The Utilization of Bacteriophage as a Biocontrol Agent to Proactively Prevent *Escherichia coli* in Microgreen Cultivations

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Impact on California Agriculture: In the past 30 years, U.S. fresh produce consumption has risen by 25%, majority in leafy greens. Foodborne disease outbreaks have also significantly increased over the same period. Pathogenic *Escherichia coli,* the common root cause for bacterial contamination, causes over 54% of outbreaks in produce. Current disinfection methods including chlorine, peroxyacetic acid and hydrogen peroxide were shown with limitations due to interactions with organic matter, formation of disinfection by-products, and environmental impacts. Lack of food safety awareness and unstandardized farm training also aggregate produce safety challenges. Nonetheless, bacteriophage, a natural biocontrol agent, is gaining popularity for its effectiveness against foodborne pathogens.

Rationale/Introduction: Microgreens, predominantly cultivated in greenhouses, have gained popularity in urban agriculture due to their nutritional value. However, their unique conditions make them susceptible to foodborne pathogens and bacterial colonization. To overcome the challenge, phage, known for its antimicrobial potential, has been recognized as one of the biocontrol agents to eliminate *E. coli* on produce to potentially enhance produce safety. Moreover, a farm training checklist that ensures adherence to Good Manufacturing Practices (GMPs) was created to promote consistent food safety practices. This systematic approach covers facility defects, equipment uses, cleaning, and waste management to mitigate contamination risks.

Experimental Approach: Three 3cm x 3cm pre-trimmed microgreen leaves were washed for one minute with sterile deionized (DI) water to remove soil particles and sanitized with 70% ethanol to eliminate background flora. After a rapid burn-dry to remove the residual alcohol and the air-dry process, 15 μl of 8 log CFU/ml of *E. coli* BL21 (surrogate for pathogenic *E. coli*) was spot-inoculated onto a single spot on each leave, followed by a 30-minute incubation, respectively. Post-incubation, treatment groups were inoculated with T7 phage at a 1:5 ratio of multiplicity of infection (MOI). Treatment groups were inoculated with the bacteria and 75 μl of 9 log PFU/ml of T7 phage, while the control groups were inoculated with the pathogen and sterile saline. Both sets of samples underwent incubation for 0, 2, and 4 hours at 25°C and 37°C with added sterile DI water. Subsequently, both samples underwent bead beating with silica beads, followed by serial dilution for bacteria enumeration.

Major Conclusion: Recovered *E. coli* BL21 population remained at 6 log CFU/ml in all control samples. A reduction ($p<0.05$) in *E. coli* BL21 was observed in microgreens treated with T7 phage across all 24 replicates. Quantitative analysis revealed a consistent 3-log reduction ($p<0.05$) of *E. coli* BL21 in the treatment group compared to the control group during the 2-hour and 4-hour intervals at 25°C and 37°C, respectively, with the reduction rate persisting up to 4 hours. Additionally, consistent 1.5 to 2-log reductions of *E. coli* BL21 were shown in all the treatment groups from 0-hour to 4-hour intervals.

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