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Post-Harvest Application of Bacteriophages on Beef as a Natural Intervention against E. coli O157

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Objectives

Despite the use of chemical interventions in a multi-hurdle approach against *Escherichia coli* O157, this pathogen remains a great concern for the beef industry. Bacteriophages are the natural enemy of bacteria, and able to specifically kill *E. coli* O157, for example. Together with the fact that phages are harmless to human, phages have the potential to form an additional safe and effective intervention against *E. coli* O157.

The objective of this study was to determine the efficacy of bacteriophages as natural intervention against *Escherichia coli* O157 on refrigerated beef.

Materials and Methods

Bacteriophages were isolated from sewage water, after which lysis activity of the isolated phages was assessed by spotting serial dilutions on 88 *E. coli* O157 strains. For efficacy assays on beef, 2 cold (4°C/39.2°F) beef cuts of 9 cm^2 were contaminated per treatment with *E. coli* O157 at a rate of 1×10^5 cfu/cm^2. Subsequently, samples were treated with 3×10^7 or 3×10^8 Plaque Forming Units (PFU)/cm^2 by applying 5 µL/cm^2 of a cocktail containing 2 selected phages. Controls were treated with similar volumes of tap water. Beef samples were then incubated for 24 h at 4°C, after which bacteria were retrieved for enumeration. Bacterial reductions on phage treated samples were calculated relative to tap water treated controls. Reductions of 4 different *E. coli* O157 strains obtained in 3 independent experiments were used for statistical analysis (Unpaired *t* test).

Two separate time trials were done in which beef samples were contaminated and treated as described above. In one time trial, beef samples were stored at 4°C during the complete experiment. In the second time trial, all samples were initially stored at 4°C for 24 h, after which all remaining samples were transferred to an abusive temperature of 20°C (68.0°F) for the remainder of the experiment. For both time trials, 2 beef samples per treatment were retrieved for bacterial enumeration at 2, 6, 24, 30, 48, and 54 h post phage treatment. Results were confirmed in 3 independent experiments on which statistical analysis per time point was done (Unpaired *t* test).

Results

After assessing the lysis activity of the isolated phages on 88 *E. coli* O157 strains, 2 phages were selected that showed a complementing hostrange activity, lysing 90% of all *E. coli* O157 strains tested. A cocktail of the 2 selected phages showed bacterial reductions between 1.5 and 1.9 $\log_{10}$ ($P < 0.05$) when cold beef was treated with 3×10^8 PFU/cm^2, while 0.8– 1.5 $\log_{10}$ ($P < 0.05$) reductions were observed with 3×10^7 PFU/cm^2. In both time trials, we observed that the maximum reduction was already achieved 6 h post phage application. Furthermore, in the first time trial at 4°C, we observed no further reduction in bacterial load after the 6 h time point (1.25 to 2.25 Log_{10} reduction at all time points, $P < 0.05$). Transferring the treated samples to an abusive temperature of 20°C, resulted in outgrowth of the remaining *E. coli* O157 at a similar rate as the non-treated controls.

Conclusion

The 2 phage cocktail described above significantly reduces *E. coli* O157 on refrigerated beef. Furthermore, the time trials show that phages work fast on cold beef, and that they can be regarded as processing aid. All in all, we show that bacteriophages provide a natural, safe, and effective intervention for the beef industry to fight *E. coli* O157.

