Louisville KENTUCKY
IAFP 2019

Application of bacteriophages on beef and leafy greens

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Escherichia coli O157 remains a great concern for the beef and leafy greens industry.

Bacteriophages have the potential to be an additional safe and effective intervention against E. coli O157.

● **Purpose:**
The objective was to determine the efficacy of a commercially available bacteriophage cocktail (Phage-Guard E) as an intervention against E. coli O157 on refrigerated beef and vegetables.

● **Methods:**
Lysis activity of natural phage isolates was assessed by spotting serial dilutions on 88 E. coli O157 strains.

For beef, two cold (4˚C) beef cuts of 9cm^2 were contaminated with 1×10^5 CFU/cm^2 E. coli O157, while for romaine lettuce, zucchini and spinach 18 cm^2 areas were contaminated with 1×10^6 CFU/cm^2 E. coli O157.

Subsequently, samples were treated with 3×10^7 or 3×10^8 PFU/cm^2.

Controls were treated with tap water. Samples were then incubated at 4˚C, after which bacteria were retrieved at 2, 6, 24, 30, and 54 hours post phage treatment. Bacterial reductions on phage-treated samples were calculated relative to controls at the corresponding time point. Reductions in three independent experiments were used for statistical analysis (Unpaired t-test).

● **Results:**
A cocktail of two selected phages, lysing 90% of all E. coli O157 strains tested, showed bacterial reductions from 1.5 to 1.9 log ($P<0.05$) on three different strains when cold beef was treated with 3×10^8 PFU/cm^2, while 0.8 to 1.5 log$_{10}$ ($P<0.05$) reductions were observed with 3×10^7 PFU/cm^2 at 24 hours post phage treatment.

Similarly, reductions between 1.45 to 2.97 log ($P<0.05$) and 2.33 to 3.86 log ($P<0.05$) were observed after 24 hours on contaminated vegetables treated with 3×10^7 or 3×10^8 PFU/cm^2, respectively.

In all experiments, the maximum reduction was already achieved six hours post phage application.

● **Significance:**
The phage cocktail described above can be used by the industry as a natural, safe, and effective intervention to fight E. coli O157. ■

Isolation and serotyping of Vibrio vulnificus and Vibrio cholerae in seafood in Korea

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Vibrio species are abundant in seafood and pose a risk to human health. Shellfish and molluscs have potential to accumulate Vibrio species in gills and digestive glands, but there are only a few studies for enumerating and characterising Vibrio vulnificus and Vibrio cholerae in shellfish and molluscs.

● **Purpose:**
The purpose of this study was to investigate the contamination levels of V. vulnificus and V. cholerae in shellfish and mollusk, and to serotype the isolates.

● **Methods:**
Ninety-six seafood samples (66 shellfish samples and 30 mollusc samples) were collected from seafood markets in South Korea from July to December in 2018.

To use the most probable number (MPN) method, 10ml, 1.0ml and 0.1ml of sample were inoculated in 10ml alkaline peptone water (APW). The tube was incubated at 35°C for 14 hours, and 1.0ml aliquots of the cultures were used to extract the DNA for the identification and the serotyping by PCR.

The amplified DNA products were electrophoresed and visualised under UV light.

● **Results:**
V. vulnificus was detected in six samples (6.3%), and V. cholerae was detected in two samples (2.1%). The highest prevalence month of V. vulnificus contamination was September (6.3%), and the highest contamination level was 530 MPN/g detected from cuttlefish and salted oyster.

V. cholerae was detected only in November from date mussel and common orient clam, and both samples showed the same contamination level (36 MPN/g).

The V. cholerae isolates were further analysed for serotyping, and the results showed that all isolates were negative for both V. cholerae O1 and V. cholerae O139.

● **Significance:**
This result indicates that V. vulnificus and V. cholerae contaminated some shellfish and mollusc samples, and the highest prevalence month was September in S. Korea. ■

The microbial ecology and resistome of raw and pasteurised retail milk

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Raw milk is advertised as having multiple benefits for human health, but the microbiome and resistome differences between raw and pasteurised milk remain elusive.

● **Purpose:**
The study aimed to assess the population of viable bacteria and profile the microbiome and resistome in various types of retail cow's milk sold for human consumption.

● **Methods:**
A total of 1,920 milk samples were collected from eight milk brands including ultra-pasteurised milk (n=2), HTST-pasteurised milk (n=3), vat-pasteurised milk (n=1) and raw milk (n=2).

Sampling occurred between March and August 2017 at Davis, CA through eight independent purchases for all brands of milk.

After each purchase, samples were aliquoted into three tubes and incubated for zero, two, four, six, 12, and 24 hours at both 4˚C and 23˚C. All milk samples were used to quantify the total aerobic counts, coliforms and E. coli using standardised methods.

Concurrently, DNA was extracted from all milk for 16S-rRNA sequencing.

In addition, 24 milk DNA samples, which includes both raw and HTST milk before and after 24 hours incubation at 23˚C were subjected to shotgun metagenomic sequencing.

● **Results:**
Different types of milk possess distinct microbiome structure ($P=0.04$), and raw milk has significantly more viable bacteria than other retail milk ($P<0.05$).

Remarkably, the raw milk microbiota was dominated with pseudomonadaceae and enterobacteriaceae with minimal to no detection of probiotic groups.

Raw milk carries more antimicrobial resistance genes (ARGs) than pasteurised milk ($P<0.001$).

Specifically, 138 individual ARGs conferring resistance to 11 classes of antibiotics were observed in raw milk compared with 25 ARGs found in HTST milk.

Incubation at 23˚C drives the bloom of viable bacteria in milk which also significantly enriches the population of ARGs ($P<0.001$).

● **Significance:**
Raw milk harbours more viable bacteria and ARGs than pasteurised milk, and incubation of raw milk at 23˚C dramatically increases such risk. ■

Fate of antibiotic resistance in the environment

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Knowledge gaps exist regarding the transfer of antibiotic-resistant bacteria from livestock to humans via environmental pathways, which hinders a system's assessment of the impact of antibiotic uses during food-producing animal husbandry on public health.

● **Purpose:**
The purpose of this study was to evaluate the survival and transfer of antibiotic-resistant bacteria and genes in the environment in a continuum of beef cattle primary production, cattle manure storage and land application.

● **Methods:**
Different in-feed antibiotic treatments (control, tylan and chlortetracycline) were introduced to beef cattle on feedlot (32 animals per treatment).

Samples of rectal faeces, hides and pen surface on feedlot (five months); manure during stockpiling (three months; 12 samples per pile per sampling); and amended the soil at land application sites (three months; 16 samples per site per sampling) were collected.

Changes in prevalence and concentration of generic and macrolide- and tetracycline-resistant E.coli, Salmonella and Enterococcus were determined over the project lifespan.

Antibiotic-resistant genes were characterised and quantified following shotgun metagenomics sequencing using the Illumina HiSeq platform.

● **Results:**
No statistically significant difference in antibiotic-resistant bacteria load and gene abundance was detected across antibiotic treatments throughout the study from beef cattle production to manure application.

During the three-month period of manure storage as stockpiles, the concentration of generic E. coli and Enterococcus dropped from ~five log CFU/g to a maximum of two to three log CFU/g. Manure storage as static piles significantly reduced antibiotic-resistant bacteria and genes in three months.

● **Significance:**
Our results indicate antibiotic use during beef cattle production might not be associated with extra risk of contamination of antibiotic-resistant bacteria and genes in animal wastes and the following manure and amended soil.

Stockpiling with sufficient time can effectively eliminate resistant bacteria and genes in manure before land application, highlighting the importance of manure management in controlling the transfer of antibiotic resistance through the environment. ■

Decontamination of Salmonella enterica in low-moisture foods

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Cold atmospheric plasma (CAP) offers a dry, non-thermal, and rapid process for surface decontamination of food products.

● **Purpose:**
The purpose of this study was to evaluate the effects of treatment time, treatment distance from the plasma actuator, and pre-conditioning of the product on the inactivation efficiency of CAP on Salmonella enterica on low-moisture foods.

● **Methods:**
In-shell pecans (in triplicate) and black peppercorns (one gram in triplicate) were spot-inoculated with a mixture of five strains of S. enterica (10^7 CFU/ml), and air dried.

The inoculated pecans and black peppercorns were treated by CAP for two, five, and 10 minutes at one, two, and five centimeters from the plasma actuator.

Similarly, inoculated pecans and black peppercorns moistened after air-drying were treated by CAP for two and five minutes at two centimeters from the actuator.

Experiments were repeated at least two times. Mean values of log reduction of S. enterica cells after treatments were compared using ANOVA.

● **Results:**
Treatment time had a significant effect on the reduction of the pathogen on both pecans and black peppercorns.

With 10-minute CAP treatment, 4.04 and 3.63-log CFU reductions of S. enterica were observed at all distances on pecans and black peppercorns, respectively.

Moistening of inoculated pecans or black peppercorns prior to treatment achieved an additional one-log reduction of the pathogen compared to the treatment without moistening.

● **Significance:**
These results show that cold atmospheric plasma (CAP) can be a viable and flexible technology for inactivation of foodborne pathogens on low-moisture foods such as tree nuts and spices. ■

Cultural and genetic characterisation of Escherichia phage OSYSP

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Interest in isolating and characterising new phages for controlling foodborne bacterial pathogens is increasing. This was favoured by regulatory approvals of phage applications and researchers' easy access to next-generation sequencing technologies.

● **Purpose:**
In this study, we investigated the cultural and genomic traits of the previously isolated Escherichia phage OSYSP to assess its suitability for food applications.

● **Methods:**
Bacteriophage host range was determined against Escherichia and Salmonella strains by spot-on-lawn test and double layer plaque assay technique.

Pure phage suspension was stored at 4°C for up to 24 months to determine its shelf life stability. To assess pH sensitivity and survival at different incubation temperatures, OSYSP was held at pH values in the range of 2-12 and temperatures from 4-47°C for 30 minutes. The whole genome of Escherichia phage OSYSP was sequenced using the combination of Illumina Miseq and Ion Torrent sequencing platforms. Ion Torrent reads along with conventional PCR were used for phage OSYSP genome arrangement confirmation.

● **Results:**
Phage OSYSP inactivated pathogenic and non-pathogenic strains of E. coli and S. enterica. The phage was very effective against all tested O157:H7 strains. Phage stock titers were relatively stable throughout the 24-month storage period at 4°C.

Incubation at 4-47°C and pH 4-11 had no significant detrimental effect (P>0.05) on phage infectivity. OSYSP showed close relation with T5-like phages; however, confirmed genome arrangements proved the novelty of OSYSP.

In silico analysis of conventional PCR products and phage, the whole genome showed the presence of lysis-related genes and the absence of undesirable virulence, lysogeny, allergenicity, and antibiotic resistance elements.

● **Significance:**
Desirable genomic traits and physiological stability at adverse conditions suggest that bacteriophage OSYSP is a promising biocontrol agent for foods processed and stored under various conditions. ■