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Persistence and inactivation of bacteriophages in the environment and possible consequences for application in foods

The purpose of Listex-P100 application is the eradication of contamination with *Listeria monocytogenes* during food processing. When an infective phage particle encounters a susceptible host bacterium, the encounter will eventually result in the death of the bacterium. In order to achieve this purpose on a food surface, a critical number of phages which ensures the likelihood of the phage-host encounter has to be applied at a specifically selected point in time. It is clear that the highest level of efficacy is obtained before phages become gradually inactivated, and this obviously is shortly after application/addition of phages.
Once the number of infective phages drops below a critical value due to inactivation the efficacy of this type of processing aid is no longer maintained. A summary of the various inactivation factors and their relevance in a food environment is given further below.
Reaching and maintaining the critical number of phages is essential because of the spatial distributions on the sub-microscopic scale as illustrated in Figure 1.

As an example, it is assumed that a 100 cm^2 area is treated with 10^7 PFU/cm^2 P100. Approximately 100 *Listeria* cells present (a likely contamination scenario) are now infected by phages and each produces 50 progeny phages. This would result in a net increase of 49 phages per infection and a total increase of 4900. This would constitute a total increase of approximately 0.0005% of phages present, and can be considered negligible.

Numerous environmental factors can contribute to inactivation of functional phages. Among these are: adsorption of phages to particles, proteolytic degradation of the phage virion by chemicals and enzymes, temperature, salts and also light which damages the DNA (Suttle and Chen 1992; Garza and Suttle 1998; Hurst *et al.,* 1980).

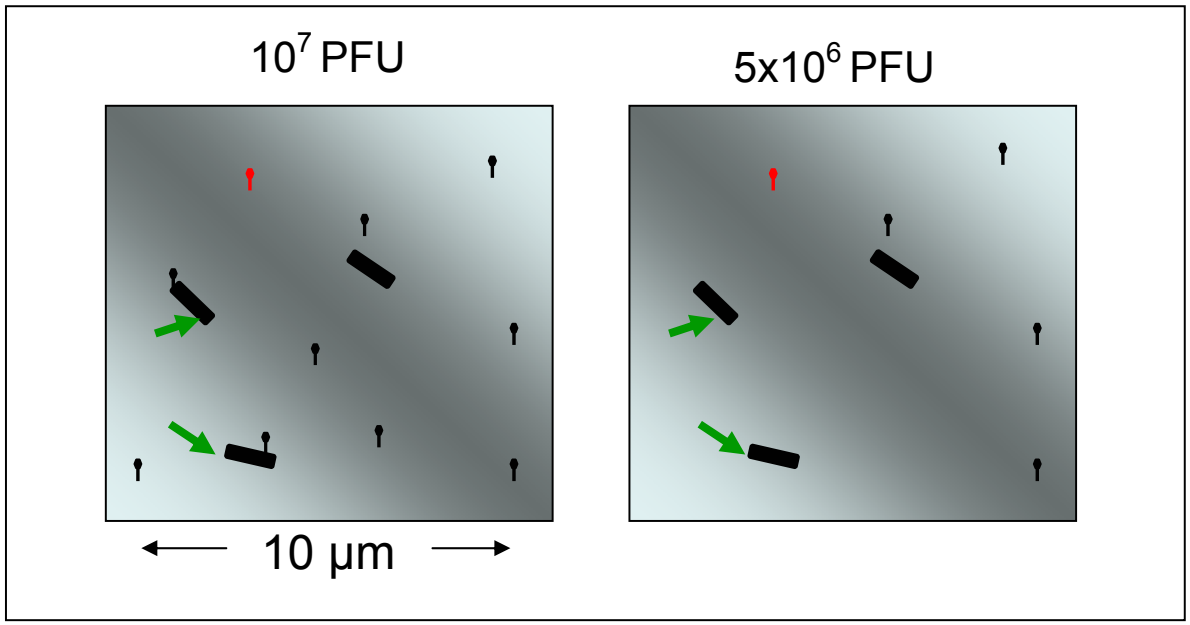

Figure 1. The figure shows possible distributions of *Listeria* cells and phages on 1 millionth of a square centimeter, with two phage concentrations differing only by a factor 2 (drawn to scale indicated). The red phage represents a phage already inactivated by adsorption to a particle. The two bacterial cells indicated by arrows face very different situations in the two panels. Those in the right panel will likely be able to replicate, leading to outgrowth while those in the left panel are likely to encounter an active phage and thus be eradicated. Since bacterial growth is not linear but exponential the drop in phage numbers below the critical value results in exponentially diminished efficacy over time. The progeny phages released from cells infected in or on the food will not significantly contribute to the number already present.

The decay in infectivity rates of phages infecting cyanobacteria in seawater are typically measured at around 1% h^{-1} (Noble and Fuhrman 1997), which indicates a rather rapid loss of infectivity. In marine and soil environments, adsorption to particles constitutes a major factor inactivating phages (Suttle and Chen 1992; Garza and Suttle 1998; Hurst *et al.,* 1980). While the phages remain structurally intact they are no longer physically able to interact with their host bacteria. This phenomenon will also occur in foods, regardless of its nature.

Enzymes from the microbial flora in soil also contribute to more rapid inactivation of phages (Nasser *et al*., 2002). In fermented foods, proteolytic enzyme levels may be especially high and even non-fermented foods of animal or plant origin may contain enzymes from the organism as well as organic acids and other inhibitory agents.

Other acids and chemicals may be present due to production techniques inherent to certain foods. Whatever the individual contributions of the various mechanisms in any particular food, the number of active phages will constantly decline from the moment of application. While absorption to particles will constitute a relatively similar drain in two closely related products, variations in the microbial flora found in two different cheese factories will result in different

enzymes and different enzyme levels. This makes any prediction on the various speeds of inactivation almost impossible and will differ from case to case.

In summary it can be concluded that active phage numbers will decline from the moment of application. This necessitates achieving the critical dose before packaging. Increasing this dosage would not make sense because:

a) It would increase cost of application.

b) *Listeria* is an opportunistic pathogen and not a spoilage-associated bacterium, and Listex P100 is not designed to help in extending shelf-life in any way.

References

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