PhageGuard
THE POWER OF NATURE

Biofilms with Listeria poses a persistent threat in food production

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In nature, microorganisms thrive by attaching to and growing upon living and inanimate surfaces. These surfaces may take many forms, including those found in a factory and processing equipment such as machines and conveyors. The common denominator of this attached growth state is that the cells develop a biofilm. Biofilm formation is a process whereby microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that facilitate attachment and matrix formation.

The biofilms can form persistent reservoirs that can release or excrete bacteria into the food processing environment. So when pathogenic bacteria form biofilms this has great significance for public health. With regards to human health, biofilm-associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents. This susceptibility may be intrinsic (as a natural outcome of growth in the biofilm) or acquired (due to transfer of extrachromosomal elements to susceptible organisms in the biofilm). Biofilms containing Listeria can be a huge menace and serious threat that the operation when it continues to contaminate food.

The process of biofilm formation

Biofilm formation is a process which involves a series of steps. Initial attachment is via weak electrostatic forces and occurs rapidly. The surface is “conditioned” for bacterial attachment and Listeria biofilm growth begins with physical attachment of the cells to the surface with complex polymers.

The second step of biofilm formation is actual attachment of microorganisms to the conditioned surface. At this point bacteria can still easily be removed. The irreversible attachment of cells happens in the third step, and the removal of cells requires much stronger forces. During this period, the attached cells also produce additional extracellular polymeric substances (EPs) that help anchor the cells to the surface. Layers of bacterial cells entrapped within the EPs-containing matrices start to develop within the biofilm. Biofilms are composed of proteins, nucleic acids, and lipids, which provide mechanical stability, surface adhesion, and scaffold formation for the three-dimensional architecture that interconnects and immobilizes biofilm cells. Biofilms contain highly permeable water paths. At some point after Listeria attachment, some bacteria are released in order to allow the cells to survive and colonize new niches. this release leads to contamination of the product stream. The vegetative cells may reattach in downstream parts of the plant and initiate biofilm formation, completing the cycle.

Biofilms therefore can manifest themselves where manufacturing plant cleaning is ineffective. Environmental surfaces such as floors and doors can also be indirect sources of contamination. Transfer to food products can take place by vectors such as air, people, dripping condensation and cleaning systems. As intricate food processing machines become increasingly complex the risk increases that they are improperly cleaned and present important niches of contamination. Other common sources involved in biofilm accumulation include doors, waste water pipes, bends in pipes, rubber seals, conveyor belts and stainless steel surfaces. In meat and dairy processing industries, the presence of *Listeria* has been found on equipment and utensil surfaces.

Natural solutions to prevent Listeria biofilm formation in food processing

A 2010 study by Soni and Nannapaneni investigated the effect of Phageguard Listex on *Listeria monocytogenes* mono-species biofilms growing on stainless steel and in 96 well plastic plates. Isolates of all 13 *L. monocytogenes* serovars were tested. PhageGuard Listex was able to eliminate the biofilms biomass in 96 well plates almost completely regardless of serovar. It should be noted that the ability of the different strains to form biofilms varied greatly with 2 strains of serovar 1/2a showing highest levels of biofilm formation.

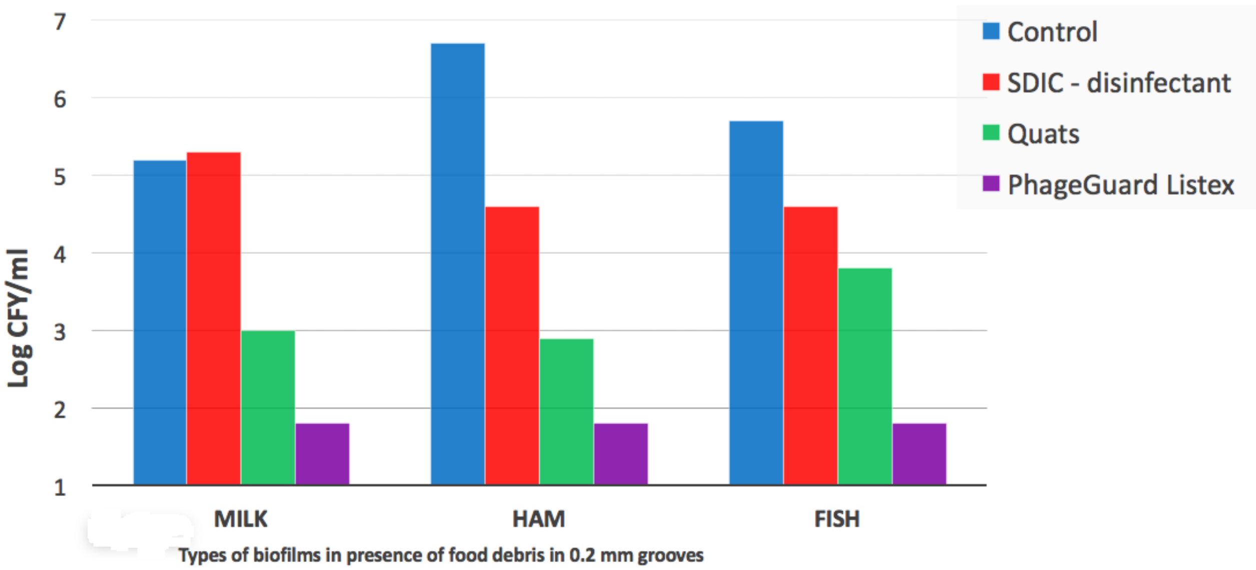

Fig. 1 Removal of *Listeria monocytogenes* biofilms with phage
K.A. Soni, R. Nannapaneni, Journal of Food Protection, 2010

Two experiments were performed on stainless steel. In the first a high number of Listeria cells were allowed to attach to stainless steel coupons for 2 days under nutrient limiting conditions. After phage treatment a 5.4 log reduction of viable cells was observed compared to the saline treated control. In the second experiment a multilayer biofilm was allowed to develop over 7 days from low initial cell numbers. Treatment with phages after this period resulted in a 3.5 log reduction in viable cells compared to the control.

Chaitiemwong et al published another study comparing the efficacy of PhageGuard Listex with traditional sanitizers on *L. monocytogenes* growing on stainless steel in the presence of 10% food debris. After 14 days of growth the phage suspension drastically outperformed a quarternary ammonium compound and sodium dichloroisocyanurate in killing cells grown in the presence of milk, ham and fish. This shows that sanitizers react away with proteinaceous/fatty matter making them less effective than experiments performed under normal conditions would suggest. Phages are not affected by the presence of food debris.

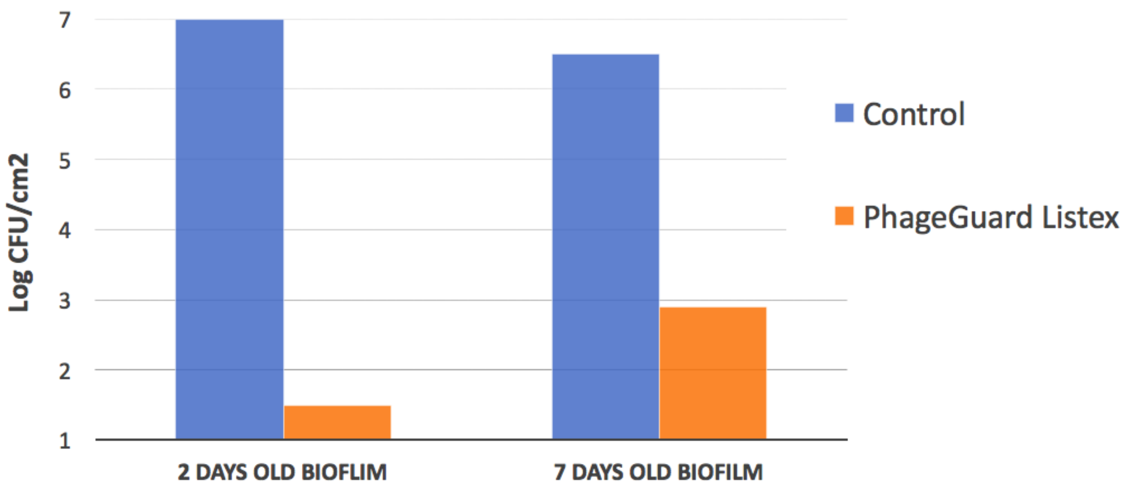

Fig. 2 Removal of Listeria monocytogenes biofilms by disinfectants, quats and phage
N. Chaitiemwong, W.C. Hazeleger, R.R. Beumer, Journal of Food Protection, 2014

The oxidizing sanitizers are quickly neutralized on the proteins of these biofilms and only scratch the surface of the multilayer biofilm. The deeper laying bacteria are unaffected and can continue the formation and excretion of contamination in the future. The publications show that PhageGuard Listex is able to travel permeable water ways into the deeper layers of the biofilm for a more effective kill.

Based on these results the use of Listex phage can provide a useful tool in a food processors effort to eradicate and prevent the formation of mature biofilms in a processing environment.

References:

Chaitiemwong N, Hazeleger WC, Beumer RR (2014) Inactivation of *Listeria monocytogenes* by disinfectants and bacteriophages in suspension and stainless steel carrier tests. J Food Prot 77 (12):2012-2020.

Soni KA, Nannapaneni R (2010) Removal of *Listeria monocytogenes* biofilms with Bacteriophage P100. J Food Pot 73 (8):1519-1524.

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