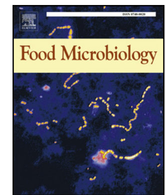
Food Microbiology 76 (2018) 434–442

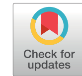
Contents lists available at ScienceDirect

Food Microbiology

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



Evaluation of a novel cocktail of six lytic bacteriophages against Shiga toxin-producing *Escherichia coli* in broth, milk and meat



David Tomat[a,*], Cecilia Casabonne[a], Virginia Aquili[a], Claudia Balagué[a], Andrea Quiberoni[b]

[a] *Área de Bacteriología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK, Rosario, Argentina*
[b] *Instituto de Lactología Industrial (UNL - CONICET), Facultad de Ingeniería Química, Santiago del Estero 2829, 3000, Santa Fe, Argentina*

ARTICLE INFO

Keywords:
Phage cocktail
Escherichia coli
Food safety

ABSTRACT

Phages are potentially useful as antimicrobial agents in food, especially cocktails of different phages which may prevent the development of bacterial resistance. Biocontrol assays with a six-phage cocktail, which is lytic against DH5α, an enteropathogenic (EPEC) and two Shiga-toxigenic (STEC) *Escherichia coli* strains, were performed in Hershey-Mg broth, milk and meat at refrigerated (4 °C), room (24 °C) and abusive (37 °C) temperatures. At 4 °C, cell counts were significantly lower (2.2–2.8 $\log_{10}$ CFU/mL) when *E. coli* strains (~10^9 CFU/mL) were challenged against the phage cocktail (~10^9 PFU/mL) in Hershey-Mg broth after 24 h. However, reductions were higher (3.2–3.4 $\log_{10}$ CFU/mL) after a 48 h exposure for all the strains tested. In addition, reduction values reached up to 3.4 $\log_{10}$ CFU/mL (24 °C) and 3.6 $\log_{10}$ CFU/mL (37 °C) in challenge tests after 24 h, though the reductions achieved were slightly lower after 48 h for the four *E. coli* strains tested. In milk, the cocktail was highly effective since bacterial counts were below the detection limit (< 10^1 CFU/mL) at 4 °C, while the reductions ranged from 2 to 4 $\log_{10}$ CFU/mL at 24 °C after a 24 h exposure. At 37 °C, DH5α was eliminated within 2 h, and an average cell decrease of 4 $\log_{10}$ CFU/mL was observed for the three pathogenic strains tested. When the assays were performed in meat, biocontrol values ranged from 0.5 to 1.0 $\log_{10}$ CFU/mL after 48 h at 4 °C, while a higher cell inactivation was achieved at 24 °C (2.6–4.0 $\log_{10}$ CFU/mL) and 37 °C (3.0–3.8 $\log_{10}$ CFU/mL). Furthermore, higher inactivation values for O157:H7 STEC (1.55 ± 0.35 $\log_{10}$ CFU/mL) at 4 °C were obtained in meat when incubation was extended up to 6 days. As a conclusion, our six-phage cocktail was highly effective at 24 °C and 37 °C, though less effective at 4 °C in both food matrices evaluated. Thus, it might be applied against pathogenic EPEC and STEC strains to prevent foodborne diseases especially when the cold chain is lost.

1. Introduction

Foodborne pathogens such as enteropathogenic *Escherichia coli* (EPEC) and Shiga-toxigenic *Escherichia coli* (STEC) strains are the main causes of diarrhea and hemolytic uremic syndrome (HUS) in our country (Rivas et al., 2008). STEC infections are transmitted to humans through contaminated foods such as meat (Rivas et al., 2003), milk (Farrokh et al., 2013) and water (Swerdlow et al., 1992), while infection by EPEC is related to fecal contamination due to unhygienic handling of food (Hernandes et al., 2009).

The use of bacteriophages as biocontrol agents seems to be a promising alternative against several foodborne pathogens (O'Flynn et al., 2004; O'Flaherty et al., 2005; Bigwood et al., 2008; Mukhopadhyay and Ramaswamy, 2012). Phages are highly active and specific and have been extensively and safely used in clinical applications in Europe (Garcia et al., 2008). In addition, phages have a highly versatile use along the food chain since they have been employed for therapy, biosanitation and biopreservation (Modi et al., 2001; Gill et al., 2006; Raya et al., 2006; Kim et al., 2007).

Bacterial resistance may be a potential problem when using phages for the control of unwanted bacteria in food (Madera et al., 2004; O'Flynn et al., 2004; Endersen et al., 2013). Bacteriophage insensitive mutants (BIMs) mainly arise from the loss or mutation of phage receptors on targeted bacteria (Tanji et al., 2004). Further studies found that BIMs emerged when *E. coli* cells were challenged against coliphages (O'Flynn et al., 2004) as well as for other pathogens (Garcia et al., 2007) and bacteria, such as lactic acid ones (Chirico et al., 2014). On the other hand, the limitation of phages as pathogen control agents, *i.e.* limited host range and limited diffusion in solid food, may be bypassed by using a phage cocktail composed by many different phages

* Corresponding author. Área de Bacteriología. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (U.N.R). Suipacha 531, S2002LRK, Rosario (Santa Fe), Argentina. Tel.: +54 341 4804592.
E-mail addresses: dtomat@fbioyf.unr.edu.ar, dami231@hotmail.com (D. Tomat).

https://doi.org/10.1016/j.fm.2018.07.006
Received 15 December 2017; Received in revised form 3 July 2018; Accepted 16 July 2018
Available online 17 July 2018
0740-0020/ © 2018 Elsevier Ltd. All rights reserved.

and by adding large amounts of phage (O'Flynn et al., 2004), respectively.

The use of phage cocktails to control foodborne pathogens has been explored in milk (Garcia et al., 2007; Zuber et al., 2008), meat (O'Flynn et al., 2004), fruits and vegetables (Leverentz et al., 2003; Viazis et al., 2011). Preparations composed by several phages which use different receptors in the targeted bacteria may have the advantage of infecting a mutant resistant to another phage present in the cocktail. Thus, the use of several different phages which are lytic for the same pathogen will reduce the probability of selecting mutants (Sulakvelidze and Barrow, 2005), enhancing the effectiveness of biological control against foodborne pathogens.

In addition to resistance to phages, bacteria may show resistance to several antibiotics (Yilmaz and Özcengiz, 2017). Furthermore, the use of antibiotics as additives in animal feeds, also known as growth-promoting antibiotics (GPAs), could spread resistance among bacteria (Jia et al., 2017). Unlike phages, antibiotics may select many resistant bacterial species because of their broad spectrum of activity. Also, bacterial resistance mechanisms against phages and antibiotics differ (Sulakvelidze and Barrow, 2005). Therefore, phages could be used as hurdle technology, contributing to reduce the incidence of bacterial resistance to several antibiotics currently employed.

These cocktails have proved effective against several pathogens as *Staphylococcus aureus* (Garcia et al., 2007) and *Enterobacter sakazakii* (Zuber et al., 2008) in dairy products. Yet, their efficacy against *E. coli* in milk (McLean et al., 2013) as in meat products at refrigerated (Abuladze et al., 2008), room and abusive (O'Flynn et al., 2004) temperature have been scarcely explored. In our study, phages are proposed as promising tools to be used in combination and/or alternation with other current technologies. Thus, the aim of the present work was to evaluate the potential utility of six coliphages mixed in a cocktail to control *E. coli* strains by testing their efficacy under different conditions in milk and meat products.

2. Materials and methods

2.1. Bacterial strains and phages

E. coli DH5α was used as the sensitive host strain to propagate all the bacteriophages used in this study. Three additional strains were used in the biocontrol experiments. Two of them, an enteropathogenic *E. coli* (*eae*+) (EPEC) and a Shiga toxigenic *E. coli* O157:H7 (*stx2* + and *eae*+) (O157 STEC), were previously isolated from stool samples, identified using API-20E system (Biomerieux, Buenos Aires, Argentina), and further characterized by PCR. The third strain was *E. coli* Shiga toxigenic non-O157:H7 (ARG4827; serogroup O18; *stx1* + and *stx2*+) (non-O157 STEC) (Balague et al., 2006). All the strains were maintained as frozen (−70 °C) stock cultures in Hershey broth (8 g/L Bacto nutrient broth, 5 g/L Bacto peptone, 5 g/L NaCl and 1 g/L glucose) (Difco, Detroit, Michigan, USA) supplemented with 15% (v/v) glycerol and routinely reactivated overnight at 37 °C in Hershey broth supplemented with $MgSO_4$ (5 mmol/L) (Cicarelli, San Lorenzo, Santa Fe, Argentina) (Hershey-Mg).

Myoviridae bacteriophages (T-even type) (Tomat et al., 2013a), DT1 to DT6, were previously isolated from stool samples of patients with diarrhea treated at the Hospital Centenario, Rosario (Tomat et al., 2013b) and characterized by electron microscopy, host range and PCR assays (Tomat et al., 2013a). Phages were grown to high titers as previously described (Tomat et al., 2013b). Phage stocks were enumerated (plaque-forming units per millilitre; PFU/mL) by the double-layer plate titration method (Jamalludeen et al., 2007) by mixing 0.1 mL of decimal dilutions with 0.1 mL of a log-phase culture of *E. coli* DH5α and 3 mL of warm (45 °C) Hershey soft agar (0.7% w/v) and pouring the mixture onto Hershey-Mg agar (1.5% w/v). Stocks were stored in Tris-magnesium-gelatin (0.05 mol/L Tris, 0.008 mol/L $MgSO_4$, 0.01% w/v gelatin, pH = 7.5) (TMG) buffer at 4 °C.

2.2. Biocontrol studies

2.2.1. Biocontrol in broth

Overnight cultures of the *E. coli* strains DH5α, EPEC, non-O157 STEC and O157 STEC were used to inoculate (1% v/v) fresh Hershey-Mg broth (pH 7,2), separately. When the appropriate cell density was reached (OD_{600} = 0.5), the phage cocktail (the six phages in equal proportions) was added (ca. 10^9 PFU/mL). Cultures were incubated at 4 °C, 24 °C or 37 °C with constant shaking (150 rpm) and samples (100 μL) were removed after 24 h and 48 h for viable cell count on Hershey-Mg agar plates (Tomat et al., 2013a). Phage enumeration (PFU/mL) was also carried out at the beginning and at the end of each experiment by the double-layer plate titration method. Cultures containing only bacteria and Hershey-Mg broth containing only phages were used as a control of viable cell reduction and to verify the absence of contamination, respectively. Three independent experiments and two replicates per assay were carried out.

In addition, qualitative assays were conducted using the phage cocktail and the four *E. coli* strains described above. Cultures in Hershey-Mg broth containing $\sim 10^3$ CFU either alone (strain; controls) or together with the phage cocktail ($\sim 10^9$ PFU) (strain and cocktail; treatments) were incubated in 1.5 mL micro centrifuge tubes at 4 °C, 24 °C or 37 °C. Evidence of bacterial development to the naked eye was recorded every 24 h for a period of seven (7) days. After this incubation, cultures at 4 °C and 24 °C were shifted to 37 °C. Finally, cultures where no development was observed were plated in Hershey-Mg agar plates.

2.2.2. Biocontrol in milk

Biocontrol experiments were carried out at 4 °C, 24 °C and 37 °C in parallel batches in sterile, commercial, reconstituted (10%, w/v) powder skim milk (RSM) (pH 6.7), added with $CaCl_2$ (0.28 g/L) to replace the lost during the sterilization of milk. All the batches were inoculated (except one; contamination control) with overnight cultures of the four tested *E. coli* strains (one strain by batch; final concentration ~ 10^3–10^4 CFU/mL; control assays) described above. Next, batches are split into two aliquots, one aliquot of every batch infected with each *E. coli* strain was inoculated with the phage cocktail ($\sim 10^8$ - 10^9 PFU/mL; experimental assays) in order to evaluate their potential as biocontrol agents, getting a multiplicity of infection (MOI) ranging from $\sim 10^4$ to 10^6. The other aliquot was used as control. The incubation proceeded for 24 h at 24 °C and 37 °C and for six (6) days at 4 °C. During incubation, bacterial cell counts were performed in MacConkey agar (37 °C, 18 h) and phage enumerations were carried out by the double-layer plaque titration method described above, at the beginning and at the end of each experiment. After milk assays, ten randomly selected colonies isolated from the experiments, *i.e.* bacteria exposed to phages, were checked for phage resistance against the cocktail. Assays were carried out in triplicate. Three independent experiments and two replicates per assay were carried out.

2.2.3. Biocontrol in meat

Beef was aseptically cut into pieces (1 cm^2 of surface and 0.4 cm thick; pH 5.6), placed in petri dishes and pre-equilibrated to 4 °C, 24 °C or 37 °C. Host strains employed in this study, namely DH5α, EPEC, non-O157 STEC and O157 STEC, were grown in Hershey-Mg broth for 18 h at 37 °C. Twenty μL of each diluted bacterial suspension were pipetted onto the surface of the meat sample (one strain by sample; final concentration ~ 10^3–10^4 CFU; control assays) and allowed to attach for 10 min at room temperature. Next, 20 μL of the phage cocktail (composed by six phages: DT1 to DT6 in equal proportions) were pipetted on the meat (final concentration ~ 10^8 - 10^9 PFU; experimental assays) at high MOI ($\sim 10^5$ PFU/CFU). Control assays were also inoculated with 20 μL of TMG buffer instead of the phage cocktail. Controls and treatments were incubated at 4 °C, 24 °C or 37 °C. After each incubation time, meat pieces were transferred to a sterile bag, 5 mL of TMG buffer were added and samples processed for 2 min in a Stomacher (Seward,

Download English Version:

https://daneshyari.com/en/article/8843459

Download Persian Version:

https://daneshyari.com/article/8843459

Daneshyari.com