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Feces of feedlot cattle contain a diversity of bacteriophages that lyse non-O157 Shiga toxin-producing *Escherichia coli*

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Abstract

This study aimed to isolate and characterize bacteriophages that lyse non-O157 Shiga toxin-producing *Escherichia coli* (STEC) from cattle feces. Of 37 non-O157 STEC-infecting phages isolated, O26- (AXO26A, AYO26A, AYO26B), O103- (AXO103A, AYO103A), O111- (AXO111A, AYO111A), O121- (AXO121A, AXO121B) and O145- (AYO145A, AYO145B) targeting phages were further characterized. Transmission electron microscopy showed the 11 isolates belonged to 3 families and 6 genera: the families *Myoviridae* (types rV5, T4, ViI, O1), *Siphoviridae* (type T5) and *Podoviridae* (type T7). Genome size of the phages as determined by pulsed-field gel electrophoresis ranged from 38 to 177 kb. Excluding phages AXO26A, AYO103A, AYO145A and AYO145B, all other phages were capable of lysing more than one clinically important strain from serogroups of O26, O91, O103, O111, O113, O121 and O128, but none exhibited infectivity across all serogroups. Moreover, phages AYO26A, AXO121A and AXO121B were also able to lyse 4 common phage types of STEC O157:H7. Our findings show that a diversity of non-O157 STEC-infecting phages are harbored in bovine feces. Phages AYO26A, AYO26B, AXO103A, AXO111A, AYO111A, AXO121A and AXO121B exhibited a broad host range against a number of serogroups of STEC and have potential for the biocontrol of STEC in the environment.

Keywords: Bacteriophages, biocontrol, *Escherichia coli*, non-O157, cattle

Introduction

Shiga toxin-producing *Escherichia coli* (STEC), in particular serogroups O26, O45, O103, O111, O121 and O145 are increasingly being recognized as important foodborne pathogens that cause outbreaks and sporadic cases of illnesses in humans worldwide (Johnson *et al.* 2006; Mathusa *et al.* 2010; Luna-Gierke *et al.* 2014; Smith *et al.* 2014). The severity of illnesses caused by non-O157 STEC may equal or even exceed that associated with STEC O157:H7, with clinical manifestations including diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Johnson *et al.* 2006; Hermos *et al.* 2011). Cattle are recognized as primary reservoirs of non-O157 STEC (Hussein and Bollinger 2005; Smith *et al.* 2014) and contaminated foods of bovine origin (Ethelberg *et al.* 2009; Mathusa *et al.* 2010; Robbins *et al.* 2014) or vegetable products contaminated with bovine feces (Hussein and Bollinger 2005; Smith *et al.* 2014) can be sources of infection for humans. The “top 6” non-O157 STEC (O26, O45, O103, O111, O121 and O145) have been declared as adulterants in raw beef products in the United States (USDA 2012). Having different evolutionary histories from O157 serotype, non-O157 STEC are a heterogeneous group of *E. coli* with limited information available about their virulence, fitness, and stress responses (Wang *et al.* 2013). This lack of knowledge and great heterogeneity of strains increases the complexity of developing strategies to reduce the risk of food and water becoming contaminated with these pathogens.

Bacteriophages (phages) are bacterial viruses that are abundant in the environment (Weinbauer, 2004). The capability of phages to infect specific bacterial pathogens without impacting commensal bacteria makes phage therapy a promising alternative to antimicrobials

(Kutateladze and Adamia 2010), especially with the ongoing challenges that antimicrobial resistance is presenting to the medical profession (Poole 2003; Mahony *et al.* 2011). Researchers have demonstrated that phages are potentially effective in mitigating STEC O157:H7 in ruminants (Sheng *et al.* 2006; Callaway *et al.* 2008; Raya *et al.* 2011) and decontaminating food products such as beef, broccoli, tomato, lettuce and spinach (Abuladze *et al.* 2008; Carter *et al.* 2012; Hong *et al.* 2014). Moreover, STEC O157:H7-infecting phages have been isolated from cattle feces (Callaway *et al.* 2008; Niu *et al.* 2009a; Hallewell *et al.* 2014) and phage ecology plays an important role in the fecal shedding of STEC O157:H7 in cattle and their environment (Niu *et al.* 2009a; Hallewell *et al.* 2014). To date, however, there are few reports on lytic properties of phages targeting non-O157 STEC, although phages with limited activity against non-O157 STEC serogroups have been isolated from ruminant feces (Viscardi *et al.* 2008; Dini and De Urreza 2010). Given these reports and the similarity of biological and epidemiological characteristics between STEC O157:H7 and non-O157 STEC serogroups (Smith *et al.*, 2014), we hypothesize that bovine feces would also be a natural source of phages with activity against non-O157 STEC. Consequently, the aims of the present study were to isolate and characterize endemic phages with activity against the “top 6” non-O157 STEC in cattle.

Materials and methods

Bacterial strains and growth conditions

All bacterial strains used in this study (Table 1) were obtained from Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, Guelph, ON. These strains comprised four strains of human or bovine origin of each of the “top 6” non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145, four strains of three other non-O157 serogroups implicated

in serious illness (O91, O113 and O128) and five strains of STEC O157:H7 representing the four most common phage types isolated from cattle and humans. Strains of some non-O157 STEC serogroups included serotypes with different H antigens (O111, O145, O113, O128, and O91). For isolation, purification, propagation, and titration of phages, STEC O26:H11 EC19970119, STEC O45:H2 EC19940040, STEC O103:H2 EC20010670, STEC O111:NM EC20030053, STEC O121:H19 EC20040083 and STEC O145:NM EC20020231 were singly used as hosts. All non-O157 STEC strains (n = 36) and STEC O157:H7 strains (n = 5) were used to evaluate host range and lytic capability of isolated phages. All bacterial strains were grown in tryptic soy broth (TSB, EMD Millipore, ON, Canada), and/or tryptic soy agar (TSA, Dalynn, Calgary, AB, Canada) at 37°C for 18 h.

Samples collection, phages isolation and propagation

Beef cattle fecal samples were obtained every two weeks from May 2013 to August 2013 from livestock transport trailers (A–E, ~ 47 cattle per trailer) in Alberta, Canada (Fig. 1 and Table 2). Approx. five 100-g fecal samples were collected from fresh fecal pats in each trailer compartment and pooled into a sterile whirl-Pak bag (Nasco, Salida, CA, USA) and transported to the laboratory within 12 h in an electric cooler (Model 5645, Coleman, Golden, CO, USA). To isolate phages from fecal samples, whirl-Pak bags containing feces were hand mixed to ensure homogeneity and then 20-g was transferred into 60 ml of sterile Lambda diluent (10 mmol·l⁻¹ Tris Cl, pH 7.5, 8 mmol·l⁻¹ MgSO₄) and processed in a laboratory mixer (Stomacher 400 Circulator, Seward Medical, London, UK) for 1 min at 230 rev min⁻¹. The samples were then allowed to stand for 30–60 min at room temperature to sediment large particulates and allow migration of phage particles into the buffer. A subsample of liquid (20 ml) was extracted from

the top layer, centrifuged at 11,000 *g* for 10 min, and filtered through a sterile 50-ml disposable vacuum filtration unit (0.2- μ m; Millipore, Billerica, MA, USA). The filtrate (1 ml) was added to a 5-ml early log phase (OD₆₀₀ = 0.2–0.3) culture of each single serotypes of non-O157 STEC in TSB with 10 mmol·l⁻¹ MgSO₄ and further incubated at 37°C in a rotary shaker (150 rev min⁻¹) (New Brunswick Scientific, Co., INC. Edison, NJ, USA) for 20 h. Individual phages were obtained by three cycles of plaque purification using the soft agar overlay technique (Sambrook and Russell 2001) and stock filtrates were prepared using the same non-O157 STEC strain of each serogroup as a host as described previously (Niu *et al.* 2012). Titers of phages in the stock filtrates were then determined by the soft agar overlay technique (Sambrook and Russell 2001).

Electron microscopy

For electron microscopy, phages were sedimented for 60 min at 18,000 *g* using a Microfuge[®] R centrifuge (Beckman Coulter Inc., Mississauga, ON, Canada). Samples were washed in buffer (10 mmol·l⁻¹ Tris Cl, pH 7.5, 10 mmol·l⁻¹ MgSO₄, 6.7 mmol·l⁻¹ CaCl₂, 0.5% (w/v) gelatin) and centrifuged for 60 min at 18,000 *g*. Purified phages were deposited on carbon-coated Formvar films on copper grids, stained with 2% Uranyl acetate (pH 4–4.5), and examined in a FEI Tecnai electron microscope (Tecnai G2 F20 model FEI, USA) at 200 kV.

Phage microplate virulence assay

Host range and lytic capability of the phages were assessed using a microplate phage virulence assay (Niu *et al.* 2009b). Briefly, high-titre phage stocks (10⁸–10¹⁰ PFU ml⁻¹) were serially diluted in 96-well microplates and incubated for 5 h at 37°C with overnight cultures of bacterial strains to estimate multiplicity of infection (MOI). After incubation, wells were

examined visually for turbidity and the highest dilution that resulted in complete lysis of bacteria was recorded. The MOI for each phage-host assay was calculated by dividing the initial number of phages in the highest-dilution causing complete lysis by the initial number of bacteria added, as determined by serially diluted plate counts of bacterial culture. Strains were then defined as sensitive ($0.000001 \leq \text{MOI} < 100$) or resistant ($\text{MOI} \geq 100$ as complete lysis did not occur) to the phages tested. Sensitivity to phage was further categorized as follows: extremely susceptible: ($0.000001 \leq \text{MOI} < 0.01$); highly susceptible: ($0.01 \leq \text{MOI} < 1$); moderately susceptible: ($1 \leq \text{MOI} < 10$); and minimally susceptible: ($10 \leq \text{MOI} < 100$).

Genome size estimation and restriction fragment length polymorphism (RFLP)

Bacterial nucleotides were removed from crude phage lysates (10^8 – 10^{10} PFU ml⁻¹) using DNase 1 (10 µg ml⁻¹ Sigma-Aldrich, Oakville, ON, Canada) and RNase A (10 µg ml⁻¹ Sigma-Aldrich), and the phage lysates were concentrated by use of polyethylene glycol 8000 (Sambrook and Russell 2001). Concentrated phage lysates were subjected to pulsed field gel electrophoresis (PFGE) for determination of genome size (Lingohr *et al.* 2009; Niu *et al.* 2012) using a CHEF DR II electrophoresis unit (Bio-Rad Laboratories, Mississauga, ON, Canada). For restriction enzyme analysis, phage DNA embedded in 1% SeaKem Gold agarose (Lonza, Rockland, ME, USA) was digested respectively, with Acc65I, BamHI, EcoRI, EcoRV, KpnI, HaeII, HindIII, SacI, SalI, PstI, XbaI and XhoI (New England Biolabs, Pickering, ON, Canada) for 16 h at 37°C. The plugs were then subjected to PFGE for 16–18 h using a pulse time of 1.0–15.0 s, 6 V·cm⁻¹ alongside with Low Range PFG marker (New England Biolabs). All PFGE digital images were then analyzed using BioNumerics 5.1 software (Applied Maths, Inc., Austin, TX). Restriction digestion banding patterns were analyzed using Dice similarity coefficients, unweighted pair

group methods arithmetic average algorithm with a 1% position tolerance, and 0.5% optimization.

Results

Phage isolation

A total of 37 phages infecting serogroups O26 (n = 10), O103 (n = 2), O111 (n = 2), O121 (n = 12) and O145 (n = 11) were isolated, but no phages with activity against O45 were isolated (Data not shown). For each serogroup, 2–3 phage isolates were selected for further characterization based on having a high titer against their corresponding host upon initial isolation (Tables 2 and 3). Each phage was designated as vB (bacterial virus), followed by Eco (*Escherichia coli*), M or S or P (*Myoviridae* or *Siphoviridae* or *Podoviridae*), A (Alberta), X or Y (Slaughter plants), specific serotypes of host for isolation with the suffix A or B (first or second phage isolates being characterized). For example, a phage isolated from serotype 26 is designated vB_EcoS_AXO26A, with AXO26A as the short form. Among the 11 phages, phages vB_EcoS_AXO26A, vB_EcoS_AXO103A and vB_EcoM_AXO121A were simultaneously isolated from fecal sample A of trailer A. vB_EcoM_AYO26A, vB_EcoP_AYO103A, vB_EcoM_AYO111A and vB_EcoM_AYO145A were concurrently isolated from fecal sample C of trailer C, and vB_EcoM_AYO26B were isolated along with vB_EcoM_AYO145B from fecal sample D of trailer D.

Phage Morphology

Overall, 11 phages displayed 6 distinct morphotypes upon examination using transmission electron microscopy (TEM; Table 3, Fig. 2). Structurally, excluding phages

AXO26A, AXO103A and AYO103A, all other phages had a long, contractile tail and were therefore assigned to the *Myoviridae* family. Phage AXO121A had an icosahedral head with diameter of 93 nm between opposite apices and an extended tail of 122×17 nm. Five to six thin tail fibers of 61 nm in length were occasionally seen. The morphology of AXO121A closely resembles that reported for phage rV5, in which the head measured 91 nm in diameter and the contractile tail was 121×17 nm with 70 nm tail fibers (Kropinski *et al.* 2013). Phages AYO26A and vB_EocM_AXO121B had a large elongated head with diameter of 115–117 nm. Striated contractile tails measured $109\text{--}118 \times 21\text{--}24$ nm with a collar, base plate, short spikes and long fibers (~ 133 nm). As a result, they were characterized as T4-like phages. Phages AYO26B and vB_EcoM_AXO111A possessed an icosahedral head of 95 nm in diameter and a contractile tail of $100\text{--}102 \times 16\text{--}20$ nm with terminal spikes, which corresponds to the morphology of *Salmonella* Typhi phage ViI and *E. coli* O157:H7-specific phage vB_EcoM_CBA120 (Ackermann *et al.* 1970; Pickard *et al.* 2010; Kutter *et al.* 2011). As such, they were classified as ViI-like phages. Phages AYO111A, AYO145A and AYO145B shared similar morphology, with the icosahedral heads of the former two being slightly smaller than the latter, resembling those of phage Felix O1, in which the head measured 73 nm in diameter and the non-contractile tail was 113×17 nm long (Ackermann 2007). Interestingly, ViI-like phages AYO26B and AXO111A shared the same head shape and diameter and tail length as the O1-like phage AYO111A, although different tail fiber structures were observed with the former two showing an intricate array of tail fibers and the latter displaying folded, long-tail fibers (Fig. 2C and 2G). In contrast, phages AXO26A and AXO103A had an icosahedral head 82–85 nm in diameter, but long ($178\text{--}196 \times 10$ nm) rigid, non-contractile tails that ended in a conical structure with terminal fibers (23×4 nm). This suggests that they are T5-like phages of the *Siphoviridae* family. Among

all the phages examined, phage AYO103A had the smallest head and shortest, non-contractile tail and was characterized as T7-like phage of the *Podoviridae* family.

Host range and lytic capability

With the exception of serogroup O45, at least one strain from the other 8 serogroups of non-O157 STEC tested was sensitive to one or more of the 11 phages studied. Excluding phages AXO26A, AYO103A, AYO145A and AYO145B, all other phages were capable of lysing a variety of strains across a range of non-O157 STEC serogroups (Table 1). Phages AYO26A, AYO26B, AXO103A, AXO111A, AYO111A, AXO121A and AXO121B, respectively, lysed 11, 12, 7, 10, 11, 13 and 8 of the 36 non-O157 STEC strains screened. Moreover, AYO26A, AXO121A and AXO121B were also able to lyse common phage types (2, 8, 14 and 14a) of *E. coli* O157 with moderate to extreme lytic capability.

Of the 10 STEC serogroups, O121-targeting phages (AXO121A and AXO121B) were able to lyse at least one strain out of the 8 serogroups including O26, O103, O111, O121, O113, O128, O91 and O157 with AXO121A showing broader host range and stronger lytic capability than AXO121B. Of 18 STEC strains sensitive to AXO121A, 13 from serogroups O103 (n = 2), O121 (n = 4), O113 (n = 1), O128 (n = 2) and O157 (n = 4) were classified as extremely sensitive (MOI = 0.00002–0.002) and 4 from serogroups O91 (n = 2), O103 (n = 1) and O157 (n = 1) as highly sensitive (MOI = 0.01–0.02) to phage.

Of O111-targeting phages, AXO111A and AYO111A displayed a similar host range and lytic capability (average MOI, 0.6 vs 0.9) against serogroups O26, O111, O121, O128 and O145.

Of the 21 sensitive strains to these phages, 5 from O111 and O128, 7 from O121 and O26, and 9 from O26, O111, O121 and O145 were extremely, highly or moderately susceptible, respectively. Host range and virulence of O26-targeting phages AXO26A, AYO26A and AYO26B were variable, with AYO26A exhibiting the broadest host range and lowest MOI against serogroup O26. AYO26A was also highly to extremely virulent to O145:NM and O103 strains (MOI = 0.0009–0.8). In contrast, AYO26B only infected 2 bovine strains of serogroup O26, but this phage was able to lyse 4 other non-O157 STEC serogroups including O111 (n = 1), O121 (n = 3), O145 (n = 4) as well as O128 (n = 2). Consequently, this phage exhibited a host lytic profile that was similar to AYO111A and AXO111A. Compared to AYO26A and AYO26B, host range of AXO26A was more limited and restricted to 3 of 4 O26 strains.

Phage genome sizing and RFLP analysis

Phages all possessed double-stranded DNA genomes that ranged in size from 38 to 197 kb (Table 3). Within genera T4-, ViI- and T5-like phages, phage isolates shared a similar genome size, whereas the genome size of O1-like phages differed with 89 kb for AYO145A, 68 kb for AYO145B and 151 kb for AYO111A.

In general, the DNA of phage was more sensitive to digestion by EcoRV (n = 11), HaeII (n = 9), EcoRI (n = 8), XbaI (n = 7) and HindIII (n = 6) than Acc65II (n = 4), BamHI (n = 4), KpnI (n = 4), SacI (n = 1), SalI (n = 2) and PstI (n = 2) (Table 4). All phage DNA was resistant to digestion by XhoI. The DNA of phages AXO26A, AXO103A, AYO103A and AXO121A were fragmented by most of the restriction enzymes examined with AXO26A and AXO103A sharing identical bands (Fig. 3A). Phages AYO26B, AXO111A, and AYO111A exhibited an

85–89% similar banding pattern after digestion with HaeII (Fig. 3B). In contrast, genomes of phages AYO26A and AXO121B remained intact after exposure to Acc65I, BamHI, EcoRI, HindIII, HaeII, KpnI, XbaI, SacI, SalI, PstI, XbaI and XhoI, but multiple restriction sites were found after digestion with EcoRV (Fig. 3C).

Discussion

In the current study, 37 phages lytic to non-O157 STEC strains were isolated from cattle fecal samples with further characterizing 11 of these. Based on TEM morphology, the 11 phage isolates were classified into rV5-, T4-, ViI- and O1-like phages of the *Myoviridae*, T5-like phages of the *Siphoviridae* and T7-like phages of the *Podoviridae*. The diversity of phages isolated may reflect the fact that fecal samples originated from livestock trailers which housed up to 47 of head of cattle transported from a diversity of geographical locations within western Canada. Viscardi *et al.* (2008) reported that two T4-like O157-infecting phages, isolated from bovine feces in Italy, were able to lyse serotypes O111, O113, O121 and O145 of non-O157 STEC, whereas Dini and Urraza (2010) found O113 phages of *Myoviridae* and O157 phages of *Myoviridae* and *Podoviridae* with activity against serotypes O103, O113 and O145 from bovine feces in Argentina. Accordingly, results of this study may indicate that non-O157 STEC infecting phages are ubiquitous in cattle. Moreover, multiple phages targeting various non-O157 STEC serogroups were recovered simultaneously from the same load of cattle. This observation is in agreement with the observation that a diversity of non-O157 STEC serogroups was also isolated from the same load of cattle (Stanford *et al.*, unpublished data). Probability of phage isolation increases with the presence of a susceptible host and consequently bovine feces that contains higher numbers of non-O15 STEC is also likely to be source of phages (Niu *et al.*

2009a). In the present study, phages with activity against serogroup O45 were not isolated, possibly as this serogroup was detected in only 12.6% of samples collected from May 2013 to August 2013 (Stanford *et al.*, unpublished data). Furthermore, the four strains of O45 evaluated were resistant to all 11 phage isolates, suggesting that these phages lacked the receptor for recognition and binding to the O45 serogroup.

Phages AYO26A, AYO26B, AXO103A, AXO111A, AYO111A, AXO121A and AXO121B exhibited a relatively broad host range as they lysed clinically important non-O157 STEC strains from multiple serogroups (O26, O91, O103, O111, O113, O121, O128 and O145) associated with mild to severe disease in humans (Johnson *et al.* 2006; Bettelheim 2007). Moreover, AYO26A, AXO121A and AXO121B were also able to infect several of the common phage types of *E. coli* O157:H7 (2, 8, 14 and 14a). The receptor binding proteins in phages are physically located on the long tail fibers and/or short tail spikes and interact specifically with receptors on the surface of the host cell (Marti *et al.* 2013; Samson *et al.* 2013). Different surface components have been identified as receptors for phages in bacteria, including the flagella, pili, lipopolysaccharides, capsular polysaccharides, teichoic acid, and outer membrane proteins (Hyman and Abedon 2010; Rakhuba *et al.* 2010). The lipopolysaccharide is composed of a lipid A anchored in the membrane, an oligosaccharide core and O-polysaccharide (O antigen) (Erridge *et al.* 2002). Among these receptors, O antigen of the lipopolysaccharides is considered to be a common receptor recognized by phages. Accordingly, the broad host range of phages AYO26A, AYO26B, AXO103A, AXO111A, AYO111A, AXO121A and AXO121B likely reflects their ability to target a common outer membrane protein or lipopolysaccharides region among susceptible strains. Some phages may also exhibit broader host spectrum by possessing more

than one receptor binding protein as is the case for the T7-like phage K1-5 which has two different receptor binding proteins enabling recognition and attachment to both *E. coli* K1 and *E. coli* K5 (Leiman *et al.* 2007). Schwarzer *et al.* (2012) found that the broad host range of rV5-like phage phi92 was a result of the presence of four different types of tail fibers and/or tail spikes that enabled this phage to infect both encapsulated and non-encapsulated bacteria including several strains of *E. coli* and *Salmonella*.

In addition, isolated phages varied in lytic capabilities both within and among non-O157 STEC serogroups. In general, a lower MOI was found for the serogroup of the non-O157 STEC strain originally used to isolate the phage as compared to susceptible strains from other serogroups. For example, phage AYO26A was extremely lytic against *E. coli* strains within the O26 serogroup (MOI = 0.000006–0.0007) with the exception of the O26 reference strain EC19930517 which was moderately susceptible to AYO26A (MOI = 1). Likely, physiological growth parameters of the phages such as adsorption rate, latent period and burst size differ from strain to strain within the same serogroup (Diaz-Munoz and Koskella 2014).

Phages AYO111A, AYO145A and AYO145B exhibited morphotypes characteristic of O1-like phages, but varied in genome size. The genome of O1-like phages previously identified ranged from 85 to 88 kb (Lehman *et al.* 2009; Villegas *et al.* 2009; Whichard *et al.* 2010), whereas the genome of AYO145A and AYO111A were 68 and 151 kb, respectively. More interestingly, O1-like phages AYO111A displayed a morphology and host range similar to the ViI-like phages, a phenotype that agrees with their similar genome sizes and RFLP profiles.

Further genome sequencing would reveal the degree of genetic relatedness among O1-like and ViI-like phages.

O26 phage AXO26A and O103 phage AXO103A, simultaneously isolated from the same fecal sample, were T5-like phages with the same genome size and identical DNA restriction profiles. However, their host range differed with AXO103A exhibiting activity against both serogroups O26 and O103 and AXO26A being infective only to O26. This suggests that although the genomes of AXO26A and AXO103A share similarities, there may be coding difference in long tail fibers and/or receptor binding proteins.

Of the 10 restriction enzymes tested, DNA of T4-like phages (AYO26A and AXO121B) was only digested by EcoRV, a result similar to that of T4-like *E. coli* O157-infecting phages (Raya *et al.* 2006; Hallewell *et al.* 2014). Most endonucleases do not digest T4-DNA, possibly due to the presence of glycosylated hydroxymethyl cytosine instead of cytosine (Miller *et al.*, 2003). This modification allows T4 DNA to be impervious to restriction-modification (R-M) systems that recognize specific sequences containing a cytosine (Labrie *et al.* 2010). Restriction-modification is one of strategies that bacterial cells utilize to guard against phage infection. As a result of their resistance to most restriction enzymes, T4-like phages may be more infective to STEC and thereby superior candidates for phage-based biocontrol strategies.

In conclusion, various types of phages from 6 genera across three *Myoviridae*, *Siphoviridae* and *Podoviridae* families were isolated from the feces of feedlot cattle. Multiple phages targeting different serogroups of non-O157 STEC were detected within feces collected

from the same trailer. Phages with infectivity against at least one isolate from STEC serogroups O26, O91, O103, O111, O113, O121, O128 and O145 were identified. As non-O157 STEC are a non-homogenous group, it is not surprising that none of the phages were able to infect all non-O157 STEC serogroups and a cocktail of phages will likely be required for controlling the “top 6” non-O157 STEC. As phages AYO26A, AYO26B, AXO103A, AXO111A, AYO111A, AXO121A and AXO121B exhibited a wide host range against a number of STEC serogroups, including O157:H7, these phages may be most appropriate for future use in a STEC biocontrol strategy.

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Table 1 Host range and lytic capability of 11 non-O157 STEC phages

Serogroups	Source*	Strain Ref.NO.(PT) [†]	Sensitivity to phages [‡]										
			O26 phages			O103 phages		O111 phages		O121 phages		O145 phages	
			AXO26A	AYO26A	AYO26B	AXO103A	AYO103A	AXO111A	AYO111A	AXO121A	AXO121B	AYO145A	AYO145B
O26:H11	B	EC19930517	R	1	4	R	R	R	R	R	R	R	R
O26:H11	B	EC19960464	9×10 ⁻⁵	7×10 ⁻⁴	2×10 ⁻¹	4×10 ⁻³	R	1×10 ⁻¹	2×10 ⁻¹	R	R	R	R
O26:H11	H	EC19970119	8×10 ⁻²	6×10 ⁻⁶	R	3×10 ⁻²	R	R	R	R	6	R	R
O26:H11	H	EC19990859	1	1×10 ⁻⁵	R	6×10 ⁻²	R	R	2	R	R	R	R
O45:H2	B	EC19940040	R	R	R	R	R	R	R	R	R	R	R
O45:H2	H	EC19970074	R	R	R	R	R	R	R	R	R	R	R
O45:H2	H	EC19970086	R	R	R	R	R	R	R	R	R	R	R
O45:H2	H	EC19970358	R	R	R	R	R	R	R	R	R	R	R
O103:H2	B	EC19970327	R	1×10 ⁻³	R	7×10 ⁻²	R	R	R	1×10 ⁻³	R	R	R
O103:H2	H	EC19970345	R	1×10 ⁻³	R	7×10 ⁻²	R	R	R	2×10 ⁻³	R	R	R
O103:H2	B	EC20010670	R	1×10 ⁻³	R	9×10 ⁻²	7	R	R	R	R	R	R
O103:H2	H	EC20020219	R	9×10 ⁻⁴	R	1×10 ⁻²	R	R	R	1×10 ⁻²	R	R	R
O111:H8	B	EC19930467	R	R	R	R	R	R	R	R	R	R	R
O111:H8	B	EC20000612	R	R	R	R	R	2	3	R	R	R	R
O111:NM	H	EC20000927	R	R	R	R	R	R	R	R	R	R	R
O111:NM	B	EC20030053	R	R	4×10 ⁻³	R	R	2×10 ⁻³	3×10 ⁻⁴	2	R	R	R
O121:H19	H	EC19960807	R	R	4×10 ⁻¹	R	R	1×10 ⁻¹	2×10 ⁻¹	2×10 ⁻⁴	1×10 ⁻¹	R	R
O121:H19	H	EC19990161	R	R	R	R	R	R	R	1×10 ⁻⁵	9×10 ⁻³	R	R
O121:H19	H	EC20020234	R	R	3×10 ⁻¹	R	R	1	2×10 ⁻¹	1×10 ⁻³	1	R	R
O121:H19	B	EC20040083	R	R	3×10 ⁻¹	R	R	1	2×10 ⁻¹	1×10 ⁻⁴	1×10 ⁻¹	R	R
O145:NM	H	EC19970355	R	1×10 ⁻¹	3	R	R	R	R	R	R	9×10 ⁻¹	1×10 ⁻¹
O145:H25	H	EC19990166	R	R	4	R	R	1	R	R	R	R	R
O145:NM	H	EC19990324	R	8×10 ⁻¹	3	R	R	1	2	R	R	8×10 ⁻¹	9×10 ⁻¹
O145:NM	H	EC20020231	R	8×10 ⁻¹	4	R	R	R	3	R	R	8×10 ⁻²	9×10 ⁻¹
O113:H4	B	EC19960371	R	R	R	R	R	R	R	R	R	R	R
O113:H4	B	EC19960434	R	R	R	R	R	R	R	R	R	R	R
O113:H21	H	EC19970352	R	R	R	R	R	R	R	R	7×10 ⁻⁴	R	R
O113:H21	H	EC20020170	R	R	R	R	R	R	R	1×10 ⁻³	9×10 ⁻³	R	R
O128:NM	H	EC19960949	R	R	6×10 ⁻⁴	R	R	2×10 ⁻⁴	4×10 ⁻⁴	3×10 ⁻⁴	R	R	R
O128:H2	H	EC19990162	R	R	2	R	R	1×10 ⁻²	4×10 ⁻³	1×10 ⁻⁴	R	R	R
O128:H10	H	EC20000914	R	R	R	R	R	R	R	R	R	R	R
O128:NM	H	EC20100049	R	R	R	R	R	R	R	R	R	R	R
O91:NM	O	EC19950329	R	R	R	R	R	R	R	1×10 ⁻²	R	R	R
O91:NM	O	EC19950340	R	R	R	R	R	R	R	R	R	R	R
O91:H21	H	EC20010076	R	R	R	R	R	R	R	R	R	R	R
O91:H21	B	EC20020030	R	R	R	R	R	R	R	2×10 ⁻¹	7	R	R
O157:H7	B	R508(14)	R	1	R	R	R	R	R	2×10 ⁻⁵	R	R	R
O157:H7	B	EC20110007(14a)	R	8×10 ⁻¹	R	R	R	R	R	1×10 ⁻³	R	R	R
O157:H7	H	EC19990298(14)	R	7×10 ⁻²	R	R	R	R	R	1×10 ⁻⁴	7	R	R
O157:H7	H	EC19990300(2)	R	9×10 ⁻²	R	R	R	R	R	1×10 ⁻⁴	9	R	R
O157:H7	H	EC20110005(8)	R	1×10 ⁻¹	R	R	R	R	R	2×10 ⁻²	13	R	R

* STEC strains isolated from, B: bovine; H: human; O: ovine.

[†] PT, phages types of STEC O157:H7.[‡] Sensitivity is based on multiplicity of infection (the lowest ratio of phage to bacteria that resulted in complete lysis of an overnight bacterial culture after 5 h of incubation).

R, no lysis observed.

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Table 2 Sampling information of 11 non-O157 STEC-infecting phages

Phage isolates	Targeting serogroups	Origin				Sampling date
		Geographical location*	Feedlot	Trailer	Fecal samples	
AXO26A	O26	A	A	A	A	May
AYO26A	O26	C	C	C	C	July
AYO26B	O26	D	D	D	D	August
AXO103A	O103	A	A	A	A	May
AYO103A	O103	C	C	C	C	July
AYO111A	O111	C	C	C	C	July
AXO111A	O111	E	E	E	E	July
AXO121A	O121	A	A	A	A	May
AXO121B	O121	B	B	B	B	May
AYO145A	O145	C	C	C	C	July
AYO145B	O145	D	D	D	D	August

*Capitals represent feedlots location in Mid (B) and South zone (A, C–E) of Alberta, Canada.

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Table 3 Taxonomy, genome size and morphology of 11 non-O157 STEC-infecting phages

Taxonomy		Phage isolates	Estimated genome size (kb)*	Head dimensions (nm)	Tail dimensions (nm)	
Genus	Family				Length	Width
rV5	<i>Myoviridae</i>	AXO121A	140	93	122	17
T4	<i>Myoviridae</i>	AYO26A, AXO121B	177	115–117	109–118	21–24
ViI	<i>Myoviridae</i>	AYO26B, AXO111A	151	92–95	100–102	16–20
O1	<i>Myoviridae</i>	AYO145A,	68–89	78–79	113–114	17–18
		AYO145B				
		AYO111A	151	92	105	20
T5	<i>Siphoviridae</i>	AXO26A, AXO103A	126	82–85	178–196	10
T7	<i>Podoviridae</i>	AYO103A	38	57	8	ND [†]

* Genome size was determined by pulsed field gel electrophoresis.

[†] ND, not detected.

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Table 4 Sensitivity of 11 non-O157 STEC-infecting phages to 12 restriction enzyme

Phage isolates	Sensitivity to restriction enzymes*												Total†
	Acc65 II	BamHI	EcoRI	EcoRV	HindIII	HaeII	KpnI	SacI	SalI	PstI	XbaII	XhoII	
AXO26A	+	+	+	+	+	+	+			+	+		9
AYO26A				+									1
AYO26B			+	+		+					+		4
AXO103A	+	+	+	+	+	+	+			+	+		9
AYO103A	+	+	+	+	+	+	+	+	+				9
AXO111A			+	+		+					+		4
AYO111A			+	+		+					+		4
AXO121A	+	+	+	+	+	+	+				+		8
AXO121B				+									1
AYO145A				+	+	+					+		4
AYO145B			+	+	+	+			+				5
Total‡	4	4	8	11	6	9	4	1	2	2	7	0	

*The DNA of phages was subjected to digestion of 12 restriction enzymes, respectively and “+” represent that phages was digestible with restriction enzymes.

†Total number of restriction enzymes showing digestion to phages.

‡Total number of phages sensitive to restriction enzymes.

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Figure captions

Figure 1. Geographical location of fecal samples collected for isolation of non-O157 STEC-infecting phages. Each blue pentagram represents fecal sampling from either Mid Zone (B) or South Zone (A, C–E) of Alberta, Canada.

Figure 2. Transmission electron microscopy of 11 non-O157-infecting phages. A, rV5-like phages (AXO121A); B, T4-like phages (AYO26A and AXO121B); C, ViI-like phages (AYO26B and AXO111A); D, O1-like phages (AYO145A and AYO145B); E, O1-like phage (AYO111A); F, T5-like phages (AXO26A and AXO103A); G, T7-like phages (AYO103A); bar represents 100 nm; Preparations were negatively stained with uranyl acetate. Arrowheads point to tail fibers where present.

Figure 3. Restriction fragment length polymorphisms of phage genomes alongside with low range PFG markers. A, genomic DNA of AXO26A and AXO103A digested with HaeII, HindIII and PstI, respectively B, genomic DNA of AXO121A, AYO26B, AYO111A and AXO111A digested with HaeII; C, genomic DNA of AYO145A, AYO145B, AYO103A, AXO121B and AYO26A digested with HaeII, SalI, EcoRI, EcoRV, and EcoRV, respectively.

Figure 1

Figure 1. Geographical location of fecal samples collected for isolation of non-O157 STEC-infecting phages. Each blue pentagram represents fecal sampling from either Mid Zone (B) or South Zone (A, C–E) of Alberta, Canada. The map was adapted from Alberta Zone Map published by government of Canada (http://www.labour.gc.ca/eng/standards_equity/contracts/schedules/Alberta/map.shtml). 121x172mm (300 x 300 DPI)

Figure 2

Figure 2. Transmission electron microscopy of 11 non-O157-infecting phages. A, rV5-like phages (AXO121A); B, T4-like phages (AYO26A and AXO121B); C, ViI-like phages (AYO26B and AXO111A); D, O1-like phages (AYO145A and AYO145B); E, O1-like phage (AYO111A); F, T5-like phages (AXO26A and AXO103A); G, T7-like phages (AYO103A); bar represents 100 nm; Preparations were negatively stained with uranyl acetate. Arrowheads point to tail fibers where present.
210x162mm (300 x 300 DPI)

Figure 3. Restriction fragment length polymorphisms of phage genomes alongside with low range PFG markers. A, genomic DNA of AXO26A and AXO103A digested with HaeII, HindIII and PstI, respectively B, genomic DNA of AXO121A, AYO26B, AYO111A and AXO111A digested with HaeII; C, genomic DNA of AYO145A, AYO145B, AYO103A, AXO121B and AYO26A digested with HaeII, SalI, EcoRI, EcoRV, and EcoRV, respectively.

230x119mm (300 x 300 DPI)