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Efficacy of novel phages for control of multi-drug resistant *Escherichia coli* O177 on artificially contaminated beef and their potential to disrupt biofilm formation

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ABSTRACT

Contaminated beef is a prominent source of foodborne pathogens such as *Escherichia coli* O177. Susceptibility of nine multi-drug resistant *E. coli* O177 strains against eight individual phages and six phage cocktails was assessed using polystyrene microplate titer plate. Further, 180 beef samples were independently inoculated with *E. coli* O177 cells in triplicates and treated with eight individual phages and six phage cocktails to determine their efficacy in inhibiting bacteria growth at 4 °C over a 7-day incubation period. Results revealed that all *E. coli* O177 strains were susceptible to the phages. A significant log reduction in viable *E. coli* O177 cell counts was observed on beef samples upon phage treatment over the 7-day incubation period. Two individual phages and three phage cocktails reduced *E. coli* cell counts to levels below the detection limit (1.0 $\log_{10}$ CFU/g). Log reduction of viable *E. coli* cell counts ranged from 2.10 to 7.81 CFU/g for individual phages and from 2.86 to 7.81 CFU/g for cocktails. Individual phages and phage cocktails inhibited *E. coli* O177 biofilm formation with phage cocktails showing high efficacy. Furthermore, phage cocktails showed greater efficacy in destroying pre-formed biofilm than individual phages. Based on these findings, we concluded that phage cocktails developed in this study could be used to reduce *E. coli* O177 contamination and extend the shelf-life of stored raw beef.

1. Introduction

The atypical enteropathogenic *E. coli* (aEPEC) is a heterogeneous group of foodborne pathogens, which causes infantile diarrhoea with high mortality in both developed and developing countries (Hu and Torres, 2015). More than 600 million people become ill and 420 000 die each year due to foodborne pathogens (WHO, 2015). The aEPEC strains are commonly found in the gastrointestinal tracts of animals, especially ruminants (Montso et al., 2019a). While the transmission pathways of *E. coli* O177 from ruminants to humans are complex, the most direct route is through contamination of products such as meat and milk produced during slaughter or milking, respectively (Fegan and Jenson, 2018). Indeed, Shebs et al. (2020) report that *E. coli* from the hides and gastrointestinal tract contents cause significant cross contamination of the carcass during the slaughtering process. Furthermore, pre-slaughter starvation may lead to *E. coli* proliferation in the rumen and thus posing a greater meat contamination hazard (Rasmussen et al., 1993; Tkalcic et al., 2000; Larsen et al., 2014). This may increase risks of food safety violation and lead to major repercussion for public health. In addition, decontamination of carcass via trimming process may result in heavy losses by meat producers.

Several strategies are currently being used to reduce microbial contamination of food and to preserve and extend shelf-life of food (Sillankorva et al., 2012; Tan et al., 2014; Duc et al., 2018). However, traditional physical and chemical processes used for this purpose have serious drawbacks that include corrosive effects on food processing plants and changes in organoleptic properties of food, especially meat (Sillankorva et al., 2012; Endersen et al., 2014). Residues from chemical interventions may also have detrimental effects on human health as well as the environment. In addition, chemical disinfectants are known to induce bacteria to aggregate and form biofilm structures (Carpio et al., 2019). Bacteria cells within these biofilm structures are extremely resistant to heat, cold, disinfectants and antimicrobials used in food industry (Carpio et al., 2019). As a result, biofilm formation in food processing equipment may lead to persistent contamination of food processing plants (Dzieciol et al., 2016). It is against this background

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that efforts to identify and evaluate novel, safe and sustainable control methods need to continue to improve food safety.

Bacteriophage therapy is one such promising natural and green technology for food preservation and safety (Sillankorva et al., 2012; Endersen et al., 2017; Harada et al., 2018). Application of lytic bacteriophages for the prevention and control of antimicrobial resistant foodborne pathogens is thus regarded as a potential alternative to combat microbial contamination in the food industry. However, the emergence of phage resistance in single phage therapy is a cause for concern (Labrie et al., 2010). Phage resistance may occur as a result of alteration and/or loss of bacterial cell surface receptors required for phage attachment, gene mutation and production of modified restriction endonucleases, which degrade phage DNA (Chan et al., 2013; Tsonos et al., 2014). The limitations of single phage application could be overcome using phage cocktails (Chen et al., 2018). Phage cocktails target different host cell receptors and can be used to circumvent the development of phage-resistant bacterial mutants and thus broaden the host range (Tsonos et al., 2014). A number of phage cocktails such as ListShield™, EcoShield™, SalmoFresh™ and Salmonelex™ have been demonstrated to be effective in reducing phage resistant mutants (Duc et al., 2018; Harada et al., 2018). Bacteriophages are capable of reducing foodborne pathogen on meat and have shown to prevent biofilm formation and or existing biofilm structure (García-Anaya et al., 2020). To the best of our knowledge, no study has assessed the utility of phages as inhibitors of biofilm formation and growth in *E. coli* O177 on raw meat. Therefore, this study was designed to evaluate the efficacy of individual phages and phage cocktails in reducing *E. coli* O177 cell counts on raw beef, preventing biofilm formation and destroying existing biofilm structures of *E. coli* O177.

2. Materials and methods

2.1. Bacterial culture

Ten multi-drug resistant atypical enteropathogenic *E. coli* O177 isolates were used in this study. Details on the study area, sampling, isolation procedures, and identification, virulence and multi-drug resistance profiles of atypical enteropathogenic *E. coli* O177 isolates have been described in our previous study (Montso et al., 2019a). In brief, stock cultures of *E. coli* O177 isolates from −80 °C freezer were and resuscitated on MacConkey agar. The plates were incubated at 37 °C for 24 h. After incubation, a single colony was transferred into 15 mL sterile falcon tube containing 5 mL nutrient broth and the tube was incubated at 37 °C for 24 h. After incubation, the optical density (OD_{630mn}) was measured using spectrophotometer. Nutrient broth was used to adjust the OD to 0.5 McFarland standard.

2.2. Bacteriophages propagation

Eight lytic bacteriophages (vB_EcoM_10C2, vB_EcoM_10C3, vB_EcoM_118B, vB_EcoM_11B2, vB_EcoM_12A1, vB_EcoM_366B, vB_EcoM_366V and vB_EcoM_3A1), previously isolated from cattle faeces in the North West province, South Africa (Montso et al., 2019b), were propagated using *E. coli* O177 host as previously described (Sambrook and Russell, 2001). Briefly, 100 μL (0.5 McFarland standard) of overnight culture of *E. coli* O177 was added into a 50 mL falcon tubes containing 30 mL tryptic soya broth (TSB). Aliquot of 100 μL of each phage was added to their respective tubes. The tubes were incubated at 37 °C for 24 h in a shaking incubator (80 rpm). After incubation, the tubes were centrifuged at 10 000×*g* for 10 min at 4 °C. The supernatant was filter-sterilised using 0.22 μm pore-size acrodisc syringe filter. Ten-fold serial dilutions were prepared and plaque assay was performed to determine the titer of each phage. The concentration was expressed as plaque forming unit per millimetre (PFU/mL). Phage cocktails were prepared by mixing individual phages of equal volume (1:1), depending on the number of phages mixed per cocktail and 32 cocktails with different combinations were obtained. Both individual phage and phage cocktail titers were standardised using lambda diluent buffer [5.8 g/L NaCl, 2 g/L $MgSO4.7H_2O$, 10 ml/L Tris-HCl (pH 7.5)] to obtain 1×10^8 PFU/mL.

2.3. Microplate virulence assay

2.3.1. Individual phage microplate virulence assay

Susceptibility of nine multi-drug resistant *E. coli* O177 isolates to eight individual phages was assessed using microplate virulence assay as previously described (Niu et al., 2009). In brief, 180 μL of tryptic soya broth supplemented with 10 mM magnesium sulphate (mTSB) was dispensed into 96-well polystyrene plates. In the first row (A-1-8 wells), 20 μL of each phage isolate (stock: 1×10^8 PFU/mL) was inoculated in duplicates and serially diluted in 10-folds (10^{-1} to 10^{-8}). Subsequently, aliquot of 20 μL of overnight culture of each *E. coli* O177 isolate (1×10^8 CFU/mL) was inoculated into each wells (row: 1–8; column: A-H). The ninth and tenth wells served as negative controls, as such they were only inoculated with host bacteria (20 μL) without phage treatment. The eleventh and twelfth wells (served as positive/blank control) were inoculated with mTSB (20 μL) with both phage and bacteria. The plates were incubated at 37 °C for 5 h. After incubation, phage activity was assessed by measuring Optical Density (OD; 630_{nm}) using microplate reader (Model: MB580). The highest dilution that resulted in complete lysis (as seen by the absence of turbidity in the culture medium) of the host bacteria was recorded. The data was used to calculate multiplicity of infection (MOI). The MOI for each phage was determined by dividing the initial concentration of phage (PFU/mL) in the inoculum with the initial concentration of host bacteria (CFU/mL). The sensitivity of the bacteria isolates to phages was defined as susceptible (MOI $<$ 100) or resistant (MOI $\geq$ 100) as previously described (Niu et al., 2009). Sensitivity to phages was further categorised as extremely susceptible (MOI $<$0.01); highly susceptible (0.01 $\leq$ MOI $<$ 1); moderately susceptible (1 $\leq$ MOI $<$ 10) and minimally susceptible (10 $\leq$ MOI $<$ 100). The following equation was used to calculate MOI.

$$\text{Equation : MOI (for equal volumes)} = \frac{\text{No of PFU/ mL}}{\text{No of CFU/ mL}}$$

The data was grouped accordingly (extremely susceptible, highly susceptible, moderately susceptible or non-susceptible) and used to construct a graph.

2.3.2. Phage cocktail microplate virulence assay

Susceptibility of three multi-drug resistant *E. coli* O177 isolates to 32 phage cocktails was assessed using microplate virulence assay as previously described (Niu et al., 2009). The selection criteria of host bacteria (isolates) were based on resistance to individual phages (incomplete lysis observed on microplate virulence assay). Microplate virulence assay was performed as described in Section 2.3.1 above. The MOI for each phage cocktail and sensitivity of bacterial to phages were determined as previously described (Niu et al., 2009). Phage cocktails showing high lytic activity and low MOI to *E. coli* O177 isolates were selected for further experiments.

2.4. Effect of phages on E. coli O177 on contaminated beef

2.4.1. Sample preparation and experimental design

Two experiments were performed simultaneously to evaluate the efficacy of individual phages and phage cocktails in reducing multi-drug resistant *E. coli* O177 strain on artificially contaminated beef. Eight individual phages (vB_EcoM_10C2, vB_EcoM_10C3, vB_EcoM_118B, vB_EcoM_11B2, vB_EcoM_12A1, vB_EcoM_366B, vB_EcoM_366V and vB_EcoM_3A1) and six phage cocktails [(vB_EcoMC1, vB_EcoMC2, vB_EcoMC3, vB_EcoMC4, vB _EcoMC5 and vB_EcoMC6) (selected based on lytic activity, host range and the best MOI against multi-drug resistant *E. coli* O177 strain)] were used in this experiment. Beef steak (5.5

kg) was purchased from local supermarket and the meat was placed in a cooler box containing ice packs and transported to the Antimicrobial Resistance and Phage Biocontrol Laboratory (AREPHABREG), NWU. The meat was processed within an hour of collection. Briefly, the samples were processed in the laminar flow hood and the meat was aseptically cut into small pieces (3 cm^3, weighing ~ 25 g) using a sterile pair of scissors and rat tooth forceps. For individual phage treatment, a total of 108 meat samples were placed in the sterile petri-dishes (90 mm) and were randomly assigned in triplicates to nine treatments [eight individual phages (1×10^8 PFU/mL) and one control (lambda diluent)] per incubation period. For phage cocktails, 72 meat samples were placed in sterile petri-dishes (90 mm) and randomly assigned in triplicates to seven treatments [six phage cocktails, (1×10^8 PFU/mL) and one negative control (lambda diluent)] and one control (lambda diluent buffer)] per incubation period. The remaining 1 kg of the meat sample was used to determine the pH as well as screening for the presence of background *E. coli* and/or any microbial contamination by standard culture technique.

2.4.2. Sample inoculation, bacteriophage application and bacteria enumeration

A 100 μL of exponential phase (*E. coli* O177; $OD_{630nm} = 0.5$) culture was pipetted onto the surface of each meat sample to simulate natural contamination and the plates were left in the laminar flow hood for 10 min to allow the bacteria to attach to the meat. Subsequently, 1000 μL of each individual phage and phage cocktail (1×10^8 PFU/mL, to achieve MOI of 10) was pipetted on the surface of the samples. For negative control, samples per experiment were treated with 1000 μL lambda diluent buffer (pH 7.2) (without phages). The samples were left in the laminar flow hood for 10 min to allow the phages to attach to the host bacteria. The samples were then placed in a sterile container and covered with a clean wrap to prevent contamination. The samples were incubated for 7 day at 4 °C to simulate the storage temperature. Samples were analysed after 0, 1, 3 and 7 days of incubation to determine the number of viable *E. coli* O177 cell remaining. The purpose of analysing the samples at day 0 was to confirm the presence of the inoculum on both treated and untreated samples. The analysis was done 15 min after applying *E. coli* O177 on the meat.

2.4.3. Enumeration of viable E. coli O177 cells

The remaining bacterial cells on experimentally contaminated beef after exposure to phages were analysed using plate count method. At indicated time points, (days 0, 1, 3 and 7) beef samples treated with both individual phages and phage cocktails including their respective negative controls were withdrawn separately and transferred into 250 mL sterile volumetric flasks containing 50 mL of 0.1% (w/v) sterile peptone water (PW). The flasks were placed on a shaker (200 rpm) for 2 min to facilitate the diffusion of the bacteria into the solution. For enumeration of *E. coli* O177 cell counts for both treated and untreated samples, aliquots of 10 mL were transferred into 50 mL sterile falcon tubes and centrifuged at 12 000×*g* for 5 min. The supernatant was discarded and the pellets were resuspended in 10 mL of 0.1% PW. Ten-fold serial dilutions were prepared and aliquots of 250 μL were plated in triplicate on MacConkey agar plates and the plates were incubated at 37 °C for 24 h for viable bacterial cell count. After incubation, typical *E. coli* O177 colonies were counted and the results were reported as log CFU/g. Log reduction [$(N_{d0}\text{-}n_d^*)$] was obtained by subtracting the number of viable cells recovered at eat time point (n_d^*) from the total number of viable cells at day 0 (N_{d0}).

2.4.4. Biofilm formation

Biofilm formation by *E. coli* O177 strain was evaluated using 96-well polystyrene plates as previously described (Stepanović et al., 2000). Briefly, eighty-one *E. coli* O177 isolates (selected based on virulence and antimicrobial resistance profiles) and one *Pseudomonas aeruginosa* (ATCC 27853) strain were cultured on MacConkey agar and nutrient agar plates, respectively, and incubated at 37 °C for 24 h. After incubation, a single colony of each isolate was transferred into 5 mL of sterile nutrient broth in 15 mL falcon tubes. The tubes were incubated at 37 °C for 24 h. A 1:100 serial dilutions were prepared from overnight cultures and 200 μL was dispensed in triplicates into the 96 well polystyrene plates. The first set of three wells were inoculated with 200 μL of nutrient broth (negative control) and the second set of three wells were inoculated with 200 μL of *Pseudomonas aeruginosa* (ATCC 27853) suspension (positive control; strong biofilm producer). The plates were incubated at 37 °C for 24 and 48 h. After incubation at each time point, the plates were washed three times with 300 μL of sterile phosphate buffer-saline [PBS 1X (pH 7.4)] to remove planktonic cells. The plates were dried for 30 min in the laminar flow hood cabinet at room temperature. After drying, the wells were stained with 200 μL of 0.1% crystal violet solution for 1 h. Subsequently, the plates were washed five times with 300 μL of sterile PBS. The plates were placed in the laminar flow hood to dry. After trying, 200 μL of 95% ethanol was added to fix the biofilm formation. To check the presence of biofilm formation, the plates were read at OD_{600nm} using spectrophotometer. The cut-off value (ODc) was defined as three standard deviation (SD) above the mean OD of the negative control. Biofilm formation was classified into four categories based on the ODs obtained: $OD \leq ODc$, non-adherent; $ODc < OD \leq 2$ x ODc, weak biofilm formation; 2 x $ODc < OD \leq 4$ x ODc, moderate biofilm formation; and 4 x $ODc < OD$, strong biofilm formation (Stepanović et al., 2000).

2.4.5. Effect of phages on biofilm formation

The ability of individual and phage cocktails to prevent biofilm formation by *E. coli* O177 strain was evaluated using the microplate virulence assays in two experiments that were performed separately. Ten *E. coli* O177 isolates (strong biofilm producers) were selected for this experiment as previously described (Stepanović et al., 2000; Endersen et al., 2017), with minor modifications. Briefly, 190 μL of sterile mTSB was dispensed into a 96-well polystyrene plates and as negative control, 10 μL of sterile mTSB was added to first set of three wells. For positive control, 10 μL of *Pseudomonas aeruginosa* ATCC 27853 strain (strong biofilm producer) and *E. coli* O177 isolate (strong biofilm producer from this study) were added separately to the second and third set of three wells, respectively. Instead of phage, 50 μL of sterile lambda diluent solution was added to both negative and positive control wells. A 10 μL of overnight culture of each isolate was added in triplicates to the rest of the wells. For each well (except for both controls), 50 μL of each individual phage stock (1×10^8 PFU/mL) and phage cocktail stock (1×10^8 PFU/mL) were added separately in triplicate to each host bacteria. The plates were incubated at 25 °C for 24 h. After incubation, the media containing planktonic cells was removed and the wells were washed three times with 300 μL PBS. Subsequently, the wells were stained with 200 μL of 0.1% crystal violet solution for 1 h. After 1 h, the stain was removed and the wells were washed five times with PBS. The plates were dried in the laminar flow cabinet. Two hundred microliter of 95% absolute alcohol was added to the wells. Then the OD_{600nm} was measured using spectrophotometer. The results were interpreted and biofilm formation was classified as described in Section 2.5.1 above.

2.4.6. Effect of phages on pre-formed biofilms

The ability of individual phages and phage cocktails to destruct established biofilm structure was assessed using the procedure described previously (Stepanović et al., 2000; Endersen et al., 2017), with some modifications. Ten *E. coli* O177 isolates, strong biofilm producers were selected and overnight cultures were prepared. Briefly, 190 μL of sterile mTSB was dispensed into a 96 well polystyrene plates. A 10 μL of mTSB (negative control) added to the first set of three wells and for positive control, 10 μL of *Pseudomonas aeruginosa* ATCC 27853 strain and *E. coli* O177 isolate (both strong biofilm producer from this study) were added separately to the second and third set of three wells, respectively. A 10 μL overnight culture of each isolate was added in triplicates into the rest

of the wells. The plates were incubated at 25 °C for 24 h to allow the bacteria to form biofilm. After incubation, the media containing planktonic cells was removed and the wells were washed three times with 200 μL of PBS. Subsequently, 150 μL of mTSB was added to each well. Fifty microliter of individual phages and phage cocktails stock (1×10^8 PFU/mL) were added separately in triplicate to each host bacteria. Fifty microliter of sterile lambda diluent solution was added to both negative and positive (established biofilm) controls. The plates were incubated at 25 °C for 5 h to allow the phages to disintegrate the formed-biofilm in the wells. The medium was removed and the wells were washed three times with 200 μL of PBS. After washing, the plates were stained as described above. After 1 h, the stain was removed and the wells were washed five times with PBS. The plates were allowed to dry. After drying, 200 μL of 95% absolute alcohol was added to the wells. Then the OD_{600nm} of the mixture was measured using spectrophotometer. The results were interpreted as described above (Stepanović et al., 2000).

2.5. Statistical analysis

The data for efficacy of individual phages and phage cocktails in reducing *E. coli* on artificially contaminated beef were converted to log CFU/g. The NORMAL option in the Proc Univariate statement was used to test for normality of measured parameters (SAS software version 9.4 of 2010). Phage efficacy data measured over time were analysed using the repeated measures procedures of SAS software version 9.4 of 2010. The general linear models (GLM) procedure of SAS software version 9.4 of 2010 was used to analyse cross sectional data according to the following statistical model:

$$Y_{ij} = \mu + D_i + E_{ij},$$

Where: Y_{ij} is the observation of the dependent variable (*E. coli* cell counts) *ij*; μ is the fixed effect of population mean for the variable; D_i the effect of the experimental treatments [individual phages and phage cocktails] and E_{ij} the random error associated with observation *ij*, assumed to be normally and independently distributed. The probability of difference (pdiff) option in the lsmeans statement was used to separate treatment means. Statistical significance for all statistical tests was declared at $P \leq 0.05$.

3. Results

3.1. Susceptibility of E. coli O177 isolates to individual phages

Susceptibility *E. coli* O177 isolates against individual phages was evaluated using microplate virulence assay. *E. coli* O177 isolates revealed two different susceptibility patterns against phages, Fig. 1. Generally, *E. coli* O177 isolates revealed either extreme susceptible (66.7–88.9%) or moderate susceptible (0.1–0.3%) to phages. Phages were able to inhibit bacterial growth within 5 h of inoculation at 37 °C. Phages vB_EcoM_10C2 and vB_EcoM_11B2 revealed similar lytic pattern against *E. coli* O177 isolates. Based on MOI, seven isolates were either extremely (MOI <0.01) or moderately susceptible (1 ≤ MOI < 10) to all the eight phages, Table 1. Two isolates (CF-D-D11 and CF-D-D4) were extremely susceptible (MOI <0.01) to all the phages. The MOI of phages against *E. coli* O177 isolates ranged from 1.3×10^{-7} and 73×10^{-1}. The lowest MOI was observed in samples treated with vB_EcoM_10C2, whereas the highest was seen in samples treated with vB_EcoM_3A1.

3.2. Lytic capabilities of phage cocktails against E. coli O177 strain

The susceptibility *E. coli* O177 isolates to 32 phage cocktails was assessed using microplate virulence assay. *E. coli* O177 isolates were extremely sensitive to all the tested phage cocktails. Most (18) phage cocktails were highly active at low titers (from 1×10^4 to 1×10^1 PFU/mL) while 14 were active at high titer (1×10^8 PFU/mL). Based on MOI, the isolates were classified as either extremely (MOI <0.01), highly (0.01 ≤ MOI < 1) or moderately susceptible (1 ≤ MOI < 10) to phage cocktails, Table 2. All three *E. coli* O177 isolates tested were sensitive to the 32 phage cocktails. *E. coli* O177 isolates showed extreme susceptibility to a large proportion (31/32) of the phage cocktails, while highly and moderately susceptible were exhibited by 29/32 and 5/32 of the phage cocktails, respectively. Isolate CF-H246 was moderately susceptible to four phage cocktails with their MOI ranging between 1.4 and 1.5 while isolates CF-A27 and CF-G202 were moderately susceptible to phage cocktails vB_EcoM_118B_ vB_EcoM_3A1 and vB_EcoM_10C2_ vB_EcoM_12A1 (MOI = 2.8 and 1.8, respectively). Phage cocktails, vB_EcoM_10C2_ vB_EcoM_366B and vB_EcoM_11B_ vB_EcoM_118B_EC12A1, had the lowest MOI values (average MOI = 0.00001615 and 0.000701, respectively).

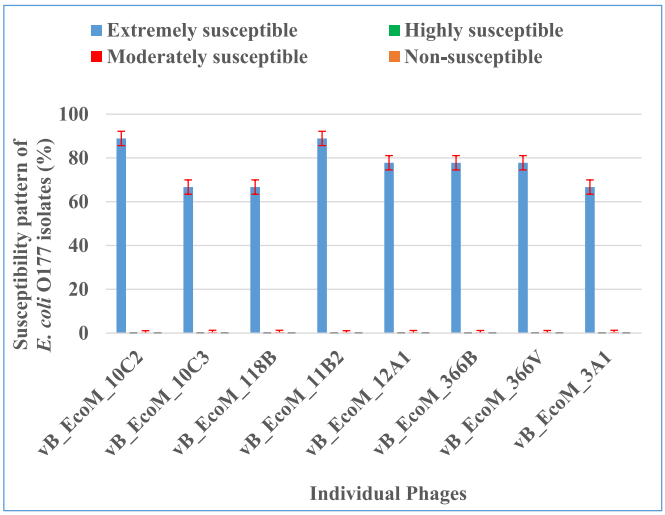

Fig. 1. Susceptibility pattern of *E. coli* O177 isolates against individual phages. The error bars represent the standard deviation.

3.3. Effect of individual phages on E. coli O177 cells on beef at 4 °C

Based on microbial screening, no bacteria (natural contamination) was detected on the meat prior to artificial contamination. The pH of the meat sample before inoculation of *E. coli* O177 was 6.3. As illustrated in Fig. 2A, there was a significant reduction of viable *E. coli* O177 cell counts following exposure to individual phages. Log reduction of *E. coli* O177 cell counts ranged between 2.10 and 7.81 CFU/g –throughout the incubation period, Fig. 2B. On day 1, log reduction in viable *E. coli* O177 cell counts ranged from 5.66 to 7.81 $\log_{10}$ CFU/g. Phages vB_EcoM_118B, vB_EcoM_11B, vB_EcoM_366B and vB_EcoM_366V reduced *E. coli* O177 cell counts to below the detection limit level (1.0 $\log_{10}$ CFU/g) after 1 day of incubation whereas phages vB_EcoM_12A1 and vB_EcoM_3A1 were able to inhibit bacteria to below the detection limit throughout day 1–7. After day 3 and 7, viable *E. coli* O177 cell counts revealed increasing trend in samples treated with individual phages vB_EcoM_118B, vB_EcoM_11B, vB_EcoM_366B and vB_EcoM_366V, Fig. 2A. Their log reduction ranged from 4.39 to 5.80 $\log_{10}$ CFU/g and 2.10 to 4.28 $\log_{10}$ CFU/g (day 3 and 7, respectively).

3.4. Effect of phage cocktails on E. coli O177 cells on beef at 4 °C

Six phage cocktails (vB_EcoMC1, vB _EcoMC2, vB _EcoMC3, vB _EcoMC4, vB_EcoMC5 and vB _EcoMC6) revealed significant efficacy of inhibiting *E. coli* O177 growth on artificially contaminated meat at 4 °C over a 7-day period, Fig. 3A. Log reduction of *E. coli* O177 cell counts ranged between 2.89 and 7.81 CFU/g from day 1–7, Fig. 3B. Three

Table 1
The sensitivity of *E. coli* O177 strains based on multiplicity of infections.

Bacteria ID	MOIs of the phages[a]							
	vB_EcoM_10C2	vB_EcoM_10C3	vB_EcoM_118B	vB_EcoM_11B2	vB_EcoM_12A1	vB_EcoM_366B	vB_EcoM_366V	vB_EcoM_3A1
CF-G202	1.2×10^{-3}	2.3×10^{-6}	4.1×10^{-3}	1.9×10^{-3}	4.5*	3.4×10^{-5}	1.8×10^{-4}	5.2×10^{-3}
CF-D-D11	1.4×10^{-4}	4.4×10^{-6}	4.4×10^{-3}	2.9×10^{-4}	1.8×10^{-5}	2.1×10^{-6}	4.4×10^{-5}	3.8×10^{-3}
CF-H1	6.8*	1.4×10^{-3}	4.1*	1.3×10^{-5}	5.4×10^{-5}	1.8×10^{-3}	2×10^{-3}	5.9*
CF-D-D4	3.8×10^{-3}	2.1×10^{-6}	2.7×10^{-6}	3.6×10^{-3}	2.2×10^{-3}	1.9×10^{-5}	3.3×10^{-4}	1.4×10^{-3}
CF-C29	3.5×10^{-5}	7.2*	4.1	2.4×10^{-4}	3.4×10^{-4}	4.6×10^{-3}	3.4×10^{-4}	3.2×10^{-6}
CF-D-D32	1.3×10^{-7}	6.4*	1.9×10^{-5}	1.1×10^{-3}	4.4×10^{-3}	1.4×10^{-3}	5.2*	4.2×10^{-6}
CF-C1	1.9×10^{-4}	5.1×10^{-3}	4.4×10^{-3}	2.3×10^{-3}	5.1×10^{-7}	7.1*	3.3×10^{-3}	7.3*
CF-A27	1.6×10^{-3}	1.7×10^{-3}	6.1*	4.2×10^{-4}	5.5*	5.4×10^{-3}	4.6*	2.8×10^{-5}
CF-H246	4.5×10^{-3}	6.6*	3.1×10^{-3}	5.6*	2.5×10^{-6}	4.7*	1.7×10^{-3}	6.1*

Key: ID = Identity; "a" superscript = multiplicity of infection (the lowest ratio of the phage to bacteria resulting in complete lysis of overnight culture bacteria culture within 5 h of incubation), asterisk (*) denotes incomplete lysis.

phage cocktails (vB _EcoMC3, vB _EcoMC4, and vB _EcoMC6) reduced viable *E. coli O177* cell counts to below the detection limit (1.0 $\log_{10}$ CFU/g) on contaminated meat –throughout the incubation period, Fig. 3A. On day 1, phage cocktails (vB _EcoMC1, vB _EcoMC2 and vB _EcoMC5) significantly ($P \leq 0.001$) reduced bacterial cell counts (4.28, 3.99 and 3.8 $\log_{10}$ CFU/g, respectively) when compared to their respective viable cell counts at day 0. At days 3 and 7, viable *E. coli* O177 cell counts showed increasing trend and the log reduction ranged from 3.75 to 3.90 $\log_{10}$ CFU/g and 2.86 to 3.13 $\log_{10}$ CFU/g, respectively, Fig. 3A and B. The extent of reduction of *E. coli* O177 cell counts at days 3 and 7 was lower after the incubation period for phage cocktails (vB _EcoMC1, vB _EcoMC2 and vB _EcoMC5) as shown in Fig. 3B.

3.5. Biofilm formation by E. coli O177 on polystyrene plate

Biofilm assay was performed to assess the ability of *E. coli* O177 to form biofilms when incubated at 37 °C and 25 °C for 24 and 48 h. The results revealed that *E. coli* O177 was able to form biofilm on polystyrene plate surface when incubated at different temperatures, Fig. 4AandB. Strong biofilm formation was relatively high at 25 °C (22.2%) than 37 °C (11.1%) after 24-h incubation period ($P < 0.05$). Similarly, after 48 h incubation period, 29.6% and 17.3% of the isolates produced strong biofilm at 25 °C and 37 °C, respectively.

3.6. Effect of phages on biofilm formation

The ability of individual phages and cocktails to prevent biofilm formation was evaluated against ten *E. coli* O177 isolates that were classified as strong biofilm producers. As shown in Fig. 5, individual phages significantly ($P < 0.05$) prevented biofilm formation. All individual phages were able to inhibit strong biofilm formation by *E. coli* O177. Most of the isolates (80%) did not form biofilms in the presence of phages vB_EcoM_10C3, vB_EcoM_10C2 and vB_EcoM_366V when incubated at 25 °C for 24 h while only 20% of the isolates were able to form weak and moderate biofilms in the presence of the phages. Phage cocktails showed greater efficacy in preventing biofilm formation by *E. coli* O177 isolates when incubated at 25 °C for 24 h, Fig. 6. None of the *E. coli* O177 isolates formed biofilms in the presence of phage cocktails.

3.7. Destruction of E. coli O177 biofilm mass by individual phages

The potential of individual phages to destruct biofilm mass formed by ten *E. coli* O177 isolates was evaluated at 25 °C for a period of 6 h. Individual phages revealed various patterns in disintegrating established biofilm structure, Fig. 7. Generally, all individual phages revealed significant ($P < 0.05$) destruction of biofilm mass formed by CF-A13 isolate to non-biofilm producer category (OD ≤ ODc). Five individual phages were able to disintegrated biofilm mass formed by CF-H14 isolate to weak category. All the phages revealed similar destruction patterns on biofilm mass formed by CF-D39, CF-G2 and CF-G3 isolates and their biofilm mass were classified under moderate category. However, phages were not able destruct the biofilm mass of four isolates (CF-G4, CF-G5, CF-G6 and CF-G34), Fig. 7.

3.8. Destruction of E. coli O177 biofilm mass by phage cocktails

The ability of phage cocktails to destruct biofilm mass formed by ten *E. coli* O177 isolates was assessed at 25 °C for a period of 5 h. Phage cocktails revealed high efficacy in disintegrating established biofilm mass formed by *E. coli* O177 isolates, Fig. 8. All phage cocktails were able to destruct biofilm mass to no, weak and moderate producer categories. Biofilm mass from isolate CF-G34 was classified as moderate upon treatment with phage cocktail vB _EcoMC6.

4. Discussion

Microplate-based approach is the most reliable, efficient and quick method to determine lytic capabilities and multiplicity of infection MOI of the phages against a given bacteria host (Niu et al., 2009; El-Dougdoug et al., 2019). In this study, microplate virulence assay was performed to evaluate lytic capabilities of individual phages and cocktails against a panel of nine multi-drug resistant *E. coli* O177 isolates obtained from cattle faeces. Individual phages revealed different lytic patterns against *E. coli* O177 isolates tested. Interestingly, all the *E. coli* O177 isolates tested were highly susceptible to all individual phages. These results are similar to those reported in a previous study, which reported the sensitivity of *E. coli* O157 to *E. coli* O157-specific phages (Niu et al., 2009). Phages vB_EcoM_10C2 and vB_EcoM_11B2 revealed similar lytic effect on *E. coli* O177. This could be attributed to the presence of common receptors such as lipopolysaccharides and O-antigen on host bacterial cell wall (Niu et al., 2009). Based on MOI, *E. coli* O177 isolates were classified as either extremely or moderately susceptible to individual phages. However, incomplete lysis of the bacteria by phages was observed and this could be due to the presence of phage-resistant *E. coli* mutants (Chan et al., 2013).

The emergence of phage resistance may affect the efficacy of single phages to control infections (Chan et al., 2013), which is why phage cocktails have been used to overcome this problem (Tsonos et al., 2014). In this study thirty-two phage cocktails were successfully designed and assessed for their lytic activities against three phage-resistant *E. coli* O177 mutants. Optimized phage cocktails revealed high lytic capabilities against all the tested isolates, which were resistant to individual phages. Interestingly, phage cocktails displayed high lytic activity at low titer (1×10^1 PFU/mL) as compared to their respective individual phages (1×10^8 PFU/mL). Based on the MOI, phage cocktails made up of two to five individual phages displayed the highest lytic capabilities against *E. coli* O177 isolates. However, cocktails made up of six phages produced similar lytic capabilities as the five-phage cocktails. A similar observation was reported in previous studies (Chen et al., 2018; Duc et al., 2020). This could be attributed to the fact that high phage cocktail

Table 2
Susceptibility of *E. coli* O177 strain to phage cocktails based on multiplicity of infections.

Phage cocktails (ID)	Host Bacteria (ID)		
	CF-A27	CF-G202	CF-H246
vB_EcoM_11B2_ vB_EcoM_118B	2.3×10^{-3}	2.9×10^{-7}	1.7×10^{-3}
vB_EcoM_11B_ vB_EcoM_10C2	2.0×10^{-6}	2.6×10^{-2}	1.5*
vB_EcoM_11B_ vB_EcoM_12A1	1.8×10^{-6}	2.3×10^{-2}	1.4*
vB_EcoM_11B_ vB_EcoM_366B	1.9×10^{-4}	2.4×10^{-5}	1.4×10^{-2}
vB_EcoM_11B_ vB_EcoM_3A1	2.7×10^{-3}	3.5×10^{-6}	2.1×10^{-1}
vB_EcoM_118B_ vB_EcoM_10C2	4.1×10^{-5}	5.3×10^{-7}	3.1×10^{-2}
vB_EcoM_118B_ vB_EcoM_12A1	2.2×10^{-6}	2.5×10^{-7}	1.4×10^{-2}
vB_EcoM_118B_ vB_EcoM_366B	2.8×10^{-3}	3.6×10^{-7}	2.1×10^{-3}
vB_EcoM_118B_ vB_EcoM_3A1	2.8*	2.1×10^{-7}	1.2×10^{-1}
vB_EcoM_10C2_ vB_EcoM_12A1	1.4×10^{-6}	1.8*	1.3*
vB_EcoM_10C2_ vB_EcoM_366B	2.3×10^{-6}	3×10^{-5}	1.5*
vB_EcoM_10C2_ vB_EcoM_3A1	1.7×10^{-5}	2.2×10^{-5}	1.3×10^{-1}
vB_EcoM_12A1_ vB_EcoM_366B	1.6×10^{-7}	2.1×10^{-5}	1.2×10^{-1}
vB_EcoM_12A1_ vB_EcoM_3A1	1.8×10^{-6}	2.4×10^{-5}	1.4×10^{-1}
vB_EcoM_366B_ vB_EcoM_3A1	1.4×10^{-4}	1.8×10^{-5}	1.0×10^{-1}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_10C2	4.3×10^{-5}	5.6×10^{-7}	3.3×10^{-3}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_12A1	2.8×10^{-6}	3.6×10^{-7}	2.1×10^{-3}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_366B	2.5×10^{-3}	3.3×10^{-7}	1.9×10^{-3}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_3A1	3.3×10^{-3}	4.4×10^{-7}	2.6×10^{-3}
vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_12A1	2.5×10^{-6}	3.3×10^{-7}	1.9×10^{-2}
vB_EcoM_118B_ vB_EcoM_366B_ vB_EcoM_3A1	1.9×10^{-3}	2.5×10^{-6}	1.5×10^{-3}
vB_EcoM_10C2_ vB_EcoM_12A1_ vB_EcoM_366B	2.2×10^{-7}	2.9×10^{-5}	1.7×10^{-1}
vB_EcoM_10C2_ vB_EcoM_366B _ vB_EcoM_3A1	2.4×10^{-6}	3.2×10^{-5}	1.9×10^{-1}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_12A1	1.7×10^{-6}	2.2×10^{-6}	1.3×10^{-2}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_366B	2.9×10^{-6}	3.7×10^{-6}	2.2×10^{-2}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_3A1	3.2×10^{-5}	4.2×10^{-6}	2.4×10^{-2}
vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_12A1_ vB_EcoM_366B	3.8×10^{-5}	5×10^{-6}	2.9×10^{-2}
vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_12A1_ vB_EcoM_3A1	1.9×10^{-5}	2.5×10^{-6}	1.5×10^{-2}
vB_EcoM_10C2_ vB_EcoM_12A1_ vB_EcoM_366B_ vB_EcoM_3A1	3×10^{-5}	3.9×10^{-6}	2.3×10^{-1}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_12A1_ vB_EcoM_366B	4×10^{-5}	5.2×10^{-7}	3.1×10^{-2}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_12A1_ vB_EcoM_3A1	4.5×10^{-6}	5.9×10^{-6}	3.4×10^{-2}
vB_EcoM_11B_ vB_EcoM_118B_ vB_EcoM_10C2_ vB_EcoM_12A1_ vB_EcoM_366B_ vB_EcoM_3A1	2×10^{-6}	2.6×10^{-5}	1.6×10^{-2}

Key: ID= Identity; "a" superscript = multiplicity of infection (the lowest ration of the phage to bacteria resulting in complete lysis of overnight culture bacteria culture within 5 h of incubation). The asterisk (*) denotes in complete lysis.

titers tend to aggregate and thus attenuate phage activity. In addition, competitive interaction for a common receptor among the phages of the same or different families may also reduce their efficacy (Liu et al., 2015; Chen et al., 2018). Unlike in individual phages, the incidence of incomplete lysis of bacteria was very low with cocktails. Incomplete lysis was only observed in isolates treated with two-phage cocktails but not with three or more phage cocktails. These findings suggest that phage cocktails designed in this study were effective in lysing phage-resistant *E. coli* O177 mutants.

Foodborne pathogens contamination remains a major concern both in food industry and public health (Endersen et al., 2014). Although several studies have demonstrated the efficacy of individual phages and phage cocktails in reducing foodborne pathogens on experimentally contaminated vegetables and meat (Hungaro et al., 2013; Tomat et al., 2014; Pereira et al., 2016; Yeh et al., 2017; Huang et al., 2018; Duc et al., 2020), no study has assessed and reported the ability of phages (individual phages or cocktails) in reducing *E. coli* O177 cells on artificially contaminated beef. In this study, treatment of artificially contaminated beef with both individual phages and phage cocktails significantly reduced viable *E. coli* O177 cell counts over the seven-day incubation period at 4 °C. Both individual phages and phage cocktails displayed highest efficacy against bacterial cells within 24 h of incubation. Six individual phages (vB_EcoM_118B, vB_EcoM_11B2, vB_EcoM_12A1, vB_EcoM_366B, vB_EcoM_366V and vB_EcoM_3A1) reduced bacterial cell counts to below the detection limit (1.0 $\log_{10}$ CFU/g) on day 1. Interestingly, two individual phages (vB_EcoM_12A1 and vB_EcoM_3A1) and three cocktails (vB_EcoMC3, vB_EcoMC4 and vB_EcoMC6) showed progressive reduction of viable cell counts to below the detection limit throughout the seven days of incubation. Despite the fact that individual phages revealed high efficacy (5.66–7.81 CFU/g) than phage cocktails (3.8–7.81 CFU/g) after 1 day of incubation, bacterial regrowth was frequently observed in samples treated with individual pages thereafter up to day 7. This demonstrated that phage cocktails were more effective in inhibiting bacterial regrowth and they could be used in abattoirs to reduce bacterial load on carcasses after slaughter. Although, bacterial cell counts increased on meat treated with individual phages after day 1, the viable cell counts were significantly lower than the cell counts in the initial inoculum and respective controls. Similar observations were reported in other studies where bacteria showed regrowth after 24 h of exposure to phages (Wang et al., 2017; Bai et al., 2019; Huang et al., 2018; Tomata et al., 2018). Bacterial regrowth could be due to the beef matrix interfering with penetration and distribution of the phages and thus decreasing the likelihood of phage-host interaction (Hungaro et al., 2013; Liu et al., 2015; Duc et al., 2020). This may limit the utility of directly applying phages to beef as biocontrol agents to reduce pathogens. A possible way of improving the efficacy of phages for reducing pathogen contamination in beef is to administer them in live animals.

Biofilm formation on food processing equipment may lead to persistence food contamination in food chain (Carpio et al., 2019). Moreover, biofilm provides a protective mechanism to bacteria and thus increase the possibilities of exchange of genetic material such as virulence and antimicrobial resistance genes between bacterial species (Merino et al., 2019; Carpio et al., 2019). For the very first time, the current study assessed the ability of *E. coli* O177 to form biofilm at different temperatures. It was observed that *E. coli* O177 is capable of forming biofilm on polystyrene surface at different temperatures (25 °C and 37 °C). Biofilm formation was frequently detected at 25 °C compared to 37 °C. Interestingly, 29.6% and 17.3% of the isolates produced strong biofilm (25 °C and 37 °C, respectively). Even though some studies reported biofilm formation at low temperatures (4, 10 and 15 °C) (Han et al., 2016; Yuan et al., 2019), our preliminary study revealed that *E. coli* O177 did not form biofilm at 4 °C. This indicates that biofilm formation depends on the nature of bacteria species and environmental temperature.

Given that biofilm structures are extremely resistant to several detergents used in the food industry, bacteriophages have been proposed

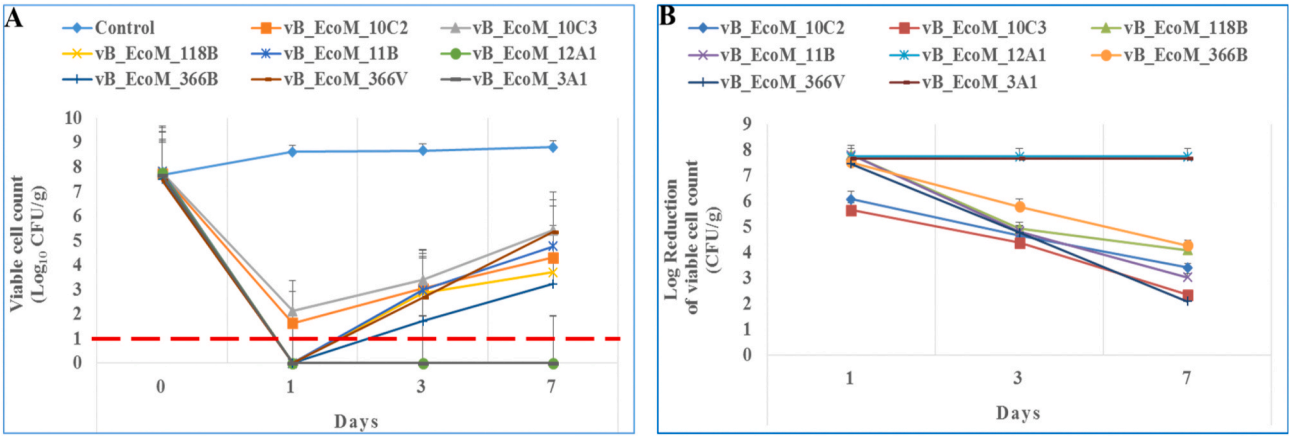

Fig. 2. The number of *E. coli* O177 cells remaining on contaminated beef after treatment with individual phages over a 7-day period (A). Log reduction (CFU/g) of *E. coli* O177 cells after treatment with phage over a 7-day period (B). The error bars represent the standard deviation. Detection limit: log 1 CFU/g (dotted red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

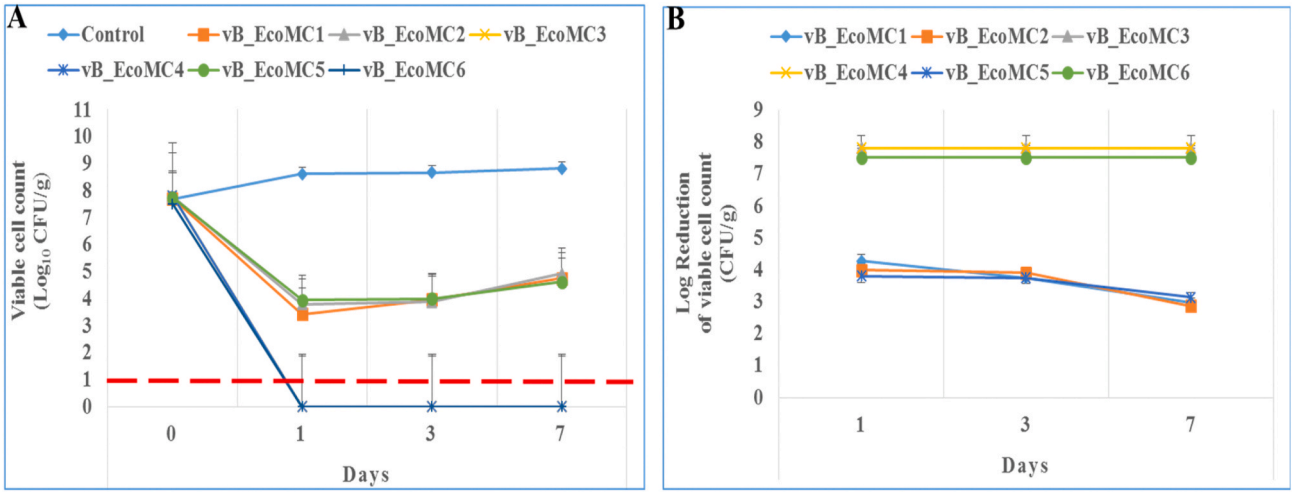

Fig. 3. The number of *E. coli* O177 cells remaining on contaminated beef after treatment with phage cocktails over a 7-day period (A). Log reduction (CFU/g)) of *E. coli* O177 cell count after treatment with phage cocktails over a 7-day period (B). The error bars represent the standard deviation. Detection limit: log 1 CFU/g (dotted red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

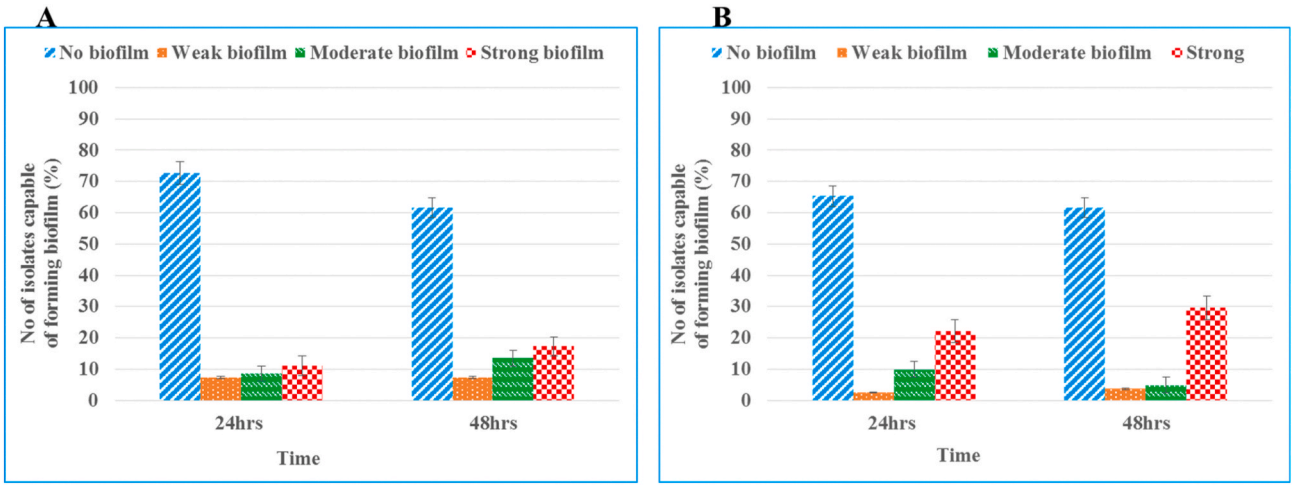

Fig. 4. Biofilm formation by *E. coli* O177 isolates on 96-well polystyrene plates. (**A**) Biofilm formation by 81 *E. coli* O177 isolates when incubated at 25 °C for 24 and 48 h. (**B**) Biofilm formation by 81 *E. coli* O177 isolates when incubated at 37 °C for 24 and 48 h. The bars indicate standard deviation. Cut-off values 0.10 and 0.14 at OD_{600nm} (for 24 h and 48 h, respectively) were used to classify the isolates as OD ≤ ODc, no biofilm formation; ODc < OD ≤ 2 x ODc, weak biofilm formation; 2 x ODc < OD ≤ 4 x ODc, moderate biofilm formation; 4 x ODc < OD, strong biofilm formation.

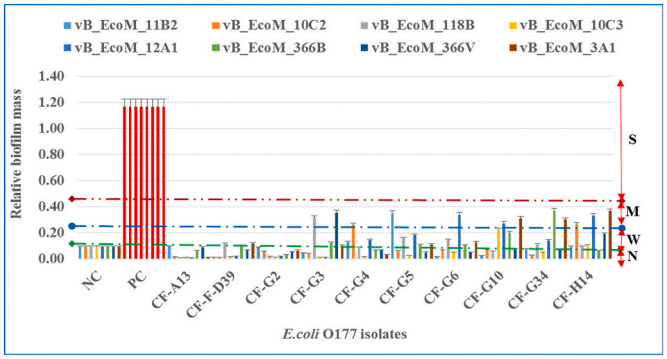

Fig. 5. Efficacy of individual phages in reducing biofilm formation by *E. coli* O177 strain at 25 °C for 24 h incubation period. The Y-axis represent the optical density of each isolates determined at OD_{600nm} while the X-axis represent *E. coli* O177 isolates. Key: S = strong (4 x ODc < OD); M = moderate (2 x ODc < OD ≤ 4 x ODc); W = weak (ODc < OD ≤ 2 x ODc); N = no biofilm formation (OD ≤ ODc); NC = negative control (phage without bacteria); PC = positive control (bacteria without phage). A cut-off value 0.10 at OD_{600nm} was used to classify the isolates. The error bars represent the standard deviation.

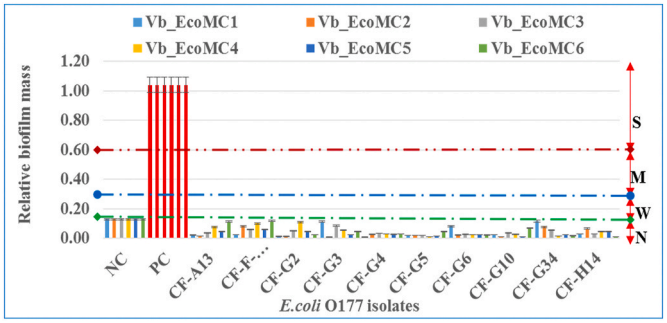

Fig. 6. Efficacy of phage cocktails in preventing biofilm formation by *E. coli* O177 strain at 25 °C for 24 h incubation period. The Y-axis represent the optical density of each isolates determined at OD_{600nm} while the X-axis represent *E. coli* O177 isolates. The error bars represent the standard deviation. Key: S = strong (4 x ODc < OD); M = moderate (2 x ODc < OD ≤ 4 x ODc); W = weak (ODc < OD ≤ 2 x ODc); N = no biofilm formation (OD ≤ ODc); NC = negative control (phage without bacteria); PC = positive control (bacteria without phage). A cut-off value 0.13 at OD_{600nm} was used to classify the isolates. The error bars represent the standard deviation.

as an alternative to prevent and eradicate biofilms (Endersen et al., 2017). This study evaluated the efficacy of individual phages and phage cocktails to prevent biofilm formation by *E. coli* O177 (strong biofilm producers) strain using microplate-based assay. Individual phages and phage cocktails inhibited biofilm formation at 25 °C over a 24-h period. When treated with individual phages, no isolates formed strong biofilm. Only weak and moderate biofilm formation were observed after 24 h of incubation. On the other hand, phage cocktails were more effective in preventing biofilm formation. Indeed, no isolate was able to form biofilm in the presence of phage cocktails. This clearly demonstrates that phage cocktails used in this study are highly effective in preventing biofilm formation by *E. coli* O177.

Another important observation was that individual phages and phage cocktails were able to destroy established biofilm structures. Individual phages revealed varied efficacy in destroying strong biofilm mass formed by *E. coli* O177 at 25 °C over a 5-h period. Remarkably, all individual phages were able to completely destroy biofilm mass of CF-A13 isolate. However, individual phages were not able destroy the biofilm mass of four isolates (CF-G4, CF-G5, CF-G6 and CF-G34). This demonstrates that individual phages were more effective in inactivating the planktonic cells than destroying pre-formed biofilm. On the other hand, phage cocktails were more effective in destroying pre-formed biofilm to below detectable levels within 5 h incubation period. Similar results were reported in a previous study that evaluated the efficacy of lytic *Pseudomonas aeruginosa*-specific phages in destroying established biofilm (Yuan et al., 2019).

5. Conclusions

In conclusion, this study revealed that phage cocktails were more potent in reducing viable *E. coli* O177 cells on raw beef than individual phages. In addition, phage cocktails reduced pre-formed biofilm mass to weak and non-biofilm category. This clearly showed that phage cocktails have greater potential as a stand-alone treatment for the control biofilm formation and reduction of *E. coli* cells in beef and possibly in food processing plants at abattoirs and butcheries. Future studies are needed to determine the possible effects of phage cocktails on sensory properties of beef as well as their effectiveness in preventing the development of biofilm structures on equipment used in beef processing.

Author contributions

V.M. and C.N.A.: Conceptualization; K.P.M., V.M. and C.N.A.: Methodology; K.P.M.: Visualization; V.M. and C.N.A.: Supervision; V.M. and C.N.A.: Project administration; V.M. and C.N.A.: funding acquisition: K.P.M: Data curation; K.P.M.: Formal analysis; K.P.M:

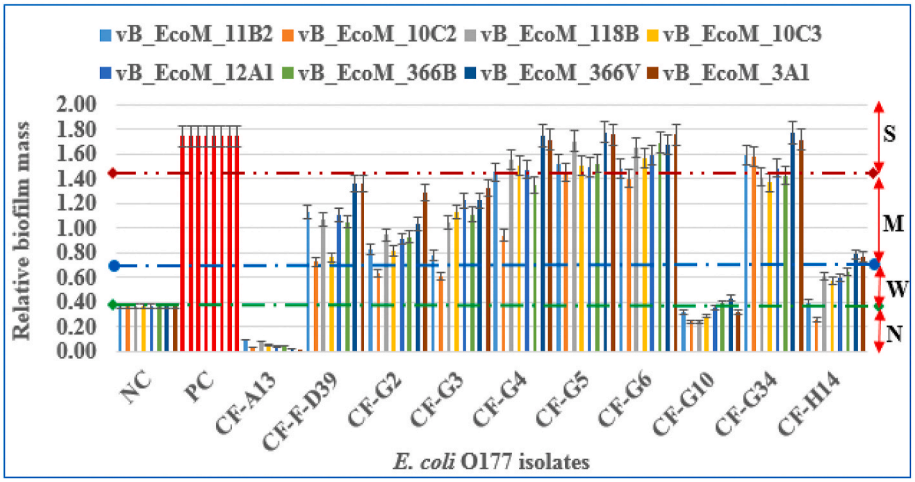

Fig. 7. Efficacy of individual phages in the destruction of pre-formation biofilm by *E. coli* O177 strain at 25 °C for 5 h incubation period. The Y-axis represent the optical density of each isolates determined at OD_{600nm} while the X-axis represent *E. coli* O177 isolates. The error bars represent the standard deviation. Key: S = strong (4 x ODc < OD); M = moderate (2 x ODc < OD ≤ 4 x ODc); W = weak (ODc < OD ≤ 2 x ODc); N = no biofilm formation (OD ≤ ODc); NC = negative control (phage without bacteria); PC = positive control (bacteria without phage). A cut-off value 0.37 at OD_{600nm} was used to classify the isolates. The error bars represent the standard deviation.

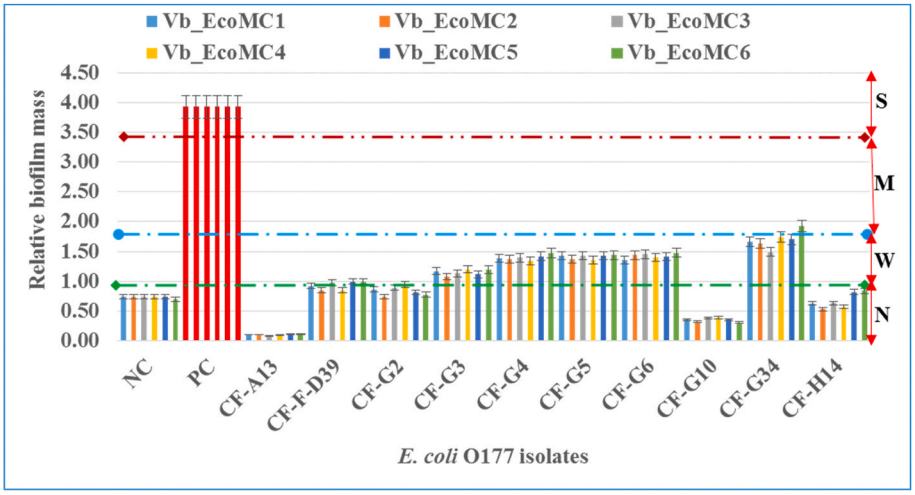

Fig. 8. Efficacy of phage cocktails in the destruction of pre-formed biofilm by *E. coli* O177 strain at 25 °C for 5 h incubation period. The Y-axis represent the optical density of each isolates determined at OD_{600nm} while the X-axis represent *E. coli* O177 isolates. The error bars represent the standard deviation. Key: S = strong (4 x ODc < OD); M = moderate (2 x ODc < OD ≤ 4xODc); W = weak (ODc < OD ≤ 2 x ODc); N = no biofilm formation (OD ≤ ODc); NC = negative control (phage without bacteria); PC = positive control (bacteria without phage). A cut-off value 0.83 at OD_{630nm} was used to classify the isolates. The error bars represent the standard deviation.

Investigation; V.M. and C.N.A.: Resources; V.M. and C.N.A.: Softwares; V.M. and C.N.A.: Validation; K.P.M.: Writing—original draft preparation, K.P.M., V.M. and C.N.A.: Writing—review and editing.

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Declaration of competing interest

The authors declare no conflict of interest.

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