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Characterization of antimicrobial properties of *Salmonella* phage Felix O1 and *Listeria* phage A511 embedded in xanthan coatings on Poly(lactic acid) films

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ABSTRACT

Beyond simply providing a barrier between food and external contaminants, active packaging technologies aim to inhibit pathogen survival and growth within the packaged environment. Bacteriophages have a proven track record as targeted antimicrobials but have yet to be successfully integrated in active packaging without serious loss of activity. We have developed two bacteriophage based xanthan coatings on poly(lactic acid) (PLA) film which significantly inhibits *Salmonella* Typhimurium and *Listeria monocytogenes* growth in culture ($P < 0.01$), and significantly reduces survival and growth of diverse cocktails of *Salmonella* sp. and *L. monocytogenes* respectively on precooked sliced turkey breast over 30 days of anaerobic packaging at 4 or 10 °C ($P < 0.05$). Specifically reductions of 0.832 log at 4 °C and 1.30 log at 10 °C for *Salmonella* sp., and 6.31 log at 4 °C and 1.52 log at 10 °C for *L. monocytogenes* were observed. The coating containing *Listeria* phage A511 also significantly inhibited growth of *L. monocytogenes* over 14 days in aerobic packaging (3.79 log at 4 °C, 2.17 log at 10 °C, $P < 0.05$). These coatings showed 99.99% phage release within 30 min for both phages. Similar approaches could be used to develop packaging inhibitory to other significant foodborne pathogens such as *Campylobacter*, and *Escherichia coli*, as well as spoilage bacteria.

1. Introduction

Salmonella sp. and *Listeria monocytogenes* are major public health concerns causing severe illness, economic losses and numerous deaths in at risk groups, including the elderly, newborn infants, pregnant women, and immunocompromised individuals (Denes et al., 2014; Woolston et al., 2013). *L. monocytogenes* is able to survive and grow under lower temperatures and pH levels than most other foodborne pathogens (Chibeu et al., 2013). As such, mitigating and preventing foodborne illnesses such as salmonellosis and listeriosis are of vital importance.

Packaging is important in sustaining food quality and safety throughout the distribution chain, while extending product shelf life (Irkin and Esmer, 2015; Kerry et al., 2006). In fresh meat proper packaging reduces weight loss, enhances tenderness, and maintains colour, while for processed meat, packaging controls dehydration, discolouration, lipid oxidation and loss of aroma (Irkin and Esmer, 2015; Kerry et al., 2006). Packaging systems range from short-term overwraps to a diversity of modified atmosphere packaging for longer term storage. Active packaging takes these properties a step further, altering the food and/or packaged environment to improve sensory properties, extend shelf life, or inhibit survival of pathogens and spoilage bacteria, without negatively effecting food quality (Ahvenainen, 2003). Antimicrobial active packaging components tested to date include various organic acids, chitosan, cranberry and oregano extracts, acetate cellulose, diverse bacteriocins and enzymes, silver zeolite, kappa-carrageenan, and allyl isothiocyanate (Ahvenainen, 2003; Cardoso et al., 2016; Gouvêa et al., 2015; Jin, 2010; Olaimat and Holley, 2015, 2016).

Abbreviations: BHI, brain heart infusion; CFU, colony forming units; OD600, optical density at 600 nm; PFU, plaque forming units; PLA, poly-lactic acid; RTE, ready-to-eat; TSA, tryptic soy agar; TSB, tryptic soy broth; WPI, whey protein isolate film.

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The active packaging compositions published to date have strengths and weaknesses, addressing only a subset of the many theoretical applications of active packaging (Ahvenainen, 2003; Irkin and Esmer, 2015). Antimicrobial stability and low specificity for pathogens are areas of particular limitation for many active packaging systems involving broad spectrum antimicrobials.

Bacteriophages are largely unexplored as embeddable antimicrobials, with unique advantages. Phages are highly selective for only infecting specific genera, species, strains or life stages of hosts, such as pathogens, while leaving other bacteria, such as symbiotic gut flora, unaffected (Khalifa et al., 2015; Mayer et al., 2008; Radford et al., 2016; Vonasek et al., 2014). Phages also tend to be inert in the absence of target hosts, can be highly stable for storage unlike many other biocontrol agents, and those tested have been 'generally recognized as safe' by national and international regulatory bodies (Chibeu et al., 2013; Knobler and Gelbart, 2009; Vonasek et al., 2014). These characteristics make them particularly promising as antimicrobials in applications where broad spectrum antibiotics are ineffective, undesirable or inappropriate, while prolonged stability or antimicrobial activities are desirable traits (Brovko et al., 2012; Chibeu et al., 2013; Hooton et al., 2011; Khalifa et al., 2015; Malik and Chhibber, 2009).

Combining safe, selective antimicrobial treatments, such as bacteriophage, with conventional packaging is expected to dramatically reduce rates of spoilage and foodborne illness by actively inhibiting these organisms and providing protection beyond the end of the production process. The issue to date has been developing an effective method of delivering and releasing phage to packaged food without significant loss of antimicrobial activity or stability (Chibeu et al., 2013; Gouvêa et al., 2015; Vonasek et al., 2014). The edible coating formulation described herein addresses both issues, by demonstrating stable incorporation of two distinct phages into an edible coating with effective phage preservation/stability in long term storage and effective release of phage to significantly control pathogen load throughout the packaged life time of the food.

2. Materials and methods

2.1. Sources for bacteria, phage, meat and media

The bacterial and viral strains used in this study are listed in Table 1 along with source locations. A cocktail of *L. monocytogenes* or *Salmonella* serotypes were used in the ready-to-eat (RTE) meat studies as recommended by the Health Canada stipulated method for accessing the efficiency of antimicrobial treatments in RTE refrigerated foods (Health Canada, 2011, 2016). The five *L. monocytogenes* strains represent the three most common serotypes associated with foodborne outbreaks of *L. monocytogenes* and also included a clinical strain associated with the 2008 Canadian listeriosis outbreak (Gilmour et al., 2010). Similarly, the six serotypes of *Salmonella* were chosen as a representative sample of the diversity of foodborne pathogenic *Salmonella*. *L. monocytogenes* strains were grown in 5.0 mL aliquots of sterile brain heart infusion (BHI) broth and incubated for 24 h at 30 °C to generate stationary phase cultures as per established methods (Radford et al., 2016). Similarly *Salmonella* strains were grown in 5.0 mL aliquots of sterile tryptic soy broth (TSB) and incubated for 24 h at 37 °C.

Freshly packaged, mechanically sliced, cooked turkey breast, containing no chemical preservatives such as sodium lactate or diacetate, was obtained directly from a certified processing facility and prepared for sampling the same day with storage at 4 °C prior to use. Sodium chloride (6.5 mg Na/g of meat), potassium chloride (8.2 mg K/g of meat), and vinegar were present as flavouring and bacteriostatic agents. A product without sodium lactate or diacetate was chosen to determine the effect of the phage based antimicrobial packaging and avoid any confusion such as additive antimicrobial effects of lactate, acetate and phages. Experiments on the meat system were performed in triplicate, using two independent packages of meat, sampling from one batch of meat for each bacterial cocktail.

2.2. Coating composition and casting

Xanthan coatings were prepared from five components. A 100 mL batch of coating solution was prepared from a mixture of 0.0–2.0 mL of 10^{12} PFU/mL *Salmonella* phage Felix O1 or *Listeria* phage A511 lysate solution prepared in our lab as detailed in Radford et al. (2016), 0.0–2.0 mL of glycerol (Fisher; Whitby, Canada), 1.0–2.0 g of xanthan gum (Sigma Aldrich; St. Louis, MO, USA), and 7.0 mL of ethanol, with 87–92 mL of deionized water, depending on the concentrations of phage, glycerol and xanthan added. Desirable coating physical properties and anti-*Salmonella* activity were observed with 2% xanthan, 1% (w/w) glycerol (with these components of the coating fixed at these values) and 1% (w/w) *Salmonella* phage Felix O1 solution. 1% (w/w) *Listeria* phage A511 coatings were prepared based on the same formulation but substituting one phage for the other. Control coatings were prepared with 2% xanthan, 1% (w/w) glycerol. The 7% (w/w) ethanol served as a dispersing aid to solubilize the xanthan powder during

Table 1
Bacterial and viral strains used.

Strain	Source
Listeria monocytogenes 08–5578 (serotype 1/2a) [Canadian 2008 outbreak] (Gilmour et al., 2010)	The National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba
Listeria monocytogenes ATCC 19115 (serotype 4b)	University of Guelph Laboratory Services Division, Guelph, ON, Canada
Listeria monocytogenes Li0512 (serotype 1/2b)	University of Guelph Laboratory Services Division, Guelph, ON, Canada
Listeria monocytogenes C6-3000 (serotype 1/2a)	Weidmann Lab, Department of Food Science, Cornell University, Ithaca, NY, USA
Listeria monocytogenes FSL F6-367(serotype 4b)	Weidmann Lab, Department of Food Science, Cornell University, Ithaca, NY, USA
Listeria phage A511 (Klumpp et al., 2008)	The Institute of Food, Nutrition and Health, ETH, Zurich, Switzerland
Salmonella Typhimurium DT104 (ATCC 700408)	The National Microbiology Laboratory, Public Health Agency of Canada, Guelph, ON, Canada
Salmonella Heidelberg ATCC 8326[clinical isolate]	American Type Culture Collection, Manassas, VA, USA
Salmonella Typhimurium 19485A96 SGI1[outbreak]	The National Microbiology Laboratory, Public Health Agency of Canada, Guelph, ON, Canada
Salmonella Enteritidis ATCC 4931	American Type Culture Collection, Manassas, VA, USA
Salmonella Newport ATCC 6962[clinical isolate]	The National Microbiology Laboratory, Public Health Agency of Canada, Guelph, ON, Canada
Salmonella Typhimurium ATCC 13311 [outbreak]	American Type Culture Collection, Manassas, VA, USA
Salmonella phage Felix O1	Felix d'Hérelle Reference Center, Université Laval, Québec, Canada