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Opinion Article

Application of Bacteriophages for Biocontrol of *E. coli* O157:H7 in Fresh Produce

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DESCRIPTION

Foodborne illnesses caused by *Escherichia coli* O157:H7 remain a major concern in the fresh produce industry, especially as global consumption of raw vegetables continues to rise. Leafy greens like spinach and lettuce are frequently implicated in outbreaks due to their large surface areas and minimal processing before consumption. Traditional sanitization techniques, including chlorine-based washes, often fall short in fully eliminating bacterial contaminants and may leave undesirable chemical residues. In response to the need for safer and more effective decontamination strategies, bacteriophages viruses that infect and destroy specific bacteria have emerged as a promising tool. This study investigates the effectiveness of a bacteriophage cocktail in reducing *E. coli* O157:H7 contamination on fresh leafy greens under post-harvest conditions, aiming to offer an alternative biocontrol method for improved food safety.

Three lytic bacteriophages specific to *E. coli* O157:H7 were isolated from wastewater sources in Singapore and screened for broad lytic activity and resistance prevention. The phages were combined into a cocktail and applied to romaine lettuce and spinach samples previously inoculated with *E. coli* O157:H7 at approximately 10^5 CFU/g. Two application methods were used: Dipping and mist spraying. The produce was treated at two phage concentrations, MOI 10 and MOI 100, and stored at 4°C to simulate cold-chain storage. Microbiological assessments were performed on days 0, 1, 3, and 7 using selective agar media to quantify bacterial survival, while plaque assays tracked phage persistence. The goal was to assess both the short-term and sustained effectiveness of the phages during refrigerated storage.

The results showed that bacteriophage application resulted in significant reductions in bacterial populations. At MOI 100, reductions between 2.5 and 3.0 log CFU/g were observed within 24 hours of treatment, and these levels were maintained over the 7-day storage period. Even at MOI 10, notable reductions of 1.2 to 1.5 log CFU/g were achieved, confirming that phage concentration influences effectiveness but lower doses can still yield measurable benefits. Spinach, with its moisture-retentive surface, appeared to support better phage retention and bacterial suppression compared to romaine lettuce. Importantly, there was no visible regrowth of *E. coli* in any of the treated samples, and the phage cocktail remained stable and active on the leaves throughout the storage duration.

Resistance screening revealed that fewer than 5% of surviving *E. coli* isolates showed reduced susceptibility to the phages. However, none were resistant to all three, highlighting the protective advantage of using a multi-phage formulation. Sensory evaluation conducted by a panel of trained assessors confirmed that the phage treated produce had no noticeable differences in taste, odor or texture when compared to untreated controls. This confirmed that phage application does not compromise produce quality and supports its use as a consumer safe treatment.

Practical application methods were also compared. Dipping provided slightly more uniform phage distribution and greater reductions in bacterial counts, though mist spraying was faster, required less water, and may be more suitable for commercial-scale operations. Both methods proved viable for integrating into post-harvest handling, making bacteriophage treatment a flexible option for different types of produce processing facilities. Additionally, the absence of chemical residues and the host-specific nature of phages offer an environmentally friendly alternative to traditional sanitizers.

In conclusion, this study demonstrates that bacteriophages offer an effective, natural and safe method for controlling *E. coli* O157:H7 in fresh produce. The phage cocktail significantly reduced bacterial loads on lettuce and spinach, remained stable during storage, and did not affect product quality. With increasing consumer demand for chemical-free, minimally processed food, bacteriophages can serve as a valuable component of modern food safety systems. Their successful integration into post-harvest protocols could greatly reduce the risk of foodborne outbreaks while meeting consumer

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expectations for clean-label produce. Further research into commercial application, cost analysis, and regulatory frameworks will be essential to advance the implementation of phage-based biocontrol in the fresh produce industry.