Effect of a bacteriophage T5virus on growth of Shiga toxigenic Escherichia coli and Salmonella strains in individual and mixed cultures

Yan D. Niu 1†, Hui Liu 2†, Roger P. Johnson 3, Tim A. McAllister 2 and Kim Stanford 4

Abstract
A previously isolated a bacteriophage, vB_EcoS_AKFV33 of T5virus, demonstrated great potential in biocontrol of Shiga toxigenic Escherichia coli (STEC) O157. This study further evaluated its potential as a biocontrol agent in broth culture against other important non-O157 serogroups of STEC and Salmonella. AKFV33 was capable of lysing isolates of STEC serogroups O26 (n = 1), O145 (n = 1) and Salmonella enterica serovars (n = 6). In a broth culture microplate system, efficacy of AKFV33 for killing STEC O26:H11, O145:NM and Salmonella was improved (P < 0.05) at a lower multiplicity of infection and sampling time (6–10 h), when STEC O157:H7 was also included in the culture. This phage was able to simultaneously reduce numbers of STEC and Salmonella in mixtures with enhanced activity (P < 0.05) against O157:H7 and O26:H11, offering great promise for control of multiple zoonotic pathogens at both pre and post-harvest.

Keywords: Bacteriophages, T5virus, Biocontrol, Shiga toxigenic Escherichia coli, Salmonella

Background
Shiga toxin-producing Escherichia coli (STEC) and Salmonella are often carried by food-producing animals and remain leading causes of foodborne illness worldwide [1]. However, few effective on-farm interventions have been established. Moreover, with emergence of STEC and Salmonella that are resistant to conventional interventions (e.g. heat, acid and chemical sanitizers [1]), novel approaches are needed to control these pathogens in both primary and secondary food production. Bacteriophages (phages) are viruses that naturally use bacteria as hosts, and when virulent, induce lysis of the infected bacteria. Commercial phage-based products have been used in the biocontrol of important foodborne bacteria including STEC and Salmonella [2]. However, several challenges remain before phages could be widely used in the food industry. One major challenge is that the host range of phages is often limited to certain species and even strains within species. Although such specificity is often desirable, phage treatment to decontaminate foods adulterated with multiple pathogenic species would often require phage cocktails, a preparation including multiple phages with each targeting a specific pathogen. However, limitations in the formulation of phage cocktails such as interference among phages and high manufacturing costs [2] make the identification of polyvalent phages that kill multiple bacterial host species particularly desirable.

Previously, we identified and systematically characterized a phage vB_EcoS_AKFV33 (AKFV33), a T5virus that possesses many of the desired features of a biocontrol agent [3]. Moreover, we found AKFV33 to be superior to phages T4virus, T1virus and rV5virus used individually or as phage cocktails for inactivating O157 STEC on refrigerated beef [4]. Since several T5virus phages have shown a diverse host range including Salmonella, non-O157

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serogroups of STEC and *Shigella* [5–10], we hypothesized that AKFV33 may have lytic activity against other serogroups of STEC and *Salmonella* strains. Consequently, the objective of this study was to evaluate the efficacy of AKFV33 in biocontrol of several selected serogroups of STEC and various *Salmonella* serovars in a broth culture system.

**Methods**

**Phage microplate virulence assay**

Host range and lytic activities of phage AKFV33 were assessed using a microplate phage virulence assay [11]. High titer phage stocks (>10⁹ plaque forming units (PUF)/ml) were propagated and filter-purified as previously described [3]. To estimate multiplicity of infection (MOI), the filter-purified phage stocks were serially diluted and incubated at 37°C without shaking for 5 h with 10-fold diluted overnight cultures of bacteria in a 96-well microplate. After incubation, wells were examined visually for turbidity and the highest dilution that resulted in complete lysis (no discernable turbidity) of bacteria was recorded. The MOI for each phage-host assay was calculated by dividing the initial number of phages in the greatest-dilution wells by the initial number of bacteria added, as determined from plate counts of serially diluted bacterial cultures. Sensitivity to phages was categorized as follows: extremely susceptible: (10⁻⁶ ≤ MOI < 10⁻⁵); highly susceptible: (0.01 ≤ MOI < 1); moderately susceptible: (1 ≤ MOI < 10); and minimally susceptible: (10 ≤ MOI < 100).

**Phage lysis kinetics**

To further assess dynamics of AKFV33 infection, a bacterial growth inhibition curve was conducted. Phage stocks (~10⁸ PFU/ml, 20 μl) were serially diluted in 96-well microplates and incubated individually for 10 h at 37°C with diluted overnight bacterial cultures (~10⁴ colony forming units (CFU)/ml⁻¹, 20 μl; Table 1), at final MOIs of 0.01, 0.1, 1, 10, 100 and 1000, respectively. Mixtures of the selected STEC and *Salmonella* strains (Table 1, ~10⁶ CFU/ml⁻¹ in total) were also set up in the same microplates and inoculated with AKFV33 at the same MOIs. Untreated control wells with only the bacteria in mTSBY (tryptic soy broth with 10 mmol l⁻¹ MgSO₄ and 0.6% yeast extract), and blank control wells containing only mTSBY were included in each microplate. The plates were incubated at 37°C and the optical density (OD₆₀₀nm) was read at 2 h intervals over 10 h using a Synergy™ HT multi-mode microplate reader (BioTek, Winooski, VT, USA). Two independent experiments were performed in duplicate. The blank values were subtracted from absorbance measures at 600 nm, to give a final corrected optical density.

**Enumeration of bacteria**

To determine if efficacies of AKFV33 against non-O157 STEC and *Salmonella* were repeatable in larger-scale broth cultures, individual and 3 mixed overnight cultures of STEC O157:H7 R508N, O26: H11 EC19960464 and *S. Typhimurium* ATCC14028 (1 mL, ~10⁵ CFU/ml⁻¹) were inoculated with AKFV33 at ~10⁹ PFU ml⁻¹ (MOI = 10⁴) in 9 mL of mTSBY and incubated at 37°C with shaking at 170 rpm. Subsamples (1.8 mL) for enumeration of the inoculated strains were withdrawn at 4, 7, 10 and 24 h centrifuged. Pellets were re-suspended in sterile PBS (phosphate-buffered saline), serially diluted and plated on tryptic soy agar with 50 g/ml nalidixic acid (Sigma, Oakville, ON, Canada; O157:H7), Rhamnose MacConkey (Innovation Diagnostics, Saint-Eustache, QC, Canada; O26:H11) and brilliant green agar (Oxoid, Toronto, ON, Canada; *S. Typhimurium*). Two independent experiments were performed in duplicate.

**Statistical analysis**

Results from phage lysis kinetics and enumeration of bacteria from larger scale broth cultures were compiled from two independent experiments, respectively. The OD values at 600 nm were square-root transformed and colony forming units were log-transformed. Influence of MOIs and time on phage efficacy were analyzed using the MIXED model with repeated measure. Least-squares were used to differentiate means (P < 0.05). The analyses were conducted with SAS (version 9.4, SAS Institute, Cary, NC).

**Results**

Of 36 non-O157 STEC strains including clinically important serogroups O26, O45, O91, O103, O111, O113, O121, O128 and O145 (n = 4 per serogroup), only STEC O26:H11 strain EC19960464 and O145:NM strain EC19970355 were extremely susceptible to AKFV33 at MOIs of 6 × 10⁻⁵ and 4 × 10⁻³, respectively. Of 39 *Salmonella* strains representing *Salmonella enterica* subspp. *Enterica* serovars Typhimurium, Enteritidis, Heidelberg, I 4 [5],12:i-, Saintpaul, Newport, Infantis, Hadar, Ago, Kumasi, Landau, Soerga and Urbana (n = 1–5 per serovar, Additional file 1: Table S1), only 1 of 5 *S. Typhimurium* strains (ATCC14028), 2 of 5 *S. I 4 [5],12:i- strains (20104603 and 20085085), 1 of 1 *S. Kumasi* strain (20015671) and 1 of 1 *S. Landau* strain (20015670) were highly or moderately susceptible to
Table 1: Effect of phage AKFV33 treated individual and mixture of STEC and Salmonella at different MOIs

| Species/Serotypes | Bacterial Source | Susceptibility\(^1\) | Mean OD\(_{600\text{nm}}\) at each MOI\(^2\) Across MOIs |
|-------------------|------------------|---------------------|--------------------------------------------------|
|                   |                  | Bacterial individual or mixed culture | Phage-free Control | 0.01 | 0.1 | 1 | 10 | 100 | 1000 | |
| STEC O157:H7      | R508N Bovine     | Individual          | 0.261            | 0.006a | 0.004a | 0.001a | 0.001a | 0.001a | 0a | 0.002 |
| STEC O26:H11      | EC19960464 Bovine| Individual          | 0.310            | 0.05a   | 0.011b  | 0.004b  | 0.002b  | 0b    | 0b   | 0.011 |
| STEC O145:NM      | EC19970355 Human | Individual          | 0.224            | 0.156a  | 0.06/7b | 0.067b  | 0.02c   | 0.015c | 0.003c | 0.055 |
| S. I 4, [5],12i   | 20104,603 Porcine| Individual          | 0.162            | 0.165   | 0.094a  | 0.097a  | 0.063a  | 0.063a | 0.048b | 0.088 |
| S. Typhimurium    | ATCC14028 Porcine| Individual          | 0.262            | 0.227   | 0.207   | 0.220   | 0.174   | 0.221  | 0.038a | 0.181 |
| S. Typhimurium    | ATCC14028 Porcine| Individual          | 0.262            | 0.227   | 0.207   | 0.220   | 0.174   | 0.221  | 0.038a | 0.181 |
| S. Typhimurium    | ATCC14028 Porcine| Individual          | 0.262            | 0.227   | 0.207   | 0.220   | 0.174   | 0.221  | 0.038a | 0.181 |

\(^1\)Susceptibility of strains to phages were determined by microplate phage virulence assay for each MOI (Multiplicity of infection) value

\(^2\)The blank values were subtracted from absorbance measures at 600 nm, to give a final corrected optical density. Mean OD\(_{600\text{nm}}\) at each MOI were calculated by averaging OD\(_{600\text{nm}}\) from 2h, 4h, 6h, 8h and 10h

Letters which differ after the mean values indicate differences \(P<0.05\) among MOIs within each bacterial culture

Asterisks *, ** and *** indicate a statistical difference between phage-treated individual and mixed culture within same MOI at \(P<0.05\), \(P<0.01\) and \(P<0.001\), respectively
AKFV33 with MOIs ranging from 0.5 to 4. Although complete lysis of other strains was not observed after 5 h of phage treatment, phage-treated cultures \( (n = 3, 1 \text{ and } 1, \text{ respectively}) \), from STEC O26, S. Ago and S. Soerenga showed complete lysis at MOI = 2–10 at 2 h (data not shown). The subsequent re-growth after 2 h may indicate rapid emergence of phage-resistant mutant strains in these cultures, which complies with previous studies of other T5viruses strains [6, 7]. The ability of AKFV33 to lyse some non-O157 STEC and Salmonella strains is consistent with other reports that T5viruses may have broad host ranges across multiple bacterial species [5–10]. The susceptibility of the Salmonella serovars Ago, Kumasi, Landau and Soerenga strains in the present study may be explained at least in part by their somatic (O) antigens, which are important phage receptors in Gram-negative bacteria [12]. All these serovars possess the Group N O30 Salmonella antigen, which is antigenically strongly related to the O157 antigen of E. coli [13], and hence may enable binding of the O157-infecting phage AKFV33 to these Salmonella serovars.

Across MOIs and times, AKFV33 inhibited growth of all the individual bacteria and their mixtures with OD600nm ranging from 0.002 to 0.181 \( (P < 0.05; \text{ Table 1}) \). Moreover, AKFV33 at MOI < 10 was more efficient at lysing STEC O145:NM strain EC19970355 \( (P < 0.01) \) in mixtures \( (\text{O145:NM + O157:H7 and O26:H11 + O145:NM + O157:H7}) \) than in individual cultures, and at MOI = 0.01, phage activity against STEC O26:H11 strain EC19960464 in mixed cultures \( (\text{O26:H11 + O157:H7 and} \text{S. Typhimurium ATCC14028}) \).

**Fig. 1** Growth curves of selected STEC and Salmonella strains in individual and mixed cultures treated and not treated with phage AKFV33 across MOIs. a O157:H7 R508N; b O26:H11 EC19960464; c O145:NM EC19970355; d S. I4, [5],12:i- 20104603; e S. Typhimurium ATCC14028; f Mixture of STEC O157:H7 R508N, O26:H11 EC19960464 and O145:NM EC19970355; g Mixture of STEC O157:H7 R508N and Salmonella S. I4, [5],12:i- 20104603 and S. Typhimurium ATCC14028. Bars present standard deviations. Asterisks *, ** and *** indicate a statistical difference of OD600nm value within same sampling time between phage-treated and non-treated individual or mixed culture at \( P < 0.05, P < 0.01 \text{ and } P < 0.001 \), respectively. For \( f \) and \( g \), *** indicates significance evident in all phage-treated 3 pathogen mixtures; For \( f \), at 6 h, ns indicates OD600nm value did not differ between phage-treated and non-treated 2 mixture of S. I4, [5],12:i- + O157:H7, whereas * and **, respectively, indicate OD600nm value differed between phage-treated and non-treated 2 mixture of S. Typhimurium + O157:H7 and mixtures of 3 pathogenic bacteria.
O26:H11 + O145:NM + O157:H7 was also increased ($P < 0.05$). AKFV33 exhibited less activity against *Salmonella* than against STEC, inhibiting growth of *S. I4*, [5],12:i- strain 20104603 at MOI > 0.01 ($P < 0.001$) and *S. Typhimurium* ATCC14028 at MOI = 1000 ($P < 0.05$). However, in 2 mixtures (*S. I4*, [5],12:i- + O157:H7 and *S. Typhimurium* + O157:H7), activity of AKFV33 against *Salmonella* was substantially greater, as growth of *S. I4*, [5],12:i- strain 20104603 at MOI > 0.01 ($P < 0.001$) and *S. Typhimurium* ATCC14028 at MOI = 1000 ($P < 0.05$). However, in 2 mixtures (*S. I4*, [5],12:i- + O157:H7 and *S. Typhimurium* + O157:H7), activity of AKFV33 against *Salmonella* was substantially greater, as growth of *S. I4*, [5],12:i- strain 20104603 at MOI > 0.01 ($P < 0.001$) and *S. Typhimurium* ATCC14028 at MOI = 1000 ($P < 0.05$).
[5],[12]:- strain 20104603 (P<0.05) was reduced at all MOIs and S. Typhimurium strain ATCC14028 (P<0.001) at MOI<1000. Furthermore, this enhanced phage activity was also evident in a 3 bacterial mixed culture (S. 1 4, [5],[12]:+ S. Typhimurium + O157:H7). In addition, AKFV33 was more active against O26:H11 (6 h) and O145:NM (6, 8 and 10 h, S. 1 4, [5],[12]: (8 and 10 h for mixture of two pathogens, 6, 8 and 10 h for 3 a mixture of two pathogens) and S. Typhimurium (6, 8 and 10 h) across all MOIs (P<0.001) in mixed cultures containing STEC O157:H7 than in individual cultures of these strains without STEC O157:H7 (Fig. 1).

For individual bacterial cultures, AKFV33 caused an overall reduction of 7.5±0.4 log_{10} CFU/ml in O26:H11, greater (P<0.001) than those in STEC O157:H7 (2.5±2.7 log_{10} CFU/ml) or S. Typhimurium (2.2±1.2 log_{10} CFU/ml, Fig. 2). The greatest efficacy of the phage (P<0.001) was at 4 and/or 7 h, but was reduced (P<0.001) thereafter. Notably, after 24 h of incubation, phage treatment had no effect (P>0.1) on the numbers of O157:H7 or S. Typhimurium. When exposed to a mixture of O157:H7, O26:H11 and S. Typhimurium, AKFV33 was able to simultaneously reduce (P<0.01) numbers of each bacteria in the mixtures by 2–8 log_{10} CFU/ml (Fig. 2). Moreover, both O157:H7 and O26:H11 in the mixture were undetectable (<300 CFU/ml) at each sampling time, even after 24 h. This indicates that AKFV33 was more active and/or the targeted STEC were more vulnerable to the phages (P<0.05) in mixed cultures. In contrast, S. Typhimurium was equally sensitive to the phages either alone or in a mixture with O157:H7.

**Discussion**

To our knowledge, this is the first study to evaluate the effectiveness of a polyvalent phages *T5ivirus* in control of STEC and *Salmonella* in a mixed culture. In our previous studies, AKFV33 was shown to be highly virulent to various phage types of STEC O157 strains [3], but its virulence for other foodborne pathogens was unknown. Here we have found that AKFV33 is virulent for a broad host range that includes some non-O157 STEC and *Salmonella* serovars, and that in mixed cultures, AKFV33 not only simultaneously reduces numbers of STEC and *Salmonella*, but in some instances also has greater efficacy. Further study is required to understand mechanism(s) underlying this improved efficacy. Potentially, replication of AKFV33 in a preferred host (O157:H7) and enhanced concentrations of phage led to improved control of non-preferred hosts (*Salmonella* and non-O157 *E. coli*). In addition, this finding was consistent with earlier reports that phage av-08 (unknown taxonomy) was able to decontaminate *S. Montevideo* and STEC O157:H7 on chicken skin [14]. Costa et al. [15] also found that single phage ELY-1 or phSE-5 (unknown taxonomy) reduced number of non-O157 *E. coli* and *S. Typhimu- rium* ATCC13311 in a mixture, although this re-duction was less than produced by a cocktail of both of these phages in broth culture. The relative con-tribution of polyvalent phages vs phage cocktails to bacte-rial biocontrol remains unclear. However, Zhao et al. [16] reported that a polyvalent phage of the *Siphoviridae* was effective in decreasing population of *E. coli* K12 and *Pseudomonas aeruginosa* in a soil-carrot system. Although less effective than a cocktail of phages against these organisms, polyvalent phages were more capable than the phage cocktail of sustain-ing the diversity of the commensal bacterial community in the system. In another study, a polyvalent phage of the *Podoviridae* in combination with biochar treatment effectively eliminated *E. coli* K12 and *P. aeruginosa* in a soil-lettuce system, while synergisti-cally enhancing indigenous bacterial communities [17]. This suggests that polyvalent phages such as AKFV33 may be used for simultaneous inhibition of various zoonotic bacterial pathogens without harming benefi-cial microbes resident in gastro-intestinal tracts of food animals or in food products.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s12985-019-1269-7.

**Additional file 1 : Table S1.** Host range and lytic activity of Phage AKFV33 against *Salmonella* strains

**Abbreviations**

MOI: Multiplicity of infection; mTSB: Tryptic soy broth with 10 mmol l^{-1} MgSO4 and 0.6% yeast extract; OD: Optical density; PBS: Phosphate-buffered saline; STEC: Shiga toxigenic *Escherichia coli*

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**Authors’ contributions**

YDN conceived and designed the study. HL performed the experiments. YDN and HL analyzed the data. YDN, HL, RPJ, TAM and KS wrote the paper. All authors read and approved the final manuscript.

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**Availability of data and materials**

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Competing interests
The authors declare no competing interest.

Author details
1Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 1N4, Canada. 2Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B1, Canada. 3National Microbiology Laboratory, Public Health Agency of Canada, Guelph, ON N1G 3W4, Canada. 4Alberta Agriculture and Forestry, Agriculture Centre, Lethbridge, AB T1J 4V6, Canada.

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References
1. Heredia N, García S. Animals as sources of food-borne pathogens: a review. Animal Nutrition. 2018;4:250–5.
2. Moye ZD, Woolston J, Sulakvelidze A. Bacteriophage applications for food production and processing. Viruses. 2018;10:205.
3. Niu YD, Stanford K, Kropinski AM, Ackermann H-W, Johnson RP, She Y-M, et al. Genomic, proteomic and physiological characterization of a T5-like bacteriophage for control of Shiga toxin-producing Escherichia coli O157:H7. PLoS One. 2012;7:e34585.
4. Liu H, Niu YD, Meng R, Wang J, Li J, Johnson RP, et al. Control of Escherichia coli O157 on beef at 37, 22 and 4°C by T5-, T1-, T4- and O1-like bacteriophages. Food Microbiol. 2015;31:69–73.
5. Rabieh M, Ma L, Wiley G, Najar FZ, Kaserer W, Schuerch DW, et al. FepA- and TonB-dependent bacteriophage H8: receptor binding and genomic sequence. J Bacteriol. 2007;189:5658–74.
6. Hong J, Kim KP, Heu S, Lee SJ, Adhya S, Ryu S. Identification of host receptor and receptor-binding module of a newly sequenced T5-like phage EPS7. FEMS Microbiol Lett. 2008;289:202–9.
7. Kim M, Ryu S. Characterization of a T5-like coliphage, SPC35, and differential development of resistance to SPC35 in Salmonella enterica serovar Typhimurium and Escherichia coli. Appl Environ Microbiol. 2011;77:2042–50.
8. Dalmasso M, Strain R, Neve H, Franz CM, Cousin FJ, Ross RP, et al. Three new Escherichia coli phages from the human gut show promising potential for phage therapy. PLoS One. 2016;11:e0156773.
9. Amrar I, Rubi-Rangel L, Cháidez C, González-Robles A, Lightbourn-Rojas L, León-Félix J. Isolation and characterization of a phiLLS, a novel phage with potential biocontrol agent against multidrug-resistant Escherichia coli. Front Microbiol. 2017;8:1355.
10. Sváb D, Falgenhauer L, Rohde M, Szabó J, Chakraborty T, Tóth I. Identification and characterization of T5-like bacteriophages representing two novel subgroups from food products. Front Microbiol. 2018;9:202.
11. Niu YD, Johnson RP, Xu Y, McAllister TA, Sharma R, Louie M, et al. Host range and lytic capability of four bacteriophages against bovine and clinical human isolates of Shiga toxin-producing Escherichia coli O157:H7. J Appl Microbiol. 2009;107:646–56.
12. Broeker NK, Barbirz S. Not a barrier but a key: how bacteriophages exploit host’s O-antigen as an essential receptor to initiate infection. Mol Microbiol. 2017;105:353–7.
13. Westerman RB, He Y, Keen JE, Littelkile ET, Kwang J. Production and characterization of monoclonal antibodies specific for the lipopolysaccharide of Escherichia coli O157. J Clin Microbiol. 1997;35:679–84.
14. López-Cuevas O, Castro-Del Campo N, León-Félix J, Valdez-Torres B, Cháidez C. Evaluation of bacteriophage av-08 for simultaneous biocontrol of Salmonella Montevideo and Escherichia coli O157:H7 in experimentally contaminated chicken skin. J Food Safety. 2012;32:305–10.
15. Costa P, Pereira C, Gomes ATPC, Almeida A. Efficiency of single phage suspensions and phage cocktails in the inactivation of Escherichia coli and Salmonella Typhimurium: an in vitro preliminary study. Microorganisms. 2019;7:94.
16. Zhao Y, Ye M, Zhang X, Sun M, Zhang Z, Chao H, et al. Comparing polyvalent bacteriophage and bacteriophage cocktails for controlling antibiotic-resistant bacteria in soil-plant system. Sci Total Environ. 2019;657:918–25.
17. Ye M, Sun M, Zhao Y, Jiao W, Xia B, Liu M, et al. Targeted inactivation of antibiotic-resistant Escherichia coli and Pseudomonas aeruginosa in a soil-lettuce system by combined polyvalent bacteriophage and biochar treatment. Environ Pollut. 2018;241:978–87.

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