Control of Campylobacter spp. and Yersinia enterocolitica by virulent bacteriophages

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ABSTRACT

The efficacy of the Campylobacter (C.) phages NCTC12684 (group II) and CP81 (group III) and of the Yersinia (Y.) phage PY100 to reduce the numbers of Campylobacter and Y. enterocolitica in meat at 4°C applying different Multiplicities of Infection (MOIs) was analyzed. Initial experiments were carried out in broth at 4°C and 37°C to compare cell number reductions under chilling and optimized growth conditions, respectively. The results showed a 1 log10 unit reduction of Campylobacter cell numbers at 37°C in broth. However, no reduction was observed in broth and meat at 4°C. In contrast, Y. enterocolitica cell numbers were reduced in broth at 4°C (up to 3 log10 units after 24 hr) and 37°C (5 log10 units after 1.5 hr) and also in meat at 4°C (2 log10 units after 48 hr). The highest cell number reductions were obtained at the highest MOIs.

KEYWORDS: Campylobacter, Yersinia, bacteriophage, food safety, biocontrol

INTRODUCTION

Campylobacter causes approximately 200,000 cases of campylobacteriosis in the European Union (EFSA, 2009) and thus is one of the most important bacterial foodborne pathogens. Yersinia infections are of secondary importance with about 7,500 cases yearly (EFSA, 2009). Complete elimination of both pathogens in the food chain is currently not feasible, but the quantitative load can be reduced by several pre- and post-harvest applications. Post-harvest approaches exploiting virulent phages have already been described and focus up to now on the control of Listeria (L.) monocytogenes and Salmonella. Due to the effectiveness and innocuousness of Listeria phages, two products were already approved by the FDA: ListShield, the LMP-102 phage preparation comprising six phages for the control of L. monocytogenes on ready-to-eat foods (Bren, 2007), and Listex P100 (phage P100) for the control of this species in meat and cheese products (Carlton et al, 2005). P100 is able to eliminate or reduce Listeria up to 3.5 log10 cfu/g under appropriate conditions (Carlton et al, 2005; Holck and Berg, 2009; Soni et al, 2009; Soni and Nannapaneni, 2010). With Salmonella, up to 4 log10 unit reductions of cell number have been described in vegetables, chicken, chicken products, sausages and cheese (Modi et al, 2001; Goode et al, 2003; Leverentz et al, 2003; Whichard et al, 2003; Higgins et al, 2005). Some studies demonstrated the efficacy of phages on C. jejuni. Cell numbers were reduced by approximately 1 log10 unit at 4-5°C on chicken skin, and on raw and cooked meat (Atterbury et al, 2003; Goode et al, 2003, Bigwood et al, 2008). No data are currently available on post-harvest application of Y. enterocolitica phages.

This work describes the potential of the Campylobacter phages NCTC12684 and CP81 and of the Yersinia phage PY100 to control their hosts in broth and meat.
MATERIALS AND METHODS

Bacterial strains and bacteriophages
Two Campylobacter strains and one Yersinia strain were used in this study: C. jejuni NCTC 11168 and C. coli NCTC 12668 were obtained from the National Collection of Type Cultures (NCTC), Health Protection Agency, United Kingdom. Y. enterocolitica 83/88/2 is a plasmid-cured derivative of the serogroup O:5,27, biogroup 2 strain 83/88 (Hertwig et al, 2003). Campylobacter strains were grown on Mueller-Hinton-blood (MHB) agar (Oxoid, Wesel, Germany), modified Charcoal-Cefoperazon-Desoxycholat (mCCDA) agar (Oxoid) or in Sodium-NZamines-Casaminoacids-Yeast-Magnesiumsulfate (NZCYM) medium (Roth, Karlsruhe, Germany) at 37°C under microaerobic conditions. Y. enterocolitica 83/88/2 was grown on Luria Bertani (LB) agar.

Figure 1. Phage-induced lysis of Campylobacter and Yersinia in broth at 37°C. (A) C. jejuni NCTC 11168 (Phage CP81). (B) C. coli NCTC 12668 (Phage NCTC12684). (C) Y. enterocolitica 83/88/2 (Phage PY100).
phage lysing strains of *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* (Schwudke et al, 2008).

**Reduction of bacterial cell numbers in broth**

Overnight cultures of bacterial strains were diluted to a final cell number of approximately 1x10^5 cfu/ml. 1ml phage lysate or SM buffer (negative control) was added to 1ml

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**Figure 2.** Phage-induced lysis of *Campylobacter* and *Yersinia* in broth at 4°C. (A) *C. jejuni* NCTC 11168 (Phage CP81). (B) *C. coli* NCTC 12668 (Phage NCTC12684). (C) *Y. enterocolitica* 83/88/2 (Phage PY100).
of the diluted culture. The mixture was incubated at 37°C (microaerobic/aerobic) or 4°C (aerobic). The initial bacterial host concentration was kept constant in all assays at approximately 1x10⁵ cfu/ml. Bacterial counts were determined after 0, 6, 24, 27, 30, 48, 72 and 168 hours. The following MOIs were applied: high MOI for C. jejuni (10²), and for C. coli and Y. enterocolitica (10⁴); low MOI for C. jejuni and C. coli (10¹), and for Y. enterocolitica (10²).

**Reduction of bacterial cell numbers in meat**

Raw chicken and pork meat was tested to be free of *Campylobacter* spp. and *Yersinia* spp. Meat was sliced aseptically into portions of 10gm, frozen at -20°C, and defrosted in a refrigerator 24hr prior to use. *Campylobacter* and *Yersinia* strains were grown overnight in NZCYM medium at 37°C under microaerobic (*Campylobacter*) resp. aerobic (*Yersinia*) conditions. Each meat portion was inoculated with the diluted culture. The mixture was incubated at 37°C (microaerobic/aerobic) or 4°C (aerobic). The initial bacterial host concentration was kept constant in all assays at approximately 1x10⁵ cfu/ml. Bacterial counts were determined after 0, 6, 24, 27, 30, 48, 72 and 168 hours. The following MOIs were applied: high MOI for *C. jejuni* (10²), and for *C. coli* and *Y. enterocolitica* (10⁴); low MOI for *C. jejuni* and *C. coli* (10¹), and for *Y. enterocolitica* (10²).

**Figure 3.** Phage-induced lysis of *Campylobacter* and *Yersinia* in meat at 4°C. (A) *C. jejuni* NCTC 11168 (Phage CP81), (B) *C. coli* NCTC 12668 (Phage NCTC12684), (C) *Y. enterocolitica* 83/88/2 (Phage PY100). (*p <0.05)
with 100μl of the diluted bacterial suspensions (approximately 1×10^8 cfu/ml). Bacteria were allowed to attach to the matrix for 30min at room temperature. Thereafter, 1ml of the corresponding phage lysate or SM buffer was added. All samples were vacuum sealed and stored either at 37°C or at 4°C, depending on the experimental setup. At each sampling time, inoculated meat pieces were diluted 1:10 with NZCYM medium, blended for 2min, and serially diluted with NZCYM medium before being spread onto mCCDA or CIN agar plates. For the enumeration of the bacteria, plates were incubated for 48hr (Campylobacter) or 24hr (Yersinia). All experiments were performed in triplicates. Statistical differences between bacterial cell numbers in the samples were assessed by using the Mann-Whitney-U test.

RESULTS

All three phages reduced the numbers of their hosts in broth at 37°C. While the Campylobacter phages CP81 and NCTC12684 yielded reductions of only 1-2 log_{10} units, respectively of the applied MOI, PY100 reduced Y. enterocolitica cell numbers at a MOI of 10^5 by up to 3 log_{10} units (after 24hr) and at a MOI of 10^5 by up to 5 log_{10} units (after 1.5hr) (Figure 1). No growth inhibition of C. jejuni and C. coli was observed at 4°C in broth at any MOI (Figure 2 A and B). At this temperature, Y. enterocolitica cell numbers were reduced by up to 1 log_{10} unit (low MOI) after 24hr and up to 3 log_{10} units (high MOI) after 24hr (Figure 2C).

Due to restricted growth of Campylobacter at 4°C, experiments with chicken meat (Campylobacter assays) and pork meat (Y. enterocolitica assays) were exclusively carried out at high MOIs allowing lysis from without. Though, both Campylobacter phages did not lyse their host in chicken meat at 4°C (Figure 3A and B). By contrast, phage PY100 reduced the Y. enterocolitica cell numbers in pork meat significantly by approximately 2 log_{10} units after 24hr (Figure 3C).

DISCUSSION

In this study the potential of the three phages to control foodborne pathogens at the post-harvest level was analysed. We ascertained that at 37°C the Campylobacter phages CP81 and NCTC12684 reduced the cell numbers of their respective host in broth, whereas no reduction was observed at 4°C, even at a high MOI (10^5 resp 10^5). The phages probably did not cause lysis from without under these conditions. Other studies demonstrated phage-induced lysis of Campylobacter at 4°C, where an up to 1 log_{10} unit reduction on chicken skin and in cooked and raw meat was achieved (Atterbury et al, 2003; Goode et al, 2003; Bigwood et al, 2008). As the lysis of Campylobacter at refrigeration temperatures is rather limited, post-harvest application of phages is apparently not a promising tool to reduce the Campylobacter load on carcasses or meat. On the other hand, high Campylobacter cell number reductions (2-5 log_{10} units) by phage were obtained in chickens at the pre-harvest level (Loc Carrillo et al, 2005; Wagenaar et al, 2005; El-Shibiny et al, 2009).

Contrary to the Campylobacter phages, Yersinia phage PY100 significantly reduced cell numbers of its host at 4°C in broth and in pork meat. The most efficient reductions occurred at the highest MOI. The data are consistent with previously published studies, in which a control of various pathogens was accomplished by application of high phage numbers (Goode et al, 2003; Leverentz et al, 2004; O’Flynn et al, 2004; Carlton et al, 2005; Guenther et al, 2009). To our knowledge, this is the first report on an application of phage to reduce Yersinia cell numbers in food.

CONCLUSION

An application of virulent bacteriophages for the control of Y. enterocolitica at the post-harvest level seems to be promising, whereas the potential of phages to control Campylobacter in food appears to be limited.

COMPETING INTERESTS

None declared.

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