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Biocontrol of *Escherichia Coli* O126:H7 in Skim Milk Using Bacteriophages

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Abstract: *Escherichia coli* (*E. coli*) is currently one of the most predominant human food-borne pathogens. In the dairy industry, recently, attention has been drawn to the potential use of bacteriophages (phages) to control bacteria in dairy products. In this study, a cocktail of three *Escherichia coli* bacteriophages (EcoM-AG2, EcoM-AG3 and EcoM-AG10) was tested to evaluate its ability to control *Escherichia coli* O126:H7 in experimentally contaminated skim milk. In Tryptic Soy Broth (TSB), there was an *E. coli* growth suppression of 1.8, 5.7 and 8.9 logs in phage treatments compared to controls over 8 days, at 4, 10 and 25°C, respectively. While in skim milk, the phage cocktail reduced *E. coli* populations by 1.2, 1.0 and 8.3 log compared to control samples over 15 days, at 4, 10 and 25°C, respectively. The phage population was relatively stable in skim milk during the experiments. The overall results in this study indicate that phages may be useful in the control of *E. coli* in dairy products by preventing growth and significant reducing of bacterial numbers. However, the phages didn't eliminate all the *E. coli* in skim milk tested at 4 and 10°C, but complete elimination of *E. coli* was verified after 3 days of phage treatment at 25°C. Therefore they are more likely to be used as a hurdle approach, incorporated to other treatments without compromising food quality. Future research about phage-bacteria interaction in milk is required.

Keywords: Bacteriophage, *Escherichia Coli*, Skim Milk

Introduction

Escherichia coli (*E. coli*) is one group of the most common bacteria found in the gastrointestinal tract of human and a majority of them are not harmful. However, there are six enteric pathotypes of *E. coli* that can cause human diseases (Bell and Kyriakides, 1999; Sussman, 1997): Enteropathogenic *E. coli* (EPEC). E.g. O126, O127, O158, Enterotoxigenic *E. coli* (ETEC). E.g. O115, O148, O153, Vero-cytotoxigenic *E. coli* (VTEC) (Includes Enterohaemorrhagic *E. coli,* EHEC). E.g. O157, O163, O168, Enteroinvasive *E. coli* (EIEC). E.g. O144, O152, O164, Enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coli* (DAEC).

Human diseases associated with pathogenic *E. coli* include: Infantile enteritis, watery and bloody diarrhea, vomiting, fever, severe abdominal cramps, Hemorrhagic Colitis (HC), Hemolytic Uremic Syndrome (HUS) and thrombotic thrombocytopenic purpura (Bell and Kyriakides, 1999; Griffin *et al*., 2002; Insel *et al*., 2004; Yoon and Hovde, 2008).

In the dairy industry, traditional thermal methods are most frequently used to control *E. coli*, including High Temperature-Short Time (HTST) pasteurization and Ultra High Temperature (UHT) sterilization. These control methods, however, tend to be power-consuming, expensive and may alter organoleptic properties. Furthermore, in history, there were quite a few of *E. coli* outbreaks related to pasteurized dairy products, indicating some potential food safety problems with the traditional thermal methods (Bell and Kyriakides, 1999). For example, in 1994, a large *E. coli* O157 outbreak associated with pasteurized milk occurred in the UK, with over 100 cases reported (Bell and Kyriakides, 1999). Even though the exact cause for this outbreak is not clear, two most likely reasons are: Inadequate pasteurization and/or post heat-processing contamination.

As a potential alternative or additional means that may circumvent the limitations of traditional food-borne pathogen control methods, recently, the use of bacteriophages (phages) has drawn people's attention

since they were discovered by Frederick W. Twort in 1915 and Felix d'Herelle in 1917 (Summers, 2005). Phages are viruses that can only infect and lyse specific bacterial hosts and do not infect mammalian or plant cells. In addition, phages generally do not cross bacterial species and therefore do not affect desirable flora present in food (Carlton *et al*., 2005). The specificity of their infection makes them suitable to be applied to different food-borne pathogen control (FDA 2006; Hagens and Loessner, 2014).

In addition, compared to traditional control methods, the use of phages is more "natural" and "green". Phages are so ubiquitous that they naturally exist in a variety of environments, including water, soil, farms, foods and even among human normal flora (Greer, 2005). Therefore, the phage biocontrol strategy is quite cost-effective.

Furthermore, there is evidence showing that, phages are remarkably stable in foods and even survive food processing (Atterbury *et al*., 2003; Greer, 2005; Holck and Berge, 2009), which assure the food safety during food storage. Greer (2005) reported that, the phage titer stayed unchanged on the surface of vacuum-packaged lean beef tissue stored for 6 months at 2°C; while Atterbury *et al*. (2003) found that, *Campylobacter jejuni* phages can survive on retail chicken under commercial poultry processing procedures.

In the food industry, some specific phage preparations are already commercially available in the market. For example, in 2006, the US FDA approved the use of *Listeria*-specific bacteriophage preparation (made from six purified phages, Intralytix Inc, USA), on Ready-To-Eat (RTE) meat and poultry products, to eliminate possible *Listeria monocytogenes* contaminations in these foods (FDA, 2006).

Many studies related to phage biocontrol of pathogenic bacteria in artificially contaminated foods have been performed. Examples of the food pathogenic bacteria include *Campylobacter*, *Listeria*, *Salmonella* and *E. coli*. For examples, one study has shown that, *L. monocytogenes*-specific phages, when combined with a bacteriocin, can significantly reduce *L. monocytogenes* populations on fresh-cut produce such as melons and apples (Leverentz *et al*., 2003). A study was conducted by Carlton *et al*. (2005), who obtained a significant reduction (3.5 logs) of *L. monocytogenes* counts on experimentally contaminated soft cheese, by applying *Listeria* phage P100 to the cheese surface during the rind washings.

Modi *et al*. (2001) investigated the effect of phages on *Salmonella Enteritidis* survival in cheese made from raw and pasteurized milk. The authors reported that, in cheese made from pasteurized milk, addition of phages reduced *Salmonella* to undetectable levels after 89 days storage. However, in cheese made from unpasteurized milk, with similar treatment, *Salmonella* populations were detected.

Using bacteriophages for bioconontrol of *Salmonella* on fresh cut fruits, was also reported (Leverentz *et al*., 2001). A cocktail of four lytic phages was used and there was a remarkable reduction of *Salmonella* populations on melons; however, similar results were not obtained from apples, in which inactivation of phages was found and the authors attributed this to the pH difference between melons (pH 5.8) and apples (pH 4.2).

O'Flynn *et al*. (2004) evaluated a cocktail of three phages for control of *E. coli* O157:H7 on beef. Small amounts of *E. coli* O157:H7 were inoculated with the phage cocktail on beef surfaces at 37°C for 1 h, resulting in disappearance of *E. coli* in 7 out of 9 samples, while the other two samples had low bacteria counts of <10 CFU/mL. However, it has been argued that, the temperature (37°C) used in the study, which gave a good result, was much higher than normal storage temperate (≤5°C) in real life.

In this study, we tested the ability and efficiency of some lytic phages against pathogenic *E. coli* in skim milk stored at 4, 10 and 25°C. To avoid the potential development of phage-resistant mutants, we used a cocktail of three different *E. coli* phages (EcoM-AG2, EcoM-AG3 and EcoM-AG10). Tryptic Soy Broth (TSB) was used a control.

Materials and Methods

Bacteria Stock Propagation

Enteropathogenic *E. coli* (EPEC) O126:H7 strain 761 (kindly provided by Canadian Research Institute for Food Safety, University of Guelph (CRIFS, Canada), was used in this study as host bacteria for *E. coli* phages.

Cultures of *E. coli* O126:H7 strain 761 was maintained at 4°C on a Tryptic Soy Agar (TSA, Difco, Becton Dickinson) plate. Two representative colonies were picked from the plate and inoculated into a Tryptic Soy Broth (TSB, Difco, Becton Dickinson) tube with 10 mL of TSB. Then the cultures were incubated overnight (20 to 24 hr) at 37°C and reached a cell level around 10^9 Colony Forming Unit (CFU)/mL (previous observation showed that for our bacteria, an overnight TSB culture contained approximately 10^9 CFU/mL, which was used as a basis for dilutions prior to inoculation and the exact bacteria population was later confirmed by plate counts). Every time before using, 100 μL of the culture was transferred to a single tube containing 10 mL of TSB, then cultured overnight to make sure that the bacteria were fresh.

Phage Stock Propagation

Three lytic phages (EcoM-AG2, EcoM-AG3 and EcoM-AG10) for *E. coli* O126:H7 were kindly provided

by Canadian Research Institute for Food Safety, University of Guelph (CRIFS, Canada).

All three phage were maintained in phage buffer (0.735 g $Cacl_2.2H_2O$, 2.5 g $MgSO_4.7H_2O$, 0.05 g gelatin, 6 mL 1M Tris-HCl, pH 7.2, per liter). To make large amounts of phage lysates, the soft-agar overlay method described by Jensen *et al*. (1998), was used. For each phage, 1 mL phage lysate (10^9 Plaque Forming Unit (PFU)/mL) and 1 mL overnight cultured host bacteria (10^9 CFU/mL) were mixed and put in a 37°C incubator for 10 min to facilitate the attachment of the phage to the host bacteria. After incubation, the mixture was added to 50 mL molten TSB overlay (0.4% agar), then plated on 10 TSA plates and incubated at 25°C overnight. The soft-agar layers on the plates were first washed by phage buffer, then scraped, vortexed and centrifuged for 15 min at 7000×g, followed by filtration (0.2 μm pore diameter syringe filters) of the supernatant fluid.

As a result of phage propagation, high-titer phage stocks for each phage were obtained, with concentrations about 10^9 PFU/mL (confirmed by diluting, plating and plaque counting).The same amount from each phage lysate was mixed together to make a phage cocktail and the concentration of the cocktail was approximately10^9 PFU/mL (the exact phage population was later confirmed by counting of plaques).

Phage Treatment in TSB Incubated 4, 10 and 25°C

Overnight cultured *E. coli* cells were diluted with TSB to about 10^4 CFU/mL immediately before application. In a phage treatment, a half milliliter of diluted bacteria cultures (10^4 CFU/mL) were mixed thoroughly with the same amount of the phage cocktail (10^9 PFU/mL) and 4 mL TSB and incubated for 8 days, at 4, 10 and 25°C, respectively. Therefore, the bacteria cell level in the mixture was ~10^3 CFU/mL, while for phages was 10^8 PFU/mL and the Multiplicity Of Infection (MOI) was 10^5.

In a positive control, the same model was used. However, instead of the phage cocktail, the same amount of phage buffer was applied. While in a negative control, neither bacteria nor phages were added to TSB and there was only 5 mL TSB in the sample.

Small amounts (350 μL) from treatments and/or controls were taken on day 0, day 1, day 3, day 6 and day 8, at 4, 10 and 25°C, respectively. Serial dilutions of the samples were made by 0.85% NaCl, then plated on selective MacConkey sorbitol agar (Difco, Becton Dickinson) followed by incubation at 37°C overnight. During the other day, the exact bacteria concentrations were determined by counting developed colonies on the plates.

For both treatments and controls, triplicate tubes were used. All plating was done in triplicate as well. The means of triplicate counts on plates were determined for one treatment or control and the means of triplicate-tube counts were determined and plotted.

Phage treatment in Skim Milk Incubated at 4, 10 and 25°C

Milk experiments were quite similar to TSB experiments. However, instead of TSB, skim milk was utilized. Liquid skim milk was made from skim milk powder (Difco, Becton Dickinson) and sterilized by heating at 121°C for 15 min. Then the skim milk was chilled and stored at 4°C.

Overnight cultured bacteria were diluted in milk to 10^4 CFU/mL. In treatments and/or controls, the same amounts of bacteria and phages as in TSB experiments were used in milk experiments. About 4 mL milk was added to a treatment and/or positive control, while in a negative control, totally 5 mL of milk was used.

Treatments and controls were incubated for 15 days (commercial shelf life for milk), at 4, 10 and 25°C, respectively. Samples were taken on day 0, day 1, day 3, day 6, day 8, day10, day 12 and day 15.

The counts of *E. coli* were determined by plating serial dilutions on selective MacConkey agar plates, with incubations at 37°C for overnight. Counts of phages 10 were determined by plating serial dilutions into agar overlays (containing overnight culture of host bacteria), incubating at 25°C for overnight and then counting plaques formed by phages.

All samples were prepared and plated in triplicate. All these experiments were replicated three times.

Statistical Analysis

Results of treatments and positive controls were compared using the *t* test (Microsoft Office Excel 2007) on the log value of the microbial population. Since 10 CFU/mL is the limit of detection, in some cases, if there was a bacteria CFU/value equal or below 10, 1 was used for the log value. A Significant difference was defined at $p<0.05$.

Results and Discussion

In this study, a negative control means that there was only TSB or skim milk in the sample. A negative control showed the baseline result therefore its value was treated as a "background" value to be subtracted from the test sample results. However, since in all experiments of this study, our negative controls (both bacteria-free and phage-free) gave 0 values over time, we didn't add the results of negative controls in the figures. Thus, the word "control" in all figures here refers to positive controls mentioned in the "Materials and Methods" section, which means that only bacteria and phage buffer, rather than phages were added to the TSB/skim milk samples.

The Effect of the Phage Cocktail on E. coli Growing in TSB

To test the lytic ability and efficiency of the phage cocktail to lyseenteropathogenic *E. coli* (EPEC)

O126:H7 stain 761, several TSB culture experiments were first conducted. These experiments were carried out at 4, 10 and 25°C.

Figure 1 shows the viable cell levels of *E. coli* O126:H7 at 4°C in TSB culture for 8 days. The results show that within the 8 days, when there was no phage added to TSB culture, *E. coli* O126:H7 could survive well at 4°C with only a 0.5 log cycle reduction.

The survival of *E. coli* at 4°C indicated that, even *E. coli* don't grow at the normal refrigeration temperature (3-5°C) that is commonly used in the food industry, *E. coli* don't die out either. Previous research shows that, *E. coli* O157:H7 can successfully survive in ground beef at -20°C for up to 9 months (US Institute of Medicine, 2002). Therefore, customers can't rely on either refrigerating or freezing to eliminate *E. coli* in contaminated foods.

However, *E. coli* populations in phage treated samples at 4°C have shown a promising *E. coli* control method (Fig. 1). When the phage cocktail was added, there was a significant ($p<0.05$) reduction of 1.8 logs in *E. coli* populations compared to the controls over 8 days, indicating that our phages lysed some of the bacteria. On day 0, there were 6350 CFU/mL in the sample; while only after 1 day, bacteria populations were under the detection limit (10 CFU/mL). This situation didn't change until day 6. On day 6, we found 57 CFU/mL, while on the last day, day 8, *E. coli* cell levels went down to 33 CFU/mL. The re-growth of *E. coli* shown on day 6 may be due to development of phage-resistant mutants, or, a low detection limit of plating count (10 CFU/mL). However, the former hypothesis was rejected because we found phage plaques on plates containing suspicious phage-resistant mutants; while the latter hypothesis was confirmed because later we detected *E. coli* in both day 1 and 3 samples, after an overnight enrichment (data not shown).

Figure 2 shows the populations of *E. coli* O126:H7 in TSB at 10°C. In phage-free controls, the counts of *E. coli* O126:H7 increased by 5.9 logs in 8 days. The bacterial growth difference between 4 and 10°C was because the minimum temperature for the growth of *E. coli* is 8°C (Bell and Kyriakides, 1999).

In phage treatments, at 10°C, the bacterial populations were approximately 5.7 logs lower than the corresponding samples without phages over 8 days, which was statistically significant ($p<0.05$). However, the cell levels of bacteria only decreased after 1 day phage treatment and then started to increase slightly. On day 8, the bacterial levels were similar to initial bacteria levels on day 1. The overall results of this experiment shows that, the phage cocktail can prevent the growth of *E. coli* in TSB at 10°C.

We observed the most vigorous bacterial growth in controls at 25°C, compared with 4 and 10°C in TSB (Fig. 3). This is most likely because, 37°C is the optimum temperature for *E. coli*, while 25°C is quite close to 37°C, compared with other temperatures used in this study (4 and 10°C). At 25°C, the populations in phage-free samples increased by approximately 6.1 logs throughout 8 days.

Contrary, the counts of *E. coli* were reduced significantly ($p<0.05$) by 8.9 log cycles in phage treatments compared to phage-free samples (Fig. 3). From day 0 to day 1, when bacteria in control samples grew rapidly, our phage cocktail reduced the counts of bacteria in treatments to less than 10 CFU/mL. Since day 1, the bacterial populations stayed less than 10 CFU/mL until day 8. To make sure that all the bacteria were killed, we enriched the treatment samples for: Day 1, day 3, day 6 and day 8. As a result, we found *E. coli* colonies formed for both day1 and day 3; while for day 6 and day 8, we couldn't find any colonies (data not shown). Therefore, phages were proven to be able to eliminate *E. coli* in TSB at 25°C, after 6 days of initial phage treatment.

The much better lytic ability of our phage cocktail at 25°C than other temperatures (4 and 10°C) is probably because of the more active bacteria at this temperature. At 25°C, *E. coli* grew well (Fig. 3); which favored phage replication which requires highly metabolic host cells (Greer, 2005).

The Effect of the Phage Cocktail on E. coli Growing in Skim Milk

Given that in the market, liquid milk usually has a shelf life of 15 days, our milk experiments were performed to determine whether the phage cocktail could be exploited to eliminate *E. coli* or reduce its incidence in skim milk, after 15 days of initial phage treatment.

As you can notice in Fig. 4, at 4°C, similar to TSB, *E. coli* survived, but did not grew in phage-free skim milk, with marginal reductions of 0.4 logs in 8 days and overall 0.8 logs in 15 days.

In the treatment group, after one day of phage addition, the bacterial numbers were significantly reduced ($p<0.05$) by 1.0 log when compared to the control. After day 1, the bacterial populations started to decline slightly, with similar decrease rate to the 16 controls. As a result, there was an overall reduction about 1.2 log cycles over 15 days compared with controls, with a final bacterial cell level about 15 CFU/mL in the milk.

It is noteworthy that, on day 8, there was a reduction of 0.9 logs in skim milk, while in TSB, the reduction was 1.8 logs. The lower count difference in skim milk containing phages compared to TSB may suggest lower phage activity in skim milk than TSB. This is due to the composition difference between them: Skim milk is much more complex and it contains various proteins, carbohydrates, calcium, phosphorus; while TSB is relatively simple, which mainly consists of tryptone (digest of casein), soytone (digest of soybean) and NaCl.

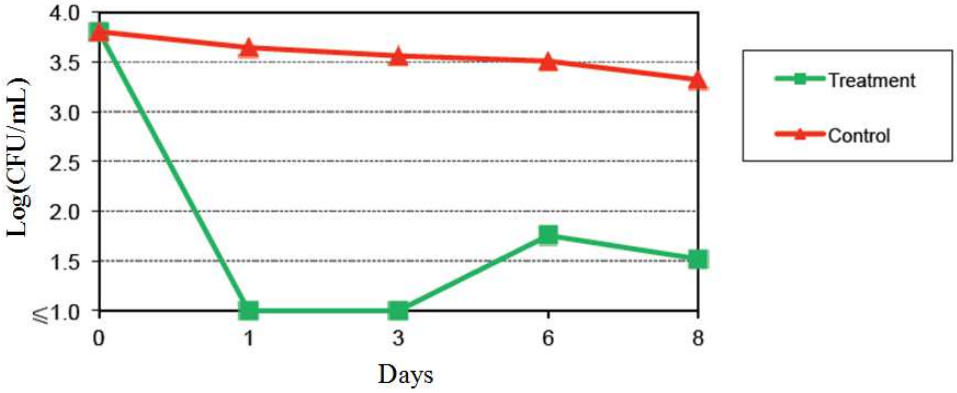

Fig. 1. Effect of phage treatment at 4°C in TSB inoculated with *E. coli* O126:H7 for 8 days. Symbols represent the averages of three replications

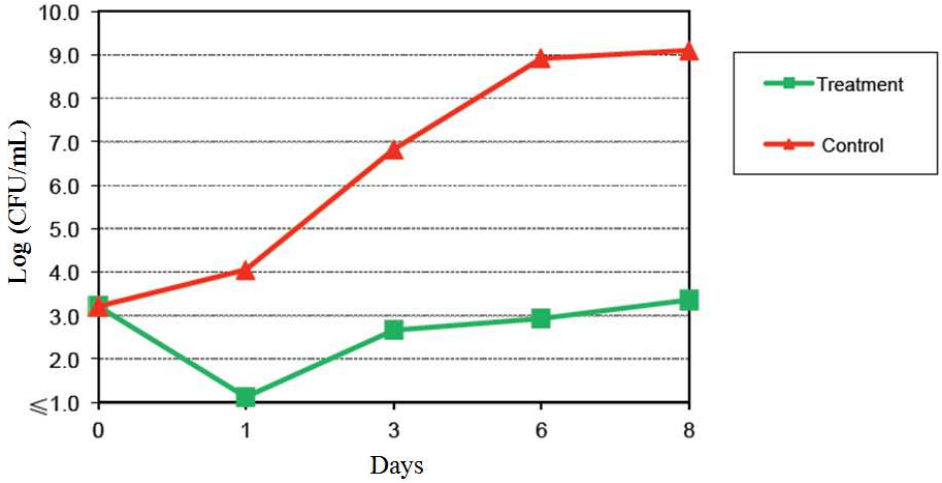

Fig. 2. Effect of phage treatment at 10°C in TSB inoculated with *E. coli* O126:H7 for 8 days. Symbols represent the averages of three replications

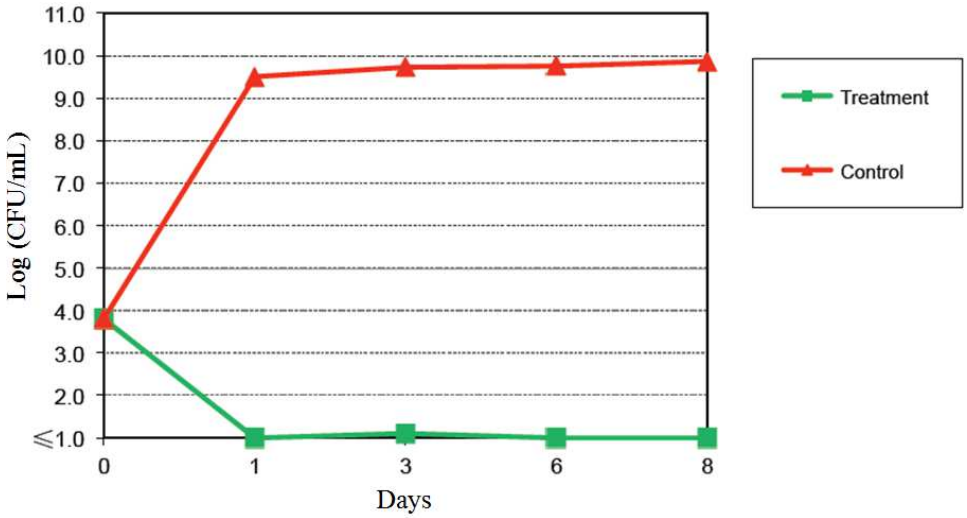

Fig. 3. Effect of phage treatment at 25°C in TSB inoculated with *E. coli* O126:H7 for 8 days. Symbols represent the averages of three replications

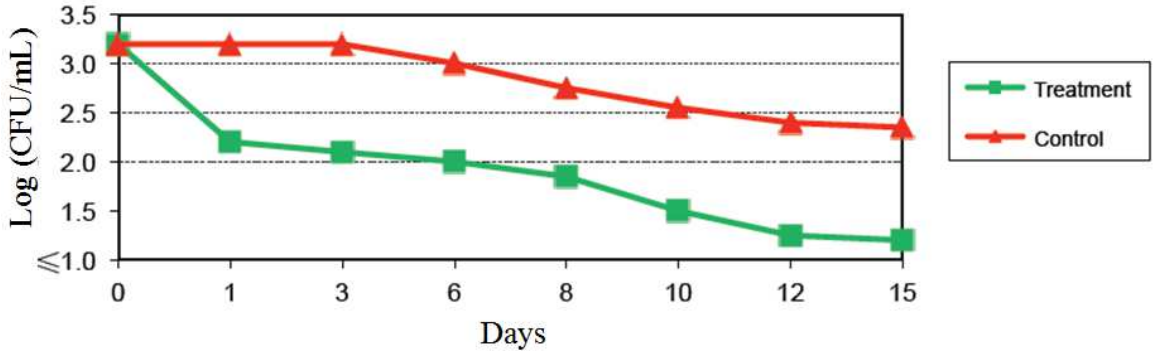

Fig. 4. Effect of phage treatment at 4°C in skim milk inoculated with *E. coli* O126:H7 for 15 days. Symbols represent the averages of three replications

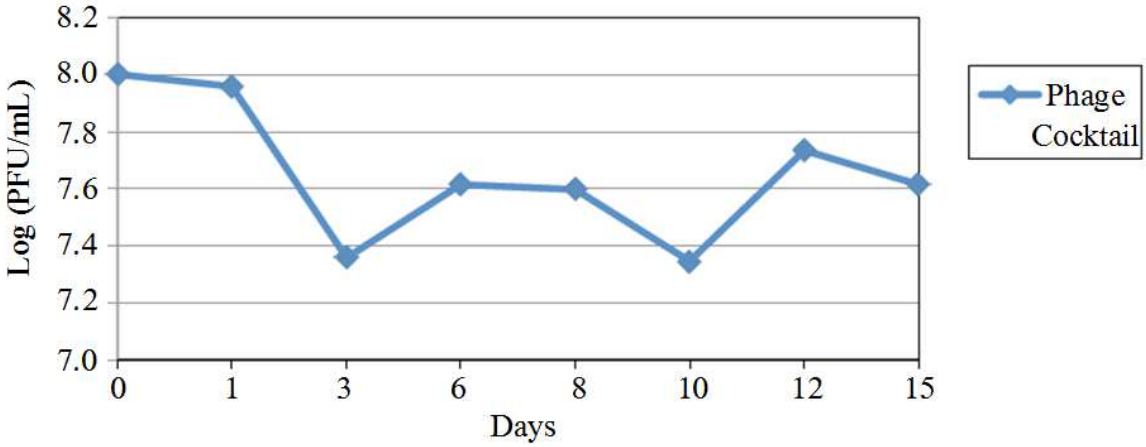

Fig. 5. Phage titer in phage treatment at 4°C in skim milk inoculated with *E. coli* O126:H7 for 15 days. Symbols represent the averages of three replications

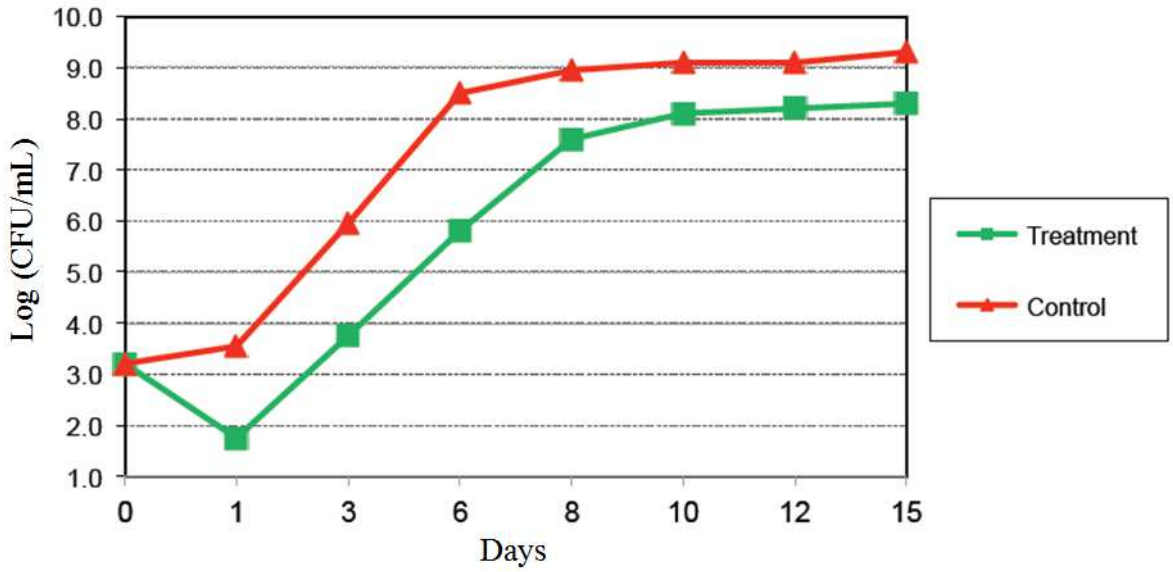

Fig. 6. Effect of phage treatment at 10°C in skim milk inoculated with *E. coli* O126:H7 for 15 days. Symbols represent the averages of three replications

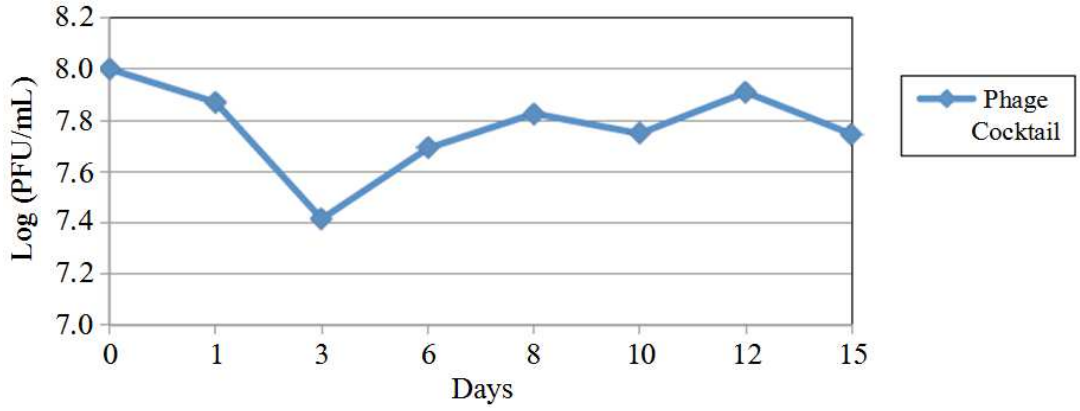

Fig. 7. Phage titer in phage treatment at 10°C in skim milk inoculated with *E. coli* O126:H7 for 15 days. Symbols represent the averages of three replications

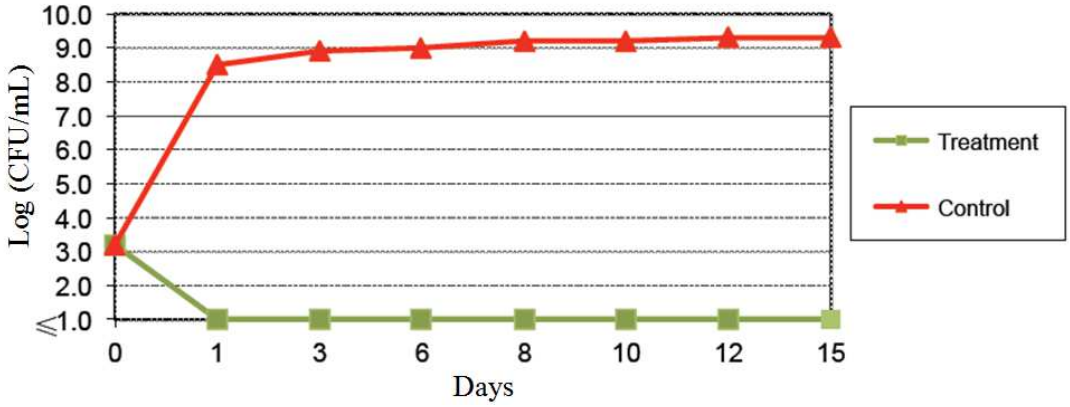

Fig. 8. Effect of phage treatment at 25°C in skim milk inoculated with *E. coli* O126:H7 for 15 days. Symbols represent the averages of three replications

In addition, it was possible that, some compounds in skim milk inhibited phage attachment. Previous report by Gill *et al*. (2006) showed the inhibitory effect of bovine whey proteins on the interaction of *Staphylococcus aureus* and bacteriophage K in raw milk. The lytic ability of phages was reduced because of adsorption of phages by whey protein and not by the *Staphylococcus aureus* cells. Garcia *et al*. (2007) reported the complete elimination of *Staphylococcus aureus* after 2 h in ultra-high temperature (whole milk at 37°C) and renneted curd within 1 and 4 h of incubation at 30 and 25°C, respectively. Incubation at higher temperature (30-37°C) and high multiplicity of infection are critical factors for the phage effective lysis of bacterial cell while Phage treatment at 4°C resulted in bacteria reduction after 5 days (Kudva *et al*., 1999; Garcia *et al*., 2007). Several Former studies have shown the feasibility of using *E. coli* specific phages for reducing *E. coli* numbers on different food matrix (O'Flynn *et al*., 2004; Carlton *et al*., 2005; Bueno *et al*., 2012; Tomat *et al*., 2013). In similar experiment, Dykes and Moorhead (2002) found no effect of bacteriophage for control of *L. monocytogenes* on contaminated beef. The authors attributed the unappreciable results due to low multiplicity of infection. On the other hand the use of an anti-Listeria phage has been approved by the FDA (2006).

Figure 5 shows phage titers at 4°C in skim milk for the 15-day period. No detectable multiplication of phage in the samples was found. Instead, there was a slight reduction of 0.4 logs on the last day, compared with the first day of study. From Fig. 4 we found that, the phage cocktail was working. However, lytic phages propagate when lysing bacterial host cells. The relative discordance between Fig. 4 and 5 may be due to: (1) the large initial phage titer we used (108 PFU/mL)-an increase is not remarkable if it is much less than the baseline; (2) mass phage adsorption to bacterial cell surface, thus they lost their viability (Bigwood *et al*., 2008); (3) possible inhibitory mechanism involved in skim milk, for example, some phages might be coated by some proteins in milk thus they lost their lytic ability. At this point, further research is required to explore the phage-bacteria interaction in the milk system.

Figure 6 shows the bacterial populations in both controls and treatments at 10°C in skim milk containing *E. coli* for 15 days. In phage-free controls, the bacterial cell level increased greatly by 6.1 logs on day 15, compared with day 1. In the first 8 days, bacteria in skim milk grow similarly to those in TSB, with an increase of 5.8 logs in skim milk and 5.9 logs in TSB. In treatments, relatively similar phage lytic ability in TSB was observed in the experiments with skim milk. Our phage cocktail worked effectively for one day, with a significant ($p<0.05$) reduction of 1.8 logs in bacterial populations, compared with controls. However, after day 1, the bacteria in treatments started to grow, with a growth rate similar to controls. Throughout the 15-day period, there was an overall 1.0-log reduction compared with controls. Although this reduction is statistically significant ($p<0.05$), the final bacterial concentration was relative high (1.8×10^8 CFU/mL). This indicates that, our phage cocktail decreased but did not eliminate the bacteria cells.

Figure 7 shows similar results to Fig. 5, as the phage titer stably measured in treatments for 15 days. No significantly ($p<0.05$) increase or decrease in phage titer was found which indicates the stability of phage in skim milk.

Figure 8 shows that at 25°C, the bacterial populations in phage-free samples increased by approximately 6.1 logs throughout 15 days.

Contrary, the counts of *E. coli* were reduced significantly ($p<0.05$) by 8.3 log cycles in phage treatments compared to control (Fig. 8). From day 0 to day 1, while bacteria in control samples grew rapidly, the phage cocktail reduced the counts of bacteria in treatments to less than 10 CFU/mL. The bacterial populations stayed less than 10 CFU/mL until day 15. To make sure that all the bacteria were killed, we enriched the treatment samples for: Day 1, day 3, day 6, day 15. As a result, we found *E. coli* colony forms for day1 but day 3, day 6 and day 15, we couldn't find any bacteria (data not shown). Therefore, phages were proven to be able to eliminate *E. coli* in skim milk at 25°C, after 3 days of initial phage treatment.

The potential use of phages as biocontrol agents in dairy products is supported by several studies including the current study that indicates absence of bacterial growth and the efficient reduction of pathogen levels in the presence of bacteriophage cocktail (Modi *et al*., 2001; Garcia *et al*., 2007; Guenther and Loessner, 2011; Bueno *et al*., 2012). Nevertheless, physical and chemical factors associated with the food matrix could compromise the successful use of phage as biocontrol agents Guenther *et al*. (2009). In addition to that there is other critical factors that are critical for cell lysis which includes aeration, incubation at 37°C, a high MOI and simultaneous infection with treatment phages (Kudva *et al*., 1999). In our case, the pathogen was not totally eliminated but its numbers were kept below the limits in the presence of phages.

Conclusion

This study evaluated the ability of a phage cocktail to inhibit enteropathogenic *E. coli* O126:H6 in contaminated TSB and skim milk, by monitoring the survival and growth of the bacteria. The overall results indicate that phage cocktail reduced *E. coli* population by 1.8, 5.7 and 8.9 logs in TSB and 1.2, 1.0 and 8.3 in skim milk at 4, 10 and 25°C. Therefore, using lytic phage as biocontrol is a feasible additional parameter to enhance the safety of dairy products. Future research is required to explore more details about phage-bacteria interaction in the complex milk system.

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Author's Contributions

All authors equally contributed in this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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