriophage Listex P100 in quantitatively reducing *L. monocytogenes* from fresh catfish fillet surfaces.

Introduction

The new 2008 farm bill legislation (PL 110–246) signed into law on June 18, 2008, extends U.S. Department of Agriculture's Food Safety Inspection Service jurisdiction to include domestic and imported farm-raised catfish (Senate and House of Representatives of the United States of America in Congress, 2008). This legislation may lead to new federal regulations on microbiological requirements across aquaculture products based on risk analysis data. A recent study by Chou *et al*. (2006) showed that 25% to 47% of fresh channel catfish fillets were contaminated with *Listeria monocytogenes*. Also, another recent study by Pao *et al*. (2008) reported that *L. monocytogenes* was present in 23.5% of catfish that was obtained from various retail stores in the United States. Although there are no documented cases of listeriosis associated with the consumption of cooked catfish products, potential risks remain for the catfish products due to a high prevalence of *L. monocytogenes*. Further, there is a possibility for other foods to become cross contaminated by *L. monocytogenes* from catfish. Such a high occurrence of *L. monocytogenes* in fresh catfish products indicates the inadequacy of current intervention strategies for *L. monocytogenes* control in raw catfish products (Kozempel *et al*., 2001; Silva *et al*., 2003). Also, due to the potential for temperature abuse of raw products during storage and shipping, *L. monocytogenes* can multiply to dangerous levels under these conditions by readily available nutrients in these food products (Fernandes *et al*., 1998). In addition, *L. monocytogenes* is able to survive in cold conditions and can actually multiply slowly even at low refrigeration temperatures of 4°C (Schmid *et al*., 2009).

Catfish are usually marketed as raw fillets that are either fresh or frozen. The typical shelf life of fresh catfish fillets range from 8 to 12 days. Current intervention measures are not adequate to control the pathogenic microorganisms such as *L. monocytogenes* in raw catfish fillets. Some catfish processors follow the practice of chilling catfish fillets in chlorine water (Silva *et al*., 2001). Recent recommendations

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revealed that dipping fish in a chlorine solution increases the chances for cross contamination since the chlorine solution quickly becomes ineffective in the absence of active management of its concentration (Eklund *et al*., 2004). Catfish fillets treated with 3–4% acetic acid or a combination of 2% acetic and 2% lactic acids for 30–60 s suppressed aerobic microorganisms for 4 days and extended the shelf life to 16 days, but combinations of acetic and lactic acid were less acceptable to sensory panels due to off-flavor, off-odor, and flesh discoloration (Kim and Marshal, 2001). Similarly, dipping of catfish fillets with of 2% acetic, citric, lactic, malic, or tartaric acid at 4°C also resulted in lighter and yellowish color changes compared with untreated controls (Bal'a and Marshall, 1998).

To combat *L. monocytogenes* contamination of food products, food industries and regulatory agencies are continuously looking for novel and promising approaches that can prevent or decrease its occurrence or persistence. To meet this challenge, one promising approach is the use of a bacteriophage as an antilisterial agent (Greer, 2005; Hudson *et al*., 2005; Petty *et al*., 2007; Hagen and Offerhaus, 2008). Bacteriophages (phages) are viruses that infect bacterial cells specific for a target genus, serotype, or a strain. All phages are obligate parasites since they solely rely on a specific host for propagation, and in its absence they represent metabolically inert state. Phages are ubiquitous in nature, and it is estimated that earth harbors approximately 10^{31} phage particles and that as many as 10^8 phage particles can be isolated from a 1 g of soil or water (Petty *et al*., 2007). Phages are naturally found in all food products. There are several examples of phages that have been isolated from food products, including ready-to-eat (RTE) foods (Whitman and Marshall, 1971), beef (Kennedy *et al*., 1986; Hsu *et al*., 2002), chicken (Hsu *et al*., 2002; Atterbury *et al*., 2003b), vegetables (Kennedy *et al*., 1986; Hernandez *et al*., 1997), and dairy products (Binetti and Reinheimer, 2000). When using bacteriophages as biocontrol agents, phages that rapidly lyse bacterial cells without integration into bacterial DNA are recommended (Plunkett *et al*., 1999; Greer, 2005; Hudson *et al*., 2005; Sakaguchi *et al*., 2005).

Recently, the U.S. Food and Drug Administration has approved a bacteriophage preparation, Listex P100, as suitable to be included on both raw and RTE food products to combat *L. monocytogenes* contamination (USFDA, 2006a, 2006b, 2007). Listex P100 bacteriophage preparation is approved for all raw and RTE foods in general at levels not to exceed 10^9 plaque forming units (PFU)/g. Currently, there are no reports on the effectiveness of phage P100 in killing *L. monocytogenes* on fresh catfish fillets and factors affecting its efficacy. In the present study, we examine the effectiveness of bacteriophage Listex P100 in reducing *L. monocytogenes* on fresh catfish fillets as a function of (1) Listex P100 contact time, (2) Listex P100 dose, (3) storage temperature, and (4) storage duration.

Materials and Methods

L. monocytogenes *strains*

Two *L. monocytogenes* strains, EGD (BUG 600) representing serotype 1/2a and the outbreak strain Scott A representing serotype 4b, were used (Czuprynski *et al*., 2002). These *L. monocytogenes* strains were grown to OD_{600} of ~1.2 (equivalent to 10^9 colony forming units [CFU]/mL) in tryptic soy broth at 37°C for 24 h for obtaining stationary phase cells. The cells were harvested by centrifugation at 10,000 *g* for 10 min, and the resulting pellet was resuspended in physiological saline (0.8% NaCl). For inoculation, a two-strain mixture of *L. monocytogenes* EGD and Scott A was prepared by mixing an equal volume of cell suspension from both of these strains, and serial dilutions were performed in sterile physiological saline for the desired cell concentration.

Bacteriophage

Bacteriophage Listex™ P100 (phage P100) approved by the U.S. Food and Drug Administration and U.S. Department of Agriculture's Food Safety and Inspection Service was obtained from EBI Food Safety (Wageningen, The Netherlands) (USDA-FSIS, 2009; USFDA, 2006a, 2007). Phage P100 is active against multiple serovars of *L. monocytogenes* (Carlton *et al*., 2005). Phage P100 stock concentration in buffered saline was approximately 10^{11} PFU/mL by plaque formation assay. Phage P100 stock solution was serially diluted in physiological saline for preparing the desired concentrations for phage application.

Bacteriophage plaque forming assay

The titer of phage P100 was determined using the soft agar overlay method (Guenther *et al*., 2009). In this assay, the bacteriophage suspension was first serially diluted in sterile buffer (100 mM NaCl, 10 mM MgSO4, and 50 mM Tris-HCL pH 7.5). Four milliliters of sterile soft agar (42°C; tryptic soy broth containing 0.4% agar) was mixed with 150 μL of overnight-grown cells of *L. monocytogenes* EGD or Scott A (OD_{600} ~1.2) and then mixed with 100 μL of the serially diluted P100 suspension. The resulting mixture was vortexed and poured onto a tryptic soy agar plate and distributed evenly by gentle rotation of the tryptic soy agar plate. Duplicate plates were maintained for each serial dilution. After solidification of agar for 30 min at room temperature, plates were inverted and incubated at 30°C for 24 h for plaque formation. The number of visible plaques were counted and multiplied with the dilution factor to quantify the plaque forming units (PFU)/mL.

Catfish fillets

Fresh channel catfish fillets were obtained from a local retail grocery store or from a local commercial processor, and were kept at 4°C for use within 72 h.

L. monocytogenes *surface inoculation of catfish fillets*

Fresh fillet pieces of 5 g (approximately 2 cm^2 top surface area) were cut using a sterile knife on a sterile cutting board. Duplicate fillet pieces of 5 g each were placed in a sterile weighing dish (Fisherbrand Hexagonal Polystyrene Weighing Dishes, 10.2 cm diameter; Thermo Fisher Scientific, Hampton, NH) with white-flesh side facing up for performing surface treatments. Fifty microliters of the serially diluted two-strain mixture of *L. monocytogenes* suspension was spotted on the white-flesh side to yield an *L. monocytogenes* inoculation level of approximately 4.3 $\log_{10}$ CFU/g. This inoculum was uniformly spread on the fillet surface by random spotting at five points of 10 μL each on the flesh side. To allow binding of *L. monocytogenes* cells, the inoculum was air-dried for 15 min on the surface of the fillet samples in a Biosafety Level-2 laminar flow hood. Alternatively, for inoculating large fillets (approximately 180–200 g), each fillet was kept with a white-

flesh side facing up on an aluminum foil in a sterile stainless steel tray in a Biosafety Level-2 laminar flow hood. The aluminum foil around each fillet was raised to form a cup inside the tray. About 2 mL of the serially diluted two-strain mixture of *L. monocytogenes* was randomly inoculated as $20\times50\,\mu$L drops for the uniform spread on the white-flesh side of the catfish fillet and air-dried for 15 min to yield *L. monocytogenes* inoculation levels of about 4 $\log_{10}$ CFU/g.

Phage P100 surface treatment of catfish fillets

After the *L. monocytogenes* inoculation step, 5 g catfish fillet samples were surface treated with 100 μL of phage P100 using a concentration of 10^9, 10^7, or 10^5 PFU/mL to yield a final application dose of 2×10^7 PFU/g (7.3 $\log_{10}$ PFU/g), 2×10^5 PFU/g (5.3 $\log_{10}$ PFU/g), or 5×10^3 PFU/g (3.3 $\log_{10}$ PFU/g), respectively. On the 5 g fillet pieces, spotting with 100 μL of phage solution was adequate for its uniform spread on the flesh side after *L. monocytogenes* inoculation. For the no phage control, the fillet pieces were surface treated with 100 μL saline. Duplicate fillet samples in each weighing dish were kept inside a Ziploc bag (16.5×14.9 cm) and sealed for incubation at 4°C, 10°C, or room temperature (22°C). After a specified incubation period ranging up to 15 min, 30 min, 1 h, 2 h, 1 day, 4 day, 7 day, or 10 days, the fillet pieces were subjected to *L. monocytogenes* enumeration. All experiments were repeated three times with two replications. The 10-day shelf-life studies were conducted at both the optimum storage temperature (4°C) and temperature abuse (10°C) conditions on the phage P100 efficacy and not at 22°C, where spoilage microflora will quickly outgrow leading to loss of fillet quality.

Alternatively, the large fillets (180–200 g) were sprayed with listex phage P100 as described below. After the *L. monocytogenes* inoculation step, each large fillet kept in a stainless steel tray was sprayed with 7.5 mL of Listex P100 suspension of 10^9 PFU/mL evenly on the entire white-flesh side using a hand-held spray bottle (2 oz mini fingertip sprayer; The Bottle Crew, West Bloomfield, MI). On large fillets, spraying with 7.5 mL phage solution was adequate for its uniform spread on the flesh side after *L. monocytogenes* inoculation. The aluminum foil around the fillet was raised to form a cup inside the stainless steel stray to hold the overflow of phage P100 from uneven fillet surfaces. In untreated control, fillets were sprayed with the same amount of saline. After 30 min or 2 h contact time with Listex P100 at room temperature, fillets were processed for *L. monocytogenes* enumeration. All experiments were repeated twice.

Enumeration of L. monocytogenes *from catfish fillets*

For enumerating *L. monocytogenes*, each catfish fillet piece (5 g) was aseptically transferred to a stomacher bag containing 25 mL peptone water (0.1% peptone and 0.02% Tween-80). For a whole fillet, each fillet was aseptically placed in a stomacher bag containing 200 mL of peptone water. Each sample was homogenized for 2 min using a stomacher (Seward Model 400C; Seward Limited, Worthing, UK) at 230 rpm. The phage P100 was removed from the homogenized sample by a centrifugation step. For this purpose, homogenized rinse (1 mL) was centrifuged at 12,000 *g* for 5 min, and supernatant that contained the phage P100 fraction was removed and the pellet containing the *L. monocytogenes* fraction was resuspended to the original volume. Subsequently, a subsample volume of 100 or 250 μL (to yield a countable plate) from resuspended pellets was plated out for *L. monocytogenes* isolation. Alternatively, further serial dilutions were performed in some cases from resuspended pellets to yield countable plates of 50–300 CFU/plate. The polymixin-acriflavine-lithium chloride-ceftazidime-aesculin-mannitol (PALCAM) agar (Difco, Becton Dickinson, Sparks, MD) containing 6 mg/L of ceftazidime was used for the selective isolation and enumeration of *L. monocytogenes*. After 48 h of incubation at 37°C, *L. monocytogenes* CFU were counted in PALCAM plates and resulting numbers were multiplied with the dilution factor for determination of CFU/g.

Enumeration of phage P100 from catfish fillets

The stability of phage P100 on catfish fillet samples was determined at both 4°C and 10°C for 10 days. For this purpose, 100 μL of 10^9 PFU/mL of phage P100 was spotted on the white-flesh side of the catfish fillet piece (5 g). Duplicate catfish fillet pieces were kept in a weighing dish, sealed in a Ziploc bag, and incubated at 4°C or 10°C. The plaque forming assay was conducted for phage P100 rinses recovered from these samples after 0, 1, 4, 7, and 10-day shelf life. At such time interval, fillet pieces were aseptically placed into a stomacher bag containing 25 mL peptone water (0.1% peptone and 0.02% Tween-80) and stomached for 2 min at 230 rpm. The homogenized stomached rinse was centrifuged at 12,000 *g* for 5 min to collect supernatant (containing P100 fraction), which was filter-sterilized by a 0.22 μm syringe filter. Subsequently, the filtered phage fraction was serially diluted for phage P100 for plaque enumeration by a soft agar overlay assay described above.

Statistical analysis

All experiments were repeated two times and all treatments were replicated at least twice in each experiment. *L. monocytogenes* CFU were first converted into $\log_{10}$ CFU/g using Microsoft Excel. The $\log_{10}$ CFU/g datasets were transferred to SPSS statistical analyses software package (SPSS version 12.0, Chicago, IL) and a one-way analysis of variance test using least square differences was performed to find out the mean significant differences among different treatment/time points. For phage P100 stability analysis, observed PFU counts were converted into $\log_{10}$ PFU/g and plotted using Microsoft Excel.

Results

Phage P100 plaque formation against L. monocytogenes *serotypes 1/2a and 4b*

The sensitivities of *L. monocytogenes* strain EGD of serotype 1/2a and of Scott A of serotype 4b to phage P100 were confirmed by the soft agar overlay assay. Phage P100 produced large plaques of approximately 2 mm in diameter against both EGD and Scott A strains, which constitute as two major serotypes (on food) of *L. monocytogenes* (Borucki *et al*., 2003).

Effect of phage P100 dose against L. monocytogenes *reduction on catfish fillets*

Figure 1 shows the effect of phage P100 concentrations of 2×10^3, 2×10^5, and 2×10^7 PFU/g against *L. monocytogenes* on

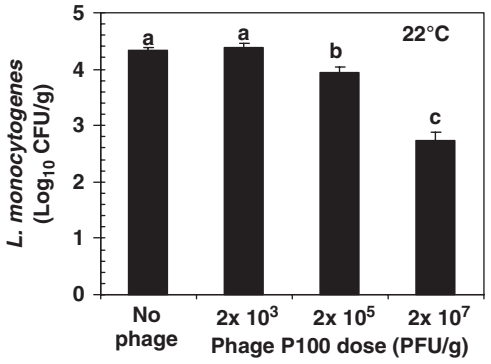

FIG. 1. Effect of phage P100 dose in reducing *Listeria monocytogenes* loads on catfish fillet samples within 2 h at room temperature. The 5 g pieces of catfish fillets were surface inoculated with $\sim 4.3 \log_{10}$ colony forming units (CFU)/g of two serotype mix (1/2a and 4b) of *L. monocytogenes* cells, and then air-dried for binding before phage application. Bars with different letters are significantly different based on least square difference one-way analysis of variance test ($p \leq 0.05$).

catfish fillet pieces within 2 h at room temperature. Phage P100 treatment of 2×10^7 PFU/g resulted in an average of $1.6 \log_{10}$ CFU/g reduction in *L. monocytogenes* counts on catfish fillet samples compared to untreated no phage control. At a density of 2×10^5 PFU/g of phage P100, there was a slight reduction of $0.4 \log_{10}$ CFU/g in *L. monocytogenes* counts, which also differed from the untreated control ($p < 0.05$). There was no reduction in *L. monocytogenes* counts at 2×10^3 PFU/g of phage P100 dose.

Effect of phage P100 contact time against L. monocytogenes *reduction on catfish fillets*

Figure 2 shows the effect of phage P100 contact time of 15, 30, 60, and 120 min at a dose of 2×10^7 PFU/g on catfish fillets

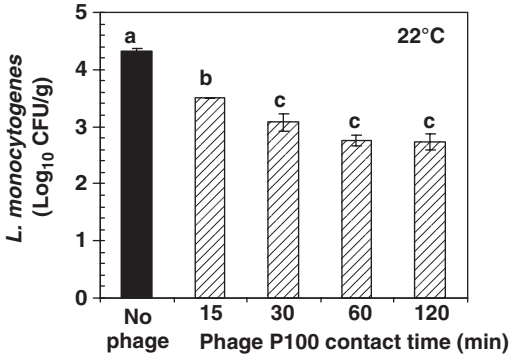

FIG. 2. Effect of phage P100 (dose of 2×10^7 plaque forming units [PFU]/g) contact time in reducing *L. monocytogenes* loads on catfish fillet surfaces at room temperature: no phage (black bar) and phage (hatched bar). The 5 g pieces of catfish fillets were surface inoculated with $\sim 4.3 \log_{10}$ CFU/g of two serotype mix (1/2a and 4b) of *L. monocytogenes* cells, and then air-dried for binding before phage application. Bars with different letters are significantly different based on least square difference one-way analysis of variance test ($p \leq 0.05$).

at room temperature. *L. monocytogenes* counts were decreased by an average of $0.8 \log_{10}$ CFU/g on catfish fillet pieces within 15 min of phage P100 treatment. Within 30, 60, and 120 min of phage P100 treatment, the maximum reduction in *L. monocytogenes* counts was in the range of 1.3–1.6 $\log_{10}$ CFU/g on catfish fillet surfaces. Such reductions in *L. monocytogenes* counts by phage P100 differed ($p < 0.05$) from the no phage control. However, there were no differences ($p > 0.05$) between phage P100 contact times of 30, 60, and 120 min, which all yielded significantly greater reductions than that of the 15 min contact time.

Effect of phage P100 in reducing L. monocytogenes *during 10-day shelf life of catfish fillets at 4°C and 10°C*

Figure 3 shows the effect of phage P100 on *L. monocytogenes* reduction during 10-day shelf life of fresh catfish fillet samples at 4°C and 10°C. Within 30 min of phage P100 treatment at 4°C, an average reduction of $1.5 \log_{10}$ CFU/g was achieved when compared to the untreated control. During subsequent storage at 4°C, *L. monocytogenes* slowly grew to $5.2 \log_{10}$ CFU/g within 10 days from the initial level of $4.3 \log_{10}$ CFU/g at day 0 in the untreated control. In phage P100 treatment, *L. monocytogenes* grew to a maximum of $3.8 \log_{10}$ CFU/g after 10 days from the initial level of $2.7 - \log_{10}$ CFU/g after 30 min of phage treatment. These findings indicate that the overall reduction of *L. monocytogenes* remained greater than $\sim 1.5 \log_{10}$

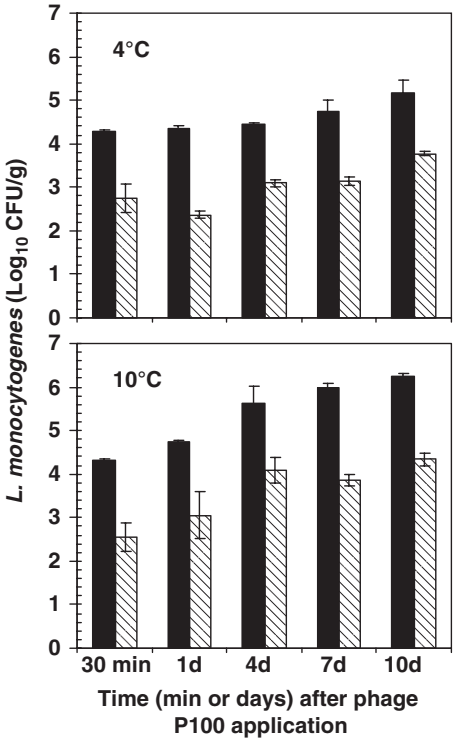

FIG. 3. Growth of *L. monocytogenes* during the 10-day shelf life of catfish fillets at 4°C and 10°C in the presence of phage P100 at a dose of 2×10^7 PFU/g: no phage (black bar) and phage P100 (hatched bar). The 5 g pieces of catfish fillets were surface inoculated with $\sim 4.3 \log_{10}$ CFU/g of two serotype mix (1/2a and 4b) of *L. monocytogenes* cells, and then air-dried for binding before phage application.

CFU/g for the phage P100 treatment when compared to the untreated control over the 10-day shelf life.

At 10°C, 30 min of phage P100 treatment led to 1.7 $\log_{10}$ CFU/g reduction in *L. monocytogenes* counts on catfish fillet pieces when compared to the untreated control. At 10°C, *L. monocytogenes* growth increased to 6.3 $\log_{10}$ CFU/g after 10 days in an untreated control. In the phage P100 treatment, *L. monocytogenes* growth increased to a maximum of 4.3 $\log_{10}$ CFU/g after 10 days from the initial 2.5 $\log_{10}$ CFU/g level with a 30 min treatment. These findings illustrate an overall reduction of 1.5–2.0 $\log_{10}$ CFU/g in *L. monocytogenes* loads after 10 days of storage for fresh catfish fillets that were treated by the phage P100 treatment when compared to the untreated control (i.e., no phage).

Effect of phage P100 spray on whole fillets against L. monocytogenes *reduction*

Figure 4 shows the effect of Listex P100 spray of whole catfish fillets after 30 min and 2 h at room temperature against *L. monocytogenes*. Compared to untreated control, Listex P100 spray led to 1.7–2.3 log reductions in *L. monocytogenes* counts within 30 min and 2 h on the whole catfish fillets at room temperature.

Stability of phage P100 on catfish fillet samples stored at 4°C and 10°C

Figure 5 shows the stability of phage P100 at both 4°C and 10°C during the 10-day shelf life of catfish fillet. Phage P100 was found to be relatively stable at both 4°C and 10°C on catfish fillet tissue, and there were no marked differences in phage stability between 4°C and 10°C. From the initial level of 7.3 $\log_{10}$ PFU/g, phage P100 numbers decreased to 5.5 and 5.2 $\log_{10}$ PFU/g during the 10-day shelf life of the catfish fillets at 4°C and 10°C, respectively. These results indicate a maximum loss of approximately 1.8–2.1 $\log_{10}$ PFU/g in phage P100 number during 10 days of storage for fresh catfish fillets.

Discussion

Host-specific bacteriophages are candidates of continuing interest for food safety applications. To date, only limited studies have been conducted on the usefulness of phages as *L. monocytogenes* biocontrol agents in raw and RTE food products. Some earlier studies include the efficacy of *Listeria* bacteriophage P100 in soft-variety cheese and RTE foods (Carlton *et al*., 2005; Guenther *et al*., 2009), in cold smoked salmon (Hagen and Offerhaus, 2008), and in frankfurters (Call *et al*., 2008). In addition, *Listeria* bacteriophage LMP-102 was evaluated for its efficacy in honeydew melon and apple (Leverentz *et al*., 2003), and *Listeria* bacteriophage LH7 was evaluated for its efficacy in raw beef (Dykes and Moorhead, 2002).

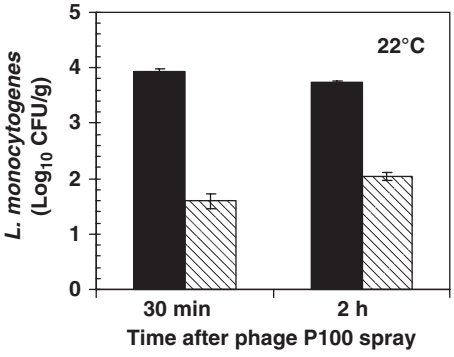

FIG. 4. Reduction of *L. monocytogenes* on whole catfish fillets by phage P100 at a dose of 2×10^7 PFU/g during 30 min and 2 h at room temperature. No phage (black bar) and phage P100 (hatched bar).

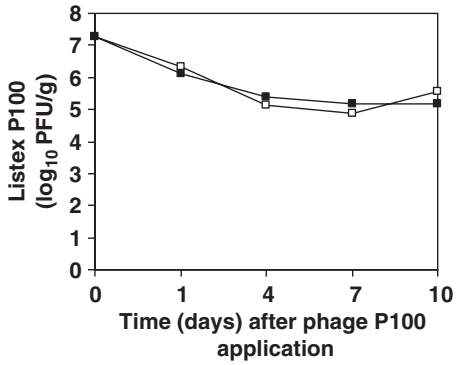

FIG. 5. Stability of phage P100 on catfish fillet samples during the 10-day shelf life of at 4°C (white square) and 10°C (black square).

In this study, the efficacy of the recently approved lytic bacteriophage Listex P100 for the quantitative reduction of *L. monocytogenes* was demonstrated on the surfaces of 5 g catfish fillet samples as a function of bacteriophage contact time, dose, storage temperature, and storage period. The phage P100 efficacy was evaluated by the surface spot method on 5 g catfish fillet samples as well as on large fillets (180–200 g) as a spray. Currently, in phage efficacy studies, the homogenized tissue extracts containing both surviving bacteria and phage particles are normally spread plated on the agar surface (Atterbury *et al*., 2003a; Hudson *et al*., 2005) for the enumeration of surviving bacterial cells. Due to the presence of phage in the rinse sample, it may interfere with host cell counts during the enumeration period. Therefore, we used a simple alternative approach to remove the phage P100 through a centrifugation step before direct plating of the stomached rinses. By this approach, majority of phage P100 particles from bacterial pellet were excluded for the accurate *L. monocytogenes* enumeration of stomached rinses. The significant removal/recovery of phage P100 in the top supernatant fraction of the stomached rinse was confirmed by determining the phage titer. This is evident in Fig. 5 at time point 0 in which the supernatant (phage fraction) was assayed and almost the entire initial amount of the 7.3 $\log_{10}$ PFU/g (i.e., 2×10^7 PFU/g) phage inoculum was recovered through the centrifugation step.

Our results show that a dose of 2×10^7 PFU/g (compared to 2×10^5 and 2×10^3 PFU/g) of phage P100 was necessary to achieve a significant reduction in *L. monocytogenes* counts on fresh catfish fillet samples. At this phage dose, the reductions in *L. monocytogenes* counts were 1.5–1.7 $\log_{10}$ CFU/g on the 5 g fillet pieces (by spot application of P100) or 1.7–2.3 $\log_{10}$ CFU/g on the whole fillets (by spray application of P100).

In line with the results of this study, phage P100 treatment at 10^8 PFU/g was required to reduce the *L. monocytogenes* population on different RTE foods by approximately 2 logs (Guenther *et al*., 2009). Also, the phage P100 was successfully used in soft cheese at a phage density of 6×10^7 PFU/cm^2 to achieve complete control when the initial low level of *L. monocytogenes* was 20 CFU/cm^2 (Carlton *et al*., 2005). Further, in other phage challenge studies against *Salmonella* and *Campylobacter*, a phage density in the range of 10^6–10^8 PFU/g or cm^2 resulted in about 1–3 log reductions of the target bacterial host (Atterbury *et al*., 2003a; Leverentz *et al*., 2004; Bigwood *et al*., 2008). In experiments conducted by Dykes and Moorhead (2002), a lower dose of bacteriophage LH7 at 3×10^3 PFU/mL did not yield any appreciable reduction in *L. monocytogenes* counts on raw beef. One of the contributing factors for this failure of efficacy was a relatively sparse distribution of bacteriophage particles ($\sim$1 phage particle/10^3 bacterial cells) (Dykes and Moorhead, 2002). Our experiments also revealed that a 30, 90 or 120 min contact time with phage P100 will yield 1.4–2.3 log reduction in *L. monocytogenes* counts on fresh catfish fillets at all temperatures evaluated. Since phage P100 was equally effective at room temperature as well as 4°C and 10°C, as observed in theses studies, this phage technology is useful in catfish processing conditions when fillets are kept at lower temperatures after the listericidal treatment. Catfish undergo deheading, evisceration, skinning, size grading, filleting, and chilling steps during processing (Silva *et al*., 2001). One effective intervention point for the lytic bacteriophage application is in between the filleting and chilling steps, which will be confirmed in future pilot plant trials.

The efficacy of phage particle on food substrate can be affected by the type of food matrix (i.e., liquid or solid), phage immobilization due to reduction in surface water content, and the ability of certain food-associated factors that lead to the structural degradation of phage particles (Hagen and Offerhaus, 2008; Guenther *et al*., 2009). This may be one of the reasons why we did not observe additional decreases in *L. monocytogenes* counts during the subsequent storage (i.e., after 1, 4, 7, and 10 days) than what was achieved within the first 30 min at 4°C or 10°C on catfish fillet pieces using the phage P100 treatment. Additionally, a decrease in phage population by 1.8–2.1 $\log_{10}$ PFU/g over a 10-day storage period may be responsible for no further reductions in *L. monocytogenes* during storage. Overall, phage P100 treatment yielded initial reduction in *L. monocytogenes* loads but did not inhibit the growth of *L. monocytogenes* during the 10-day storage period. In spite of this, *L. monocytogenes* counts remained lower ($p < 0.05$) in phage P100 treatments at the end of the 10-day storage period by about 1–1.5 $\log_{10}$ CFU/g (versus no phage), after taking into account the slow growth of *L. monocytogenes* at 4°C or 10°C. Without phage P100 treatment, *L. monocytogenes* numbers increased to $\sim$6 $\log_{10}$ CFU/g at lower temperature regimes. Our observation of 10-day storage period efficacy for P100 emphasizes that any reduction in *L. monocytogenes* count occur only during initial time period. Similar to these findings, Leverentz *et al*. (2003) observed an initial 2-log reduction in *L. monocytogenes* counts on honeydew melon surfaces by LMP-102 bacteriophage and followed by a 2-log increase in *L. monocytogenes* during the subsequent 7-day storage at 10°C. Also, on chicken tissues, *Campylobacter*-specific phage treatment resulted in an initial 1.2-log reduction of *Campylobacter jejuni* with no subsequent reduction during the 10-day storage period (Atterbury *et al*., 2003a).

There are no reported cases of *L. monocytogenes* resistance to a bacteriophage when used as biocontrol agents (Greer, 2005; Hudson *et al*., 2005). Carlton *et al*. (2005) found no resistance development in surviving *L. monocytogenes* cells after phage P100 bacteriophage treatment of cheese that was collected and repeatedly challenged with the same bacteriophage. O'Flynn *et al*. (2004) found development of bacteriophage-insensitive mutant (BIM) for *Escherichia coli* O157:H7 in broth study at a low level (1 BIM in 10^6 CFU) and suggested that such low chances for BIM development are not likely to hinder the use of these phages as biocontrol agents. Wang *et al*. (1998) observed a predominance of serotype 1 in a majority of catfish isolates of *L. monocytogenes* that were collected in his studies. Also, Liu *et al*. (2003) observed the different virulence profiles for *L. monocytogenes* that are obtained from catfish when compared to environmental and human isolates. On the other hand, a high diversity of *L. monocytogenes* isolates was demonstrated by pulsed-field gel electrophoresis (PFGE) (Chou *et al*., 2006) in catfish fillets and in processing plants. However, the ability of phage P100 in killing all such diverse isolates of *L. monocytogenes* occurring in catfish processing is yet to be established.

Conclusions

Our findings demonstrate the efficacy of bacteriophage P100 for the quantitative reduction of *L. monocytogenes* on raw catfish fillet samples as influenced by phage dose, phage contact time, and storage temperature. Since there is high diversity of *L. monocytogenes* isolates that may occur in catfish fillets and in processing plants, further work is needed to determine the ability of phage P100 in eliminating the diverse set of isolates of *L. monocytogenes* occurring in these conditions.

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Disclosure Statement

No competing financial interests exist.

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