Virus Research 236 (2017) 14–23

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Characterization of a broad host-spectrum virulent *Salmonella* bacteriophage fmb-p1 and its application on duck meat

Changbao Wang[a,b,c], Qiming Chen[a], Chong Zhang[a], Jie Yang[a], Zhaoxin Lu[a,*], Fengxia Lu[a], Xiaomei Bie[a]

[a] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Institute of Functional Food, Anhui Normal University, Wuhu 241002, PR China
[c] College of Environmental Science and Engineering, Anhui Normal University, Wuhu 241002, PR China

ARTICLE INFO

Keywords:
Bacteriophage
Siphoviridae
Salmonella
Multidrug resistant bacteria
Duck meat

ABSTRACT

The aim of this study was to find a virulent bacteriophage for the biocontrol of *Salmonella* in duck meat. A broad host-spectrum virulent phage, fmb-p1, was isolated and purified from an duck farm, and its host range was determined to include *S.* Typhimurium, *S.* Enteritidis, *S.* Saintpaul, *S.* Agona, *S.* Miami, *S.* Anatum, *S.* Heidelberg and *S.* Paratyphi-C. Electron microscopy and genome sequencing showed that fmb-p1 belongs to the family *Siphoviridae*. The genome of fmb-p1 is composed of a 43,327-bp double-stranded DNA molecule with 60 open reading frames and a total G + C content of 46.09%. There are no deleterious sequences or genes encoding known harmful products in the phage fmb-p1 genome. Phage fmb-p1 was stable under different temperature (40–75 °C), pH (4–10) and NaCl solutions (1–11%). The phage treatment (9.9×10^9 PFU/cm^2) caused a peak reduction in *S.* Typhimurium of 4.52 log CFU/cm^2 in ready-to–eat (RTE) duck meat, whereas potassium sorbate treatment (PS, 2 mg/cm^2) resulted in a 0.05–0.12 log reduction. Compared to PS treatment, there was significant difference in the *S.* Typhimurium reduction ($P < 0.05$) by phage treatment at both 4 °C and 25 °C. The results suggested that phage could be applied to reduce *Salmonella,* on commercial poultry products.

1. Introduction

Salmonellosis is one of the most common foodborne illnesses worldwide, and outbreaks are typically associated with the consumption of foods contaminated by *Salmonella* spp. *Salmonella* infections can cause abdominal pain, vomiting, inflammatory diarrhea, fever, and headache (Haraga et al., 2008). According to recent reports, in USA, there is an estimated 1 million cases of salmonellosis, resulting in 378 deaths per year (Scallan et al., 2011). In 2013, there were 82,694 confirmed human cases and 59 deaths in the EU (EFSA-ECDC, 2015). To date, more than 290 *Salmonella* serovars have been found in China, and *Salmonella enterica* subspecies *enterica*, serovar Typhimurium (*S.* Typhimurium), which is one of the most frequently reported serovars associated with human foodborne illness, causes acute gastroenteritis (Sun et al., 2014). It is estimated that 22.2% of foodborne illnesses in China were caused by *Salmonella* (Wang et al., 2007). With the increased consumption of meat in China, the potential risk of foodborne salmonellosis has also increased.

Duck is a popular meat consumed in China, and it is used to prepare many traditional dishes, such as Nanjing salted duck and Beijing roast duck. According to the FAO (2014), China is the largest producer of duck meat (3 million tons annually), and consumption is increasing every year. However, duck is one of the major environmental reservoirs for *Salmonella*. One study showed that 23 out of 121 duck meat samples obtained from conventional farms (6), abattoirs (10), and retail markets (7) in Sichuan (China) were positive for *Salmonella*, which was thought to be caused by poor hygiene (Li et al., 2013). In Malaysia, a study showed that 125 out of 531 duck meat samples were positive for *Salmonella* (Adzitey et al., 2012). *S.* Typhimurium is the predominant serovar present in duck meat and eggs (Saitanu et al., 1994; McCrea et al., 2006), and consumption of duck products has been associated with outbreaks of salmonellosis, often resulting in hospitalization and sometimes, death (Noble et al., 2012). With the increased consumption of cold dishes, like Nanjing salted duck, the potential risk of exposure to *Salmonella* has increased (Yu et al., 2016).

Substantial efforts have been made to reduce the number of *Salmonella* on poultry, from farm to fork (Capita et al., 2002; Dincer and Baysal, 2004; del Río et al., 2007; Chaine et al., 2013). Chemical decontamination techniques are widely applied to poultry worldwide (Sarjit and Dykes, 2015). In China, it is legal to use antibiotics to control

* Corresponding author.
E-mail address: fmb@njau.edu.cn (Z. Lu).

http://dx.doi.org/10.1016/j.virusres.2017.05.001
Received 21 February 2017; Received in revised form 17 April 2017; Accepted 2 May 2017
Available online 03 May 2017

Salmonella spp. in poultry, as this may increase the possibility of the emergence of antibiotic-resistant strains (Hu, 2010). In one study, 48 *Salmonella* strains were isolated from duck in Sichuan province that were resistant to 36 commonly used antibiotics, and the resistance rates to 14 of the tested antibiotics were greater than 50% (Yu, 2012). In another study, among tested isolates in Shanghai, 43.3% were multi-drug resistant, and 44 different resistance patterns were found (Zhang et al., 2015). One similar study reported that the *Salmonella* serovars isolated from ducks in Malaysia showed various resistance patterns against 13 different antibiotics (Adzitey et al., 2012). Therefore, new, safe interventions to minimize cross-contamination at all stages of food preparation, from handling live ducks and duck meat, to processing and market, are greatly needed.

Bacteriophages are very highly specific bacterial viruses, and therefore are potentially effective means of targeted biocontrol. The concept of using virulent phage against foodborne pathogens has been receiving increased interest and acceptance over the last decade. The application of phages to reduce *Salmonella* has been demonstrated for beef (Bigwood et al., 2008); pork (Wall et al., 2010); turkey breast (Guenther et al., 2012); chicken breast (Spricigo et al., 2013); hot dogs, mixed seafood, and egg yolk (Guenther et al., 2012); chicken skin (Hungaro et al., 2013); and pig skin and fresh eggs (Spricigo et al., 2013). However, few studies has been published on the use of phage against *Salmonella* in duck meat. Although these *Salmonella* phages were found and used in different foods, the narrow host specificity of phage limited its application on food industry (Guenther et al., 2012; Pereira et al., 2016a, 2016b; Sukumaran et al., 2015). The diversity of *Salmonella* strains in different areas calls for more broad host-spectrum phages to find out.

The aim of this study was to evaluate the efficiency of the new phage fmb-p1 of *Salmonella* and determine the effectiveness of using the phage for controlling *S.* Typhimurium in contaminated ready-to–eat (RTE) duck meat, to develop a non-chemical method for food decontamination and alleviate the risk of the development and spread of antimicrobial resistance to ensure food safety.

2. Materials and methods

2.1. Bacterial strains and growth conditions

For this study, 34 *Salmonella* strains and 12 non-*Salmonella* strains (Table 1) were collected from the China Center of Industrial Culture Collection (CICC), National Center for Medical Culture Collections (CMCC; Beijing, China), China Veterinary Culture Collection Center (CVCC), American Type Culture Collection (ATCC), and Guangdong Institute of Microbiology Culture Center (GIMCC; Guangdong, China). All bacterial strains were used to test the host range of the phage isolates, and one strain (CMCC50115) was used as a host for phage propagation. The cultures were stored in glycerol at −70 °C.

2.2. Selection and preparation of phages

According to the method described by Sambrook (2000), phage fmb-p1 was isolated from a sewage sample obtained at a local duck farm (Nanjing), and was incubated with *S.* Typhimurium strain CMCC50115. The double layer agar method was used to determine the titer of the phage stock. Dilutions of the phage stock (100 μL each) were made in sterile SM buffer (10 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris·HCl, pH 7.5), mixed with a suspension of exponential phase *Salmonella* (~10^9 CFU/mL, 100 μL), and added to 5 mL of molten (45 °C ≤ temperature ≤ 50 °C) LB agar (0.7%). The mixture was then poured onto surface-dried LB agar plates (LB broth + 2% agar). These soft agar overlays were allowed to set at room temperature for 5 min. Then, the plates were incubated at 37 °C for 24 h, and the number of plaques was counted on plates with 10–300 plaques. High titer stocks of phage were prepared by mixing 100 μL of the phage stock (~10^9 PFU/mL) with 100 μL of a *Salmonella* overnight culture (~10^9 CFU/mL) and 100 mL of LB broth and incubating the mixture for 12 h at 37 °C to amplify the phage. The culture was centrifuged at 10,000 × *g* for 10 min. Then, the supernatant was filtered through a disposable sterile syringe filter (0.22-μm pore size nylon membrane filter, Agela, USA), and the filtrate was stored at 4 °C until use.

Table 1
Host spectrum of phage fmb-p1.

Strain	Source	Lytic state	Strain	Source	Lytic state
S. Typhimurium	CMCC50115	+	*S.* Meleagridis	CICC21511	−
S. Typhimurium	DT104	+	*S.* Miami	CICC21509	+
S. Typhimurium	CICC21483	−	*S.* Montevideo	CICC21588	−
S. Choleraesuis	CICC21493	−	*S.* Paratyphi-A	CICC21501	−
S. Choleraesuis	ATCC13312	−	*S.* Paratyphi-B	CICC21495	−
S. Enteritidis	CVCC3374	+	*S.* Paratyphi-C	CICC21512	+
S. Enteritidis	CMCC50041	+	*S.* Potsdam	CICC21500	−
S. Enteritidis	CICC21527	−	*S.* Saintpaul	CICC21486	+
S. Adelaide	CICC21505	−	*S.* Thompson	CICC21480	−
S. Agona	CICC21586	+	*S.* Wandsworth	CICC21504	−
S. Anatum	CICC21498	+	***Non-Salmonella***		
S. Arizonae	CICC21506	−	*Listeria monocytogenes*	CICC21633	−
S. Bazenheid	CICC21587	−	*Listeria monocytogenes*	CICC21634	−
S. Blockley	CICC21489	−	*Listeria monocytogenes*	CICC21635	−
S. Bonariensis	CICC21496	−	*Listeria monocytogenes*	CICC21662	−
S. Bonn	CICC21677	−	*Escherichia coli*	ATCC35150	−
S. Bovis-morbificans	CICC21499	−	*Escherichia coli*	ATCC43889	−
S. Braenderup	ATCC19812	−	*Escherichia coli*	CICC23657	−
S. Dakar	CICC21507	−	*Escherichia coli*	ATCC25922	−
S. Dublin	CICC21497	−	*Staphylococcus aureus*	ATCC25923	−
S. Eastbourne	CICC21508	−	*Bacillus subtilis*	Fmb60	−
S. Heidelberg	CICC21487	+	*Bacillus subtilis**		−
S. Jerusalem	CICC21651	−	*Waxy bacillus**		−
S. Kentucky	CICC21488	−			

+, plaques; −, no plaques; *, keep in our lab.