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Bacteriophage Intervention Effectively Kills *Listeria* on Food Contact Surface Materials

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Objectives

Listeria is a pathogenic bacterium that is widespread in nature and can enter food processing plants through many vectors, like raw materials, process waste and personnel. Food processors work hard to keep *Listeria* out of the environment, but it can at times be found from food contact surfaces to floor drains. The sanitation can be compounded when equipment is pitted or cracked creating a harborage or niche in which *Listeria* can grow. Many control strategies for cleaning and biofilm removal have been put into place but may not suffice in eliminating *Listeria* from the food contact surface or environment. Bacteriophages are now being used to tackle these pathogens in food processing environments. Since they only target specific bacteria, they are harmless to humans, animals and plants, while effectively eliminating *Listeria*.

This study determines the efficacy of a commercially available bacteriophage product, PhageGuard Listex, against *Listeria* on commonly found materials in food processing plants (stainless steel and UHMW polyethylene). Efficacy was determined by applying two phage concentrations, as well as two exposure times.

Materials and Methods

Overnight cultures of *L. monocytogenes* ATCC13832 and *L. innocua* ATCC51742 were mixed in equal parts to create a *Listeria* cocktail (2×10^9 CFU/cm^2). Sterile coupons (100 cm^2) of stainless steel or UHMW polyethylene were artificially inoculated with the cocktail at 2.5ML/cm^2 and left to dry at 37°C until completely dry. Subsequently, coupons were treated with 2×10^7 or 1×10^8 Plaque Forming Units (PFU)/cm^2 using a spray system and incubated at room temperature for 1 and 3 h, before retrieval and enumeration of bacteria on selective agar plates. Sample size n:3. Results were analyzed using two-way ANOVA, with Dunnett's multiple comparisons test on the normalized data.

Results

A dose dependent response to the phage treatment was observed, where an increasing phage concentration resulted in an increase in *Listeria* kill on both surfaces. On stainless steel, a treatment dose of 2×10^7 PFU/cm^2 resulted in a statistically significant bacterial reduction of 1.27 log after 1 h (p value < 0.0001), while application of 1×10^8 PFU/cm^2 showed a 2.16 log reduction (p value < 0.0001). On UHMW polyethylene, a bacterial reduction of 0.47 log was observed 1 h after applying 2×10^7 PFU/cm^2, while the application of 1 ×

Table 3. Log reduction of *Listeria* cells after applicatio nof two bacteriophage concentrations, measured at 1 and 3 h post phage treatment

Treatment (PFU/cm^2)	Stainless steel (bacterial reduction, log)		UHMW polyethylene (bacterial reduction, log)	
	1 hour	3 hours	1 hour	3 hours
$2x10^7$	1.27 ± 0.13	1.42 ± 0.17	0.47 ± 0.33	0.4 ± 0.19
$1x10^8$	2.16 ± 0.16	3.38 ± 0.24	1.82 ± 0.62	1.95 ± 0.4

10^8 PFU/cm^2 led to a reduction of 1.95 log. However, these reductions were not statistically significant (p value > 0.05). After 3 h of treatment, the reductions were slightly higher in both materials (Table 3). After this time, the difference between control and 5% treatment on UHMW polyethylene obtained a p-value < 0.05.

Conclusion

Phage technology is an easy and safe intervention which can be used as an additional tool to control *Listeria* in processing environments. The above results indicate that the commercially available phage solution, PhageGuard Listex, can reduce *Listeria* contamination on food contact surfaces by 0.4 to 3.4 logs after 3 h of treatment.