The Utilization of Bacteriophage as a Biocontrol Agent for *Escherichia coli* Decontamination in Microgreens

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Impact on California Agriculture: Microgreens represent a rapidly expanding niche within California's agricultural economy, where stringent food safety measures are critical to protect public health. However, the absence of robust pre-harvest safety controls poses significant risks of bacterial contamination. Incorporating bacteriophage-based method, such as T7 phage, into microgreen production protocols could revolutionize food safety management practices. By mitigating pathogenic bacterial loads on microgreen surfaces, this strategy has the potential to enhance product quality, reduce post-harvest interventions, and provide growers with a competitive solution.

Rationale/Introduction: Despite the growing production and consumption of microgreens, there remains a significant gap in pre-harvest safety controls designed to prevent bacterial contamination. Although bacteriophages have been successfully implemented as biocontrol agents in other domains, their utility in microgreen production is underexplored. This study addresses the need for alternative, antimicrobial strategies by evaluating the efficacy and stability of T7 phage against *E. coli* BL21 in microgreen production.

Experimental Approach: The investigation was conducted using radish and pea microgreens cultivated under two systems: soil-based and hydroponic. Prior to treatment, microgreen leaf surfaces were sanitized to reduce external microbial populations. An inoculum containing 8-log CFU of *E. coli* BL21 was spot applied to the microgreen surfaces, allowing a 30-minute period for bacterial attachment. The treatment group was subsequently inoculated with a 9-log PFU T7 phage, corresponding to a multiplicity of infection of 0.2, while the control group received an equivalent volume of sterile saline. Both groups were incubated at controlled temperatures of 25°C and 37°C, and samples were collected at 0, 2, and 4-hours post-inoculation. Additionally, phage stability was examined in a greenhouse setting using a hydroponic system. T7 phage was introduced into both the growth water and directly onto leaf surfaces. Stability was monitored over a 7-day period, with treatment groups exposed to LED light (105 μmol/(m² s)) and no exposure to LED light. Phage populations were quantified at 0, 24, 72, 120, and 168 hours to assess the stability of the bacteriophage under varying conditions.

Major Conclusion: The study demonstrated that T7 phage treatment achieved a statistically significant reduction (>2.5-log reduction, $P < 0.05$) of *E. coli* BL21 within 2 hours, with antimicrobial effects persisting up to 4 hours. Notably, soil-based microgreens exhibited optimal phage efficacy across both temperature settings. Stability assessments revealed that T7 phage maintained robust populations in growth water, persisting for up to 120 hours under LED exposure and 168 hours in the absence of LED light, while stability on leaf surfaces was comparatively reduced, with a marked decrease after 24 hours under LED light and up to 72 hours without. Overall, these findings validate the potential application of T7 phage as an effective biocontrol agent to mitigate bacterial contamination in microgreens, therefore addressing a critical food safety challenge in California agriculture.