PhageGuard

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Controlling Listeria monocytogenes in Cheese

Content

1 Summary

Listeriosis is primarily a foodborne infection and is particularly a problem on foods that are not cooked just prior to consumption, including dairy products. Dairy products, in particular soft cheeses, pose a major concern to the dairy industry and public health authorities as they are the leading source of listeriosis outbreaks. It is a severe foodborne infection affecting pregnant women, children, elderly and immunocompromised people, with a high (20–30%) mortality rate. Cheeses offer a suitable environment for the survival and growth of *Listeria monocytogenes*, allowing this pathogen to display tolerance responses that can favor its presence in cheese and persistence in dairy processing plants. Extensive food safety measures towards prevention of transfer of *Listeria monocytogenes* to dairy products have been implemented. However due to the specific abilities of this pathogen to overcome the processing hurdles, its control remains a challenge [(1)]. PhageGuard Listex can help cheese producers to reduce their *Listeria* risks on the product itself as well as processing equipment and environment. 1-3 log reduction can be expected on product and 2-5 log on critical process equipment such as slicers and belts and on hot spots in the environment. PhageGuard Listex can be used during processing as it is a GRAS processing aid and it does not react away with food debris.

2 Introduction

Listeria monocytogenes may cause disease in humans and it is typically transmitted as a food-borne pathogen. *L. monocytogenes* is frequently present in the environment, in soil, vegetation and faeces of animals. The organism can be found in raw foods such as fresh meat, raw milk and fish. The ubiquitous occurrence and the increased ability to grow or survive in a chilled environment compared to most other microorganisms, makes *L. monocytogenes* a significant challenge in food production. This is especially the case for ready-to-eat (RTE) foods in which *L. monocytogenes* can grow and that will not receive a heat-treatment during production, and for foods that may be exposed via the environment, including the production environment, during their manufacture.

The foods most often implicated in both sporadic and outbreak cases are those that

- are ready-to-eat and are not heated directly before consumption;
- have been stored for some time under chilled conditions;
- are the kinds of food in which *L. monocytogenes* can grow to large numbers.

It is crucial that producers of RTE foods take actions to control *L. monocytogenes*, as well as its growth in the product until the end of shelf-life [(2)].

3 Listeriosis

Most people don't get sick when exposed to *Listeria*, which typically causes a bout of uncomfortable diarrhea. Anyone with a healthy immune system can usually ward off any serious illness. Whether foods that harbor *Listeria* cause illness depends on the virulence and concentration of the strain, the amount eaten and the susceptibility of the consumer to infection.

When the bacteria get into the bloodstream, they can spread throughout the body and cause listeriosis, a disease. Difficult to detect or cure, listeriosis may start with a fever or stiff neck and then progress to confusion and convulsions, encephalitis and meningitis.

Nearly everyone who gets invasive listeriosis requires hospitalization and a weeks-long course of intravenous antibiotics. Odds are that the disease will kill one out of every five victims, giving it the highest mortality rate of foodborne pathogens [(3)].

People most at risk are those suffering from underlying health conditions, such as diabetes, or those with weakened immune systems, such as children or those undergoing cancer treatment.

People 65 and older are four times more likely than the general population to get sick from *Listeria* poisoning, and pregnant woman are 10 times more likely to be infected. Although a pregnant woman may not develop listeriosis herself, the pathogen can attack her fetus, resulting in miscarriage, preterm birth or stillbirth [(4)].

Examination of foods implicated in both sporadic cases and outbreaks have typically had more than 1.000 cfu of *L. monocytogenes*/g. Sometimes the number of *L. monocytogenes* exceeded 1-10 million/g. There are examples where ready-to-eat foods sampled at retail outlets carried 1.000 and more cfu of *L. monocytogenes*/g without evidence of them causing human infection [(5)].

The death toll from the world's largest ever outbreak of *listeria* was 216 with 1,060 laboratory-confirmed cases reported from 01 January 2017 to 17 July 2018 in South Africa [(6)]. It was traced to processed meat.

4 Listeria in Cheese

A Listeria study (2013) in 34 dairy Italian plants collected a total of 547 of food, product contact surface and floor drain samples along the product lines. Nineteen cheese factories (55.8%) were found positive for the presence of *Listeria spp*. Of these 20.6% were *L. monocytogenes* positive. *Listeria spp*. was found in 6.8% of food samples, 11.3% of product contact surfaces and 40.6% of floor drains. *L. monocytogenes* was found in 2.4% of food samples, 4.9% of product contact surfaces and 18.8% of floor drains. This underscores that *Listeria spp*. and *L. monocytogenes* are widely spread in the dairy sector and are probably transferred to foods during the production process [17]. A review on 100 studies estimated *L. monocytogenes* prevalence in soft cheese at 2.4% [39].

Cheeses, particularly soft cheeses, have been implicated in listeriosis outbreaks worldwide. Foodborne listeriosis is a relatively uncommon but serious disease caused by *L. monocytogenes*, a pathogen that can be killed under normal cooking temperature but is able to grow slowly at refrigerated temperature as low as 0°C/32°F. A food containing low levels of *Listeria* that leaves the store has the potential to become deadly inside a home refrigerator.

The presence of *L. monocytogenes* in cheeses may originate from the ingredients particularly raw milk or can come from the processing plant environment, including the equipment, personnel or be transferred between finished products and raw materials. If the temperature as well as other conditions especially acidity and water content permit, *L. monocytogenes* can grow to high levels upon prolonged storage. In general, soft cheeses made with unpasteurized milk are of much higher *L. monocytogenes* risk than hard/ extra hard cheeses made with unpasteurized milk as the formers are likely to be less acidic and contain more moisture, which provide a favorable environment for the growth of *L. monocytogenes*, than the latter. A recent risk assessment study conducted by Food Standards Australia New Zealand also pointed out that the estimated *L. monocytogenes* risk from the consumption of certain raw milk soft cheeses i.e. feta and camembert is low in the general population but is high in the susceptible population. However, the *L. monocytogenes* risk upon the consumption of raw milk cheddar cheese (a type of hard cheese) and extra hard cheese in the general and susceptible populations is negligible and low/ very low respectively [7].

Validation and verification of the pasteurization process and avoidance of post-pasteurization bacteria transfer are key areas to control. Exposure of soft and semi-hard cheese to *Listeria* may occur during handling in ripening rooms, wrapping and packaging stages or at retail/domestic cutting stages. Surface ripened washed rinds and white mould cheeses are especially at risk because consequent rises in pH and other factors at the external crust may allow *L. monocytogenes* to grow, in contrast to the core of the same cheese which will remain at a low pH. Also there is a large risk for cross transfer from one cheese to another in these cheese types due to their processing conditions.

It is important to distinguish potential routes of spreading of *L. monocytogenes* to know how to protect food and production environment from this bacterial exposure, as well as how different processes and environmental conditions influence the growth of the bacteria at different stages of the food chain. As it was mentioned, these microorganisms are able to survive under extremely different environmental conditions [8]. Furthermore, they form biofilms on the surfaces of the processing equipment which may be a reservoir of these microorganisms for a long period, since *L. monocytogenes* is resistant to disinfectants, UV light, and desiccation [9, 10]. It is difficult to remove bacterial biofilms, which makes them one of the most important hazards in food production process. Biofilm formation is often supported by accumulation of food residues in specific niches, like meat choppers or minced meat machines [9].

It has been shown that *L. monocytogenes* is capable to form biofilm on stainless steel and glass or even polyvinyl chloride and polyethylene surfaces [13]. The structure of the biofilm shows a great variety, from monolayer of cells to knitted chain network and honeycomb-like structure. Biofilm forming is influenced by *L. monocytogenes* factors, such as biofilm-associated protein (Bap), protein SecA2, and flagella, as well as environmental conditions, like temperature and adhesion properties of the surfaces [14]. No correlation between *L. monocytogenes* molecular lineages or serovars and biofilm formation capacity has been observed [9, 13, 15]. Additionally, it has been shown that these bacteria are able to form biofilms with other microorganisms which may be present in the production environment, e.g. *Pseudomonas* or *Staphylococcus*. This may increase the strength of the biofilm structure and its resistance to the majority of cleaning and disinfection agents [14, 16].

5 Trends and Regulatory Environment

5.1 *Listeria* Incidence

CDC estimates that *Listeria* is the third leading cause of death from foodborne illness, or food poisoning, in the United States. An estimated 1,600 people get sick from *Listeria* each year, and about 260 die. This is 5 cases per million. Improved control measures starting in the 1990s have greatly reduced the prevalence of *L. monocytogenes* in many food categories. However, the rate of listeriosis has remained constant during the last decade and the more severe, systemic (invasive) form of listeriosis is now recognized as occurring more frequently in small outbreaks than previously recognized [18].

According to the ECDC in the EU in 2017 a total of 2480 cases were reported (4.8 per million) including 227 deaths. There has been a statistically significant increasing trend of confirmed listeriosis cases in the EU/EEA during the period2008–2017 as well as during the last 5 years (period 2013–2017). The EU case fatality was 13.8% among the 1,633 confirmed cases with known outcome, a slight decrease compared with 2016. *Listeria* infections were most commonly reported in the elderly population in the age group over 64 years and particularly in the age group over 84 years [19].

Most listeriosis cases are due to the consumption of ready-to-eat foods which support growth of *Listeria* and develop a high concentration of *Listeria* along the food chain. An EFSA Panel concluded that keeping *Listeria* to absence in 25 g or ≤ 100 cfu/g at the point of consumption leads to very low numbers of listeriosis cases in humans.

5.2 Regulatory Environment

Both the FDA and USDA have a zero-tolerance policy for any detectable level of *Listeria* in food. A zero tolerance policy means absence of *L. monocytogenes* in 25 gram samples. This standard is in effect whether or not a food supports the growth of *L. monocytogenes*. Detecting 1 cfu of *L. monocytogenes* in 25g of sample requires recall of the product in question and interruption of the producer activities until the microbial problem is solved.

In Canada, after a foodborne outbreak in 2008, the Canadian Inspection Agency (CFIA) has developed new guidelines for *L. monocytogenes* control in industries producing RTE meat products, including a contact surface monitoring program which involves compulsory communication about positive results for *Listeria spp*. and *L. monocytogenes* observed during the execution of environmental monitoring as part of the HACCP program in every food establishment.

By contrast, the European Union tolerates what it says are safe levels. The criteria on the presence of *L. monocytogenes* in RTE food are the same as in the Codex and they are specified in Commission Regulation (EC) No. 2073/2005 as later amended. It has been established that food business operators are responsible for application of the relevant criteria for different kinds of food. The number of *L. monocytogenes* must not exceed the limit of 100 cfu/g during the shelf life of food which is unable to support the growth of these bacteria, or *L. monocytogenes* must be absent in 25 g in RTE food which is able to support the growth of these microorganisms [20].

Examination of foods implicated in both sporadic cases and outbreaks have typically had more than 1000 cfu of *L. monocytogenes*/g.

PhageGuard Listex is considered GRAS (GRN218) by the FDA.

It is further approved as a processing aid in Canada, Australia, Israel and others. It is organic certified (OMRI USA), Halal and Kosher.

A European Court of Justice Order states that PhageGuard Listex can be used as a non decontaminating processing aid to prevent the outgrowth of *Listeria*.

6 Phage

Bacteriophages (“phages”) are the most abundant micro-organisms on this planet. Phages are 100 times smaller than bacteria. Phages cannot be seen under a normal microscope, yet their collective biomass is larger than that of all humans combined.

Phages are naturally present in significant numbers in water and foods of various origins. Phages are harmless to humans, animals, and plants. Phages use bacteria for their multiplication. Via this mechanism, phages contribute to environmental homeostasis, the situation wherein none of the bacterial species in a biosphere becomes dominant. Every 48 hours 50% of the entire global bacterial population is effectively destroyed by phages.

Every species of bacteria is thought to be the host for at least one phage type. Several phages exist that are able to recognize and lyse (kill) a number of different bacterial strains within one species; these have a ‘broad spectrum’ or a wide host range. Phages are the natural enemies of bacteria, and therefore are logical candidates for targeted control of food borne bacterial pathogens like *Listeria*.

Phage facts:

- Phage kill only bacterial target cells (and do not impact plant or animal cells);
- Phage do not cross species or genus boundaries; therefore they will not affect desired bacteria in
  - foods (e.g., starter cultures for cheese and sausage)
  - commensals in the gastrointestinal tract
  - accompanying bacterial flora in the environment – like water treatment units
- Phage are composed entirely of proteins and DNA, so their breakdown products consist exclusively of amino acids and nucleotides, both of which are present in abundance in food products.

6.1 Phages are Safe

With respect to their potential application for the biocontrol of undesired pathogens in foods, feeds, and related environments, it should be considered that phages are the most abundant micro-organisms in our environment, and are present in significant numbers in water and foods of various origins, in particular fermented foods [21]. On fresh and processed dairy and meat products, more than 10^8 viable phages per gram are often present [22]. It is a fact that phages are routinely consumed with our food in high numbers. Moreover, phages are also normal commensals of humans and animals, and are especially abundant in the gastrointestinal tract [23, 24]. It is estimated that the human gut contains around one million billion phages, or 10^{15} [25].

In conclusion, bacteriophages are known to be harmless for all other organisms and are species-specific.

Phages have been successfully used for over 10 years in the food industry. Over the past 10 years, many US companies have used *Listeria* phages to eliminate *Listeria* and reduce risk in in RTE meats, cold smoked fish and soft cheese applications as well as an environmental tool to take out biofilms.

6.2 PhageGuard Listex

PhageGuard Listex is a water based phage solution which contains a *Listeria* specific bacteriophage, P100 and is characterized by its broad spectrum toward *Listeria* strains, *L. monocytogenes* as well as *L. ivanovii*, *L. welshimeri*, *L. seeligeri* and *L. innocua* strains. PhageGuard Listex is approved for use by a/o the FDA and USDA as a processing aid.

PhageGuard Listex is an organic and natural antimicrobial intervention which kills *Listeria*. PhageGuard Listex is tasteless and odorless, it has no impact on the organoleptic properties of the treated product and there is no risk in regard to worker safety. By applying PhageGuard Listex on RTE products *Listeria* is reduced by up to 3 logs or 99.9%. PhageGuard Listex is an effective anti-*Listeria* hurdle during processing of cheese, resulting in safer products. In both laboratory and factory trials PhageGuard Listex has shown to be very effective in combatting *Listeria*. On Food Contact surfaces, the use of PhageGuard Listex gives reductions of 2 to 5 log (99 to 99,999%) even in the presence of food debris.

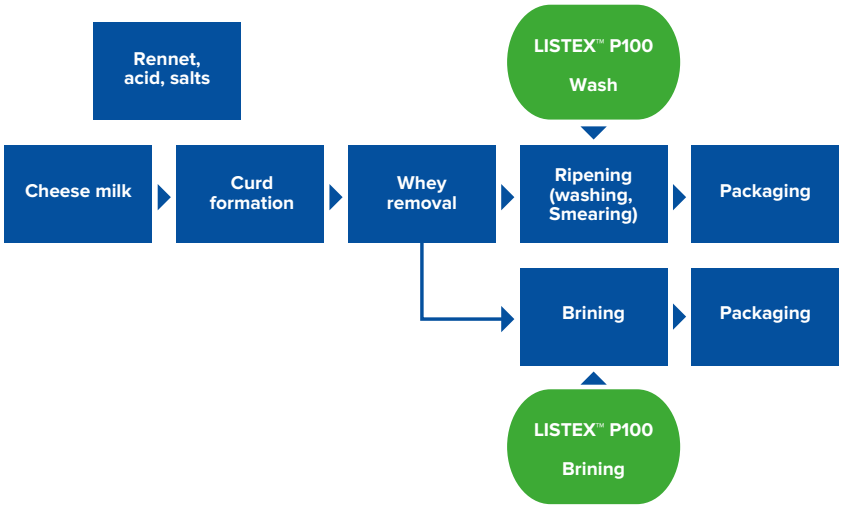
PhageGuard Listex can be applied by using either a spray, dip or wash application. A 1% dilution will typically result in $2x10^7$ pfu/cm^2. Pfu or plaque forming units is a measure of the number of particles capable of forming plaques per unit volume. A solution with a concentration of 10^7 PFU/mL indicates that 1 mL of the solution contains 10 million active phages. How many phages are required per cm^2 depends on the surface treated, the time available and the targeted reduction.

7 Effect of PhageGuard on Listeria in Cheese Processing

Cheese processing has *Listeria* risks as processing equipment gives challenges for harboring resident strains or newly brought in ones with raw materials or by movement of staff and materials.

Milk (often pasteurised) is first treated with rennet, calcium chloride or acids or combinations and the casein and fat droplets coagulate to a curd which is broken in small pieces. After curd formation the curd is put in a mould and pressed to squeeze out the whey. The young cheeses are stored for ripening. The ripening of different cheeses can be very different. Some cheeses ripen by means of a surface culture containing various moulds, yeasts or bacteria that give the cheese their specific taste, colour and texture. Some cheeses are smeared with a brine during ripening to distribute the characteristic flora on the outside of the cheese.

In the ripening stage there is a significant risk for *Listeria* due to bacterial transfer by smear, brines or simply by the turning of the cheese. PhageGuard Listex can be applied to treat the surface of a cheese without adverse effects on the ripening cultures and can also be added to a brine to prevent bacterial transfer between cheeses. Further an application on food contact surfaces or environmental can reduce the risk of transferring an environmental issue to the product.



PhageGuard therefore effectively kills *Listeria* in:

- Finished product
- Brines
- Biofilms on processing equipment
- Biofilms in the environment

Phages do not react away in the presence of food debris and contrary to common used sanitation chemicals can be used to clean up biofilms. As PhageGuard Listex is food grade it can be used on critical process equipment such as slicers during processing.

When sprayed on belts or other food contact surfaces it protects against both *Listeria* on the belt as well as *Listeria* on the product surface that touches the belt.
As PhageGuard Listex has no influence on the taste, smell or texture of the product it can be used by spray or dip applications on product.

A lot of published and unpublished work has been done that confirms the efficacy of PhageGuard Listex in various dairy and cheese application as can be seen in the table below. Depending on the specific product and application the dose will have a different effect.

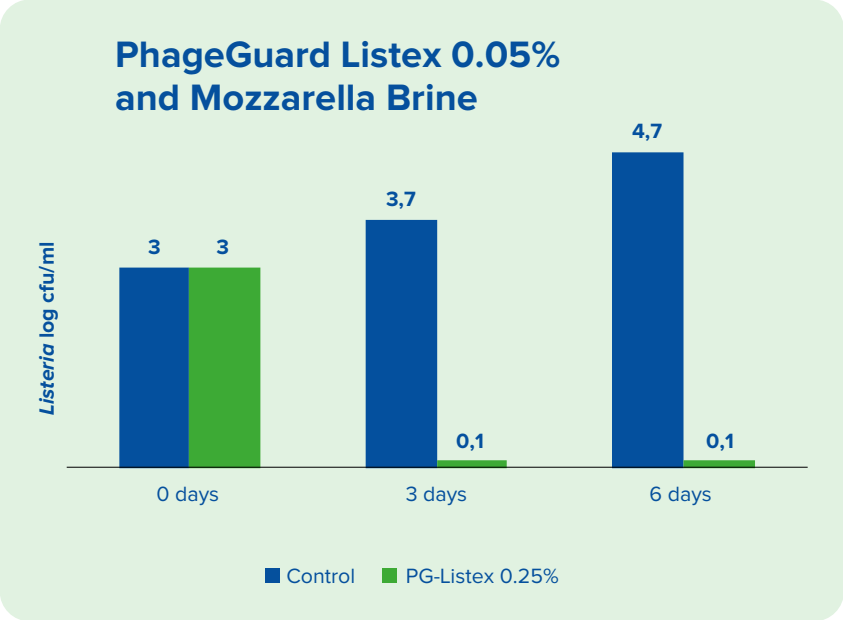
The table also shows the effect on food contact surfaces such as stainless steel.

Table 1: Efficacy of PhageGuard Listex in a number of Dairy and Cheese Application

Application/ Concentration	0.05%	0.2%	0.5%	1%	5%	Reference
Chocolate Milk 0.15%)		> 4	1.2			ETH Zürich (26)
Mozzarella Brine	4-5		1.0			Micreos, 2008 (27)
Pasteurized Cream		3	2.0			Micreos, 2019 (28)
Smear Ripened Cheese	3-8		1.1			NIZO, 2010 (29)
Camembert Cheese (15%)			1.1		1.8-2.5	ETH Zürich, 2011 (30)
Queso Fresco (2.5%)				3.5-7		Mississippi State University, 2012 (31)
Gorgonzola (2.3%)				1.5-2		CRA – Instituto Sperimentale Lattiero Caseario – Lodi, 2006 (32)
Mozzarella via the brine	2					Micreos 2008 (33)
Minas Frescal (4%)					2.3	Universidade Federal da Bahia SalvadorBA Brazil 2014 (34)
Coalho cheeses (4%)					2.1	Universidade Federal da Bahia SalvadorBA Brazil 2014 (34)
Stainless steel with food debris					4-5	F. Gao, 2010 (35)
Stainless Steel			4.0			Mississippi State University (36)
Wageningen University 2014 (37)		3	2.0			Micreos, 2019 (28)
Thermoplastic Belt				1.5	2-3	Micreos 2017 (38)

7.1 PhageGuard Listex in brines

In this experiment 0.05% (1x10^8 pfu/mL) PhageGuard Listex was added to the mozzarella brine and the development of *Listeria* was followed during a period of 6 days. The graph shows that *Listeria* is almost eradicated by the bacteriophages whereas in the untreated samples *Listeria* grows to around to 4.7 log [27].

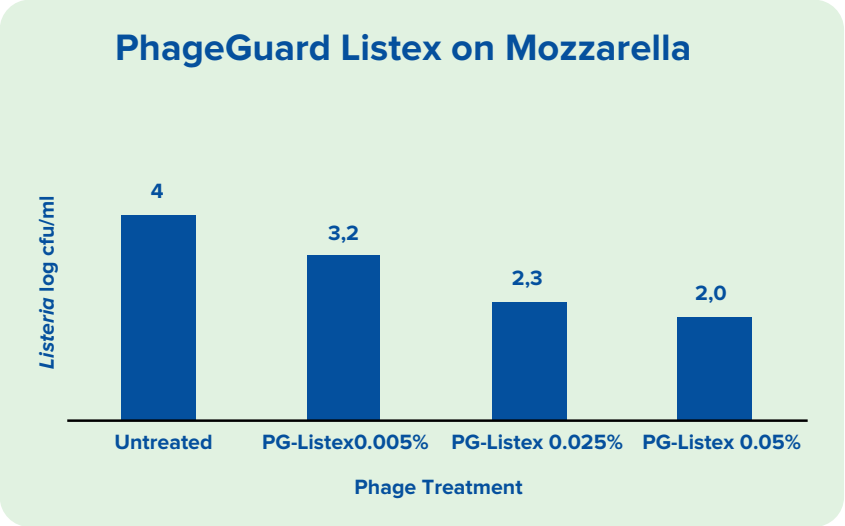


7.2 Reduction of *Listeria* load on artificially inoculated mozzarella

To show that a treatment of the brine can also eradicate *Listeria* on Mozzarella itself, Mozzarella balls were artificially inoculated at very low level (approximately 100 colony forming units per cheese) to mimic a real life exposure level.

The inoculated cheeses were then placed in brine containing PhageGuard Listex at various concentrations.

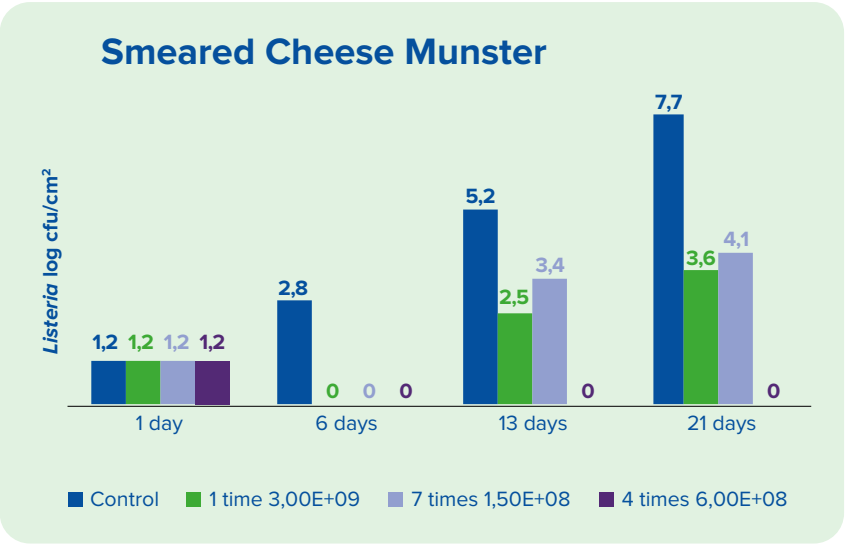
The mozzarella in brine was allowed to stand at 4°C (39°F) for 24 hours. After 24 hours the amount of *Listeria* was determined. A 99% reduction of *Listeria* load was observed after treatment of the brine with a concentration of 0.05% (1x10^8 pfu/ml). Also lower concentrations gave a significant effect. PhageGuard Listex (LISTEX™ P100 treatment of Belgioioso mozzarella 15 Aug 2008).



7.3 Phage treatment completely eradicates or prevents outgrowth of *Listeria monocytogenes* on smear cheeses

Some cheeses ripen by means of the growth of a surface culture on the cheese often consisting of moulds, yeasts or bacteria (*Brevibacterium linens*). When the surface layer harbors *Listeria*, PhageGuard Listex can provide a very elegant solution by taking out *Listeria* while having no effect on the surface ripening cultures. In the figure below the effect of PhageGuard Listex on *Listeria* on a smear ripened cheese is shown.

NIZO's scientists added very small numbers of *Listeria monocytogenes* (20 colonies per cm^2 of cheese surface) to the technical flora, so that the bacteria would be applied to the surface of a smear (spread) cheese at the same time as the technical flora itself.



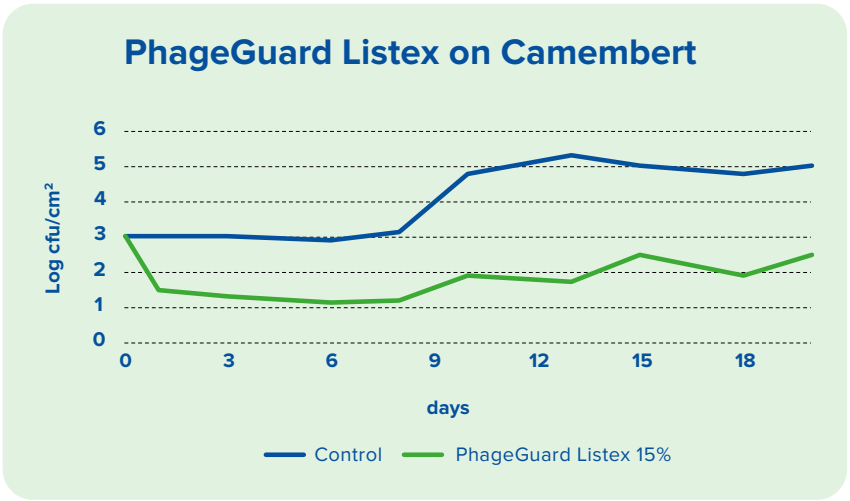
As can be seen in the graph:

- In the control cheeses *Listeria* has grown to very high titers: nearly log 8 per cm^2 of cheese surface. Bacterial levels are orders of magnitude higher than the level considered a health hazard.
- In contrast, the cheeses that received four applications of PhageGuard Listex at 0.3% ($6x10^8$ pfu/mL) showed no outgrowth at all of *Listeria*, as tested either by standard enumeration techniques or by enrichment techniques (the latter used when the numbers of bacteria are below the limits of detection).
- Treatment with every wash (7x) at 0,065% ($1.5x10^8$ pfu/mL) gave a 3.6 log reduction

Adding PhageGuard Listex at 0.065% to the culture will protect both the culture from *Listeria* and with it transfer from other cheeses as well as protecting the cheese itself.

7.4 PhageGuard Listex on Camembert

Camembert cheese was contaminated with 3 log *Listeria* and after 1 hr a 15% concentration of PhageGuard Listex was added for a concentration of $3x10^8$ pfu/cm^2. Cheeses were ripened for 10 days at 12°C and 95% relative humidity and then stored for another 10 days at 6°C. Addition of PhageGuard Listex on Camembert resulted in a decrease of more than 2 logs during the first 6-8 days of production. Regrowth occurred when the pH rose above 6.5 nevertheless compared to the controls *Listeria* growth on the phage-treated samples was significantly slower.



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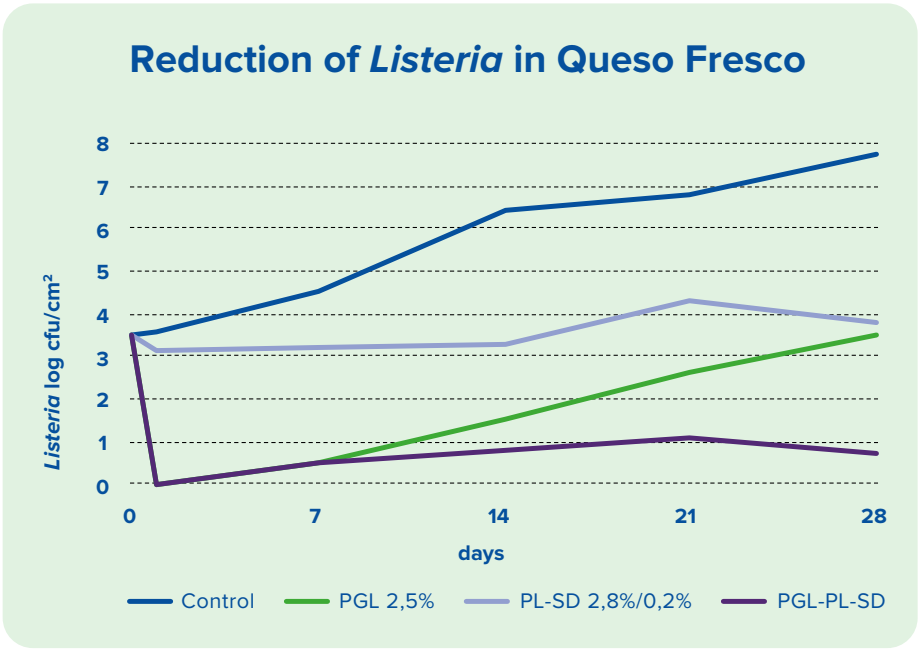
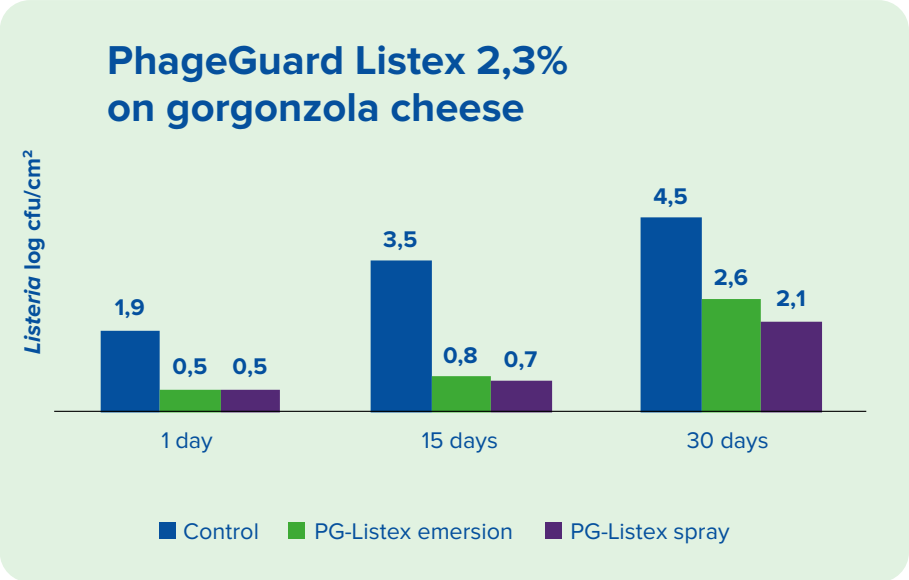
7.5 Reduction of *Listeria* in Queso Fresco by a combination of Listericidal and Listeriostatic antimicrobials

Queso Fresco (QFC) is an excellent substrate for *L. monocytogenes* growth under refrigeration temperatures. Treatment of QFC samples with 2.5% PhageGuard Listex ($5x10^7$ pfu/cm^2)reduced the *L. monocytogenes* counts to an undetectable level after 1 day storage at 4 °C, however regrowth in surviving cells occurred during subsequent cold storage. Treatment with Potassium Lactate–Sodium Diacetate alone did not result in any listericidal activity as *L. monocytogenes* counts did not decline from the original inoculum level. By contrast with PhageGuard Listex, PL–SD exerted strong listeriostatic action to completely prevent the growth of *L. monocytogenes* cells during 28 days at 4 °C. Overall, this shows that PhageGuard Listex has a strong listericidal activity initially while PL–SD has a strong listeriostatic activity towards surviving *L. monocytogenes* cells in QFC for a long time. Combined treatment of listericidal PhageGuard Listex and listeriostatic PL–SD yielded the initial reduction of *L. monocytogenes* counts by 3 log and remained at that level for the following 28 days.



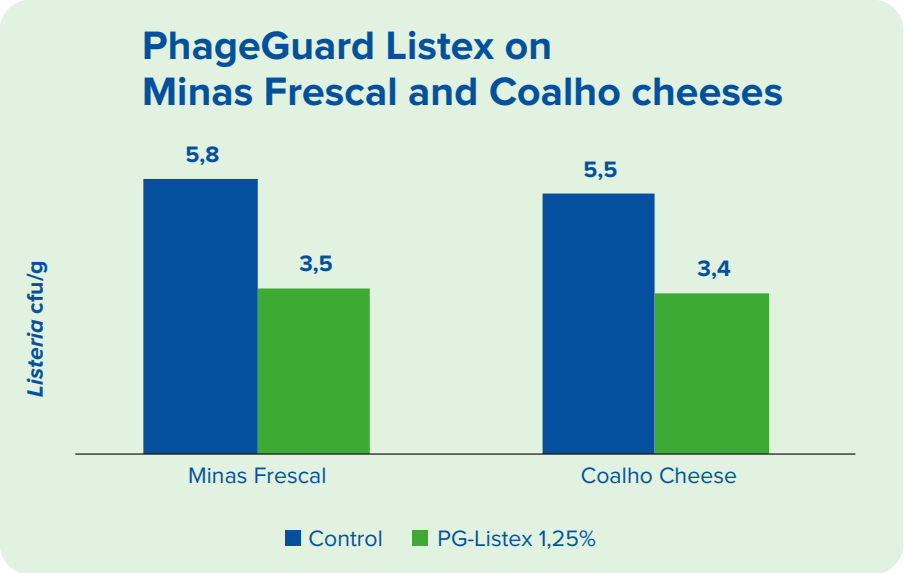
7.6 PhageGuard Listex on Gorgonzola

In this experiment 25 day old gorgonzola cheese was inoculated with low levels of *Listeria* and left to air dry for 24 hours. At half ripening the conditions for growth of *Listeria* are the best so this simulated the worst possible conditions. A 2.3% PhageGuard Listex solution ($4.6x10^9$ pfu/mL) was used to either dip ((20 seconds) or spray the gorgonzola cheese. Phages on the submerged surface were $4x10^7$ pfu/cm^2 and for the sprayed surface $1.1x10^8$ pfu/cm^2. Both methods give a significant reduction in *Listeria* with the spray method due to the higher levels of phage that were put on the cheese surface also giving a better result [32].



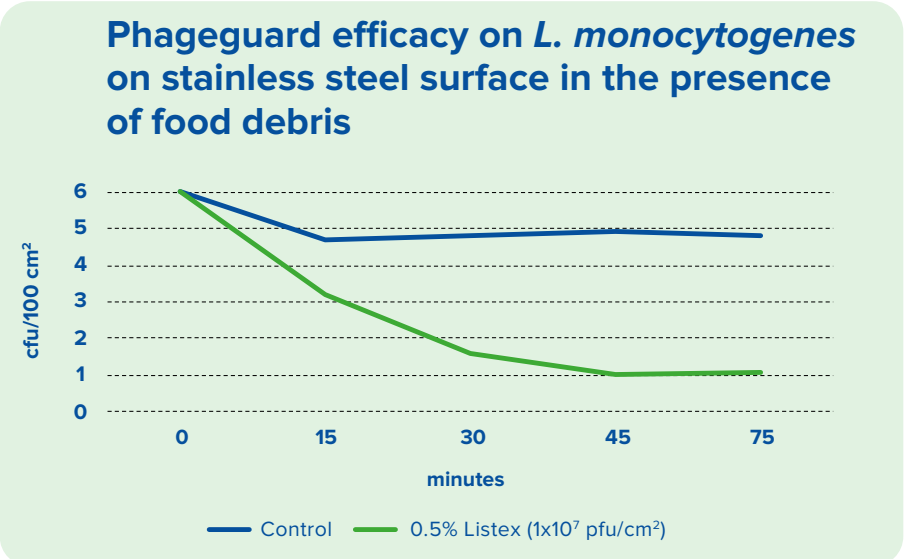
7.7 Control of *Listeria monocytogenes* growth in soft cheeses

1.25% of PhageGuard Listex (2.5 x10^7 pfu/cm^2) was used 30 minutes post infection and obtained an immediate kill of more than 2 logs. The results indicate that the P100 bacteriophage reduces the number of *Listeria* cells in soft cheeses.



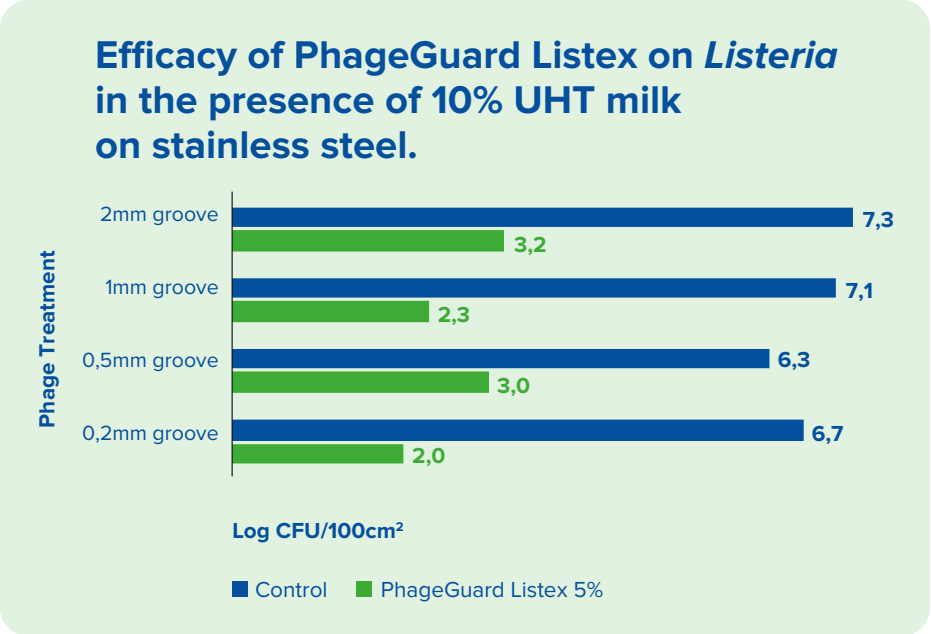
7.8 PhageGuard kills *Listeria* biofilms on stainless steel surface in the presence of food debris

Spraying PhageGuard Listex at 2x10^7 pfu/cm^2 (1%) reduces *Listeria* numbers by 3-4 log on stainless steel surfaces in the presence of food debris [35]. It works immediate and reaches its maximum result in 45 minutes. This makes PhageGuard Listex a perfect application to be used on critical process equipment during processing such as slicers, where it protects against bacterial transfer. Soni at al. [36] found 3.5 to 5.4 log reduction after 2 to 7 days attachment time of the Listeria to the surface.



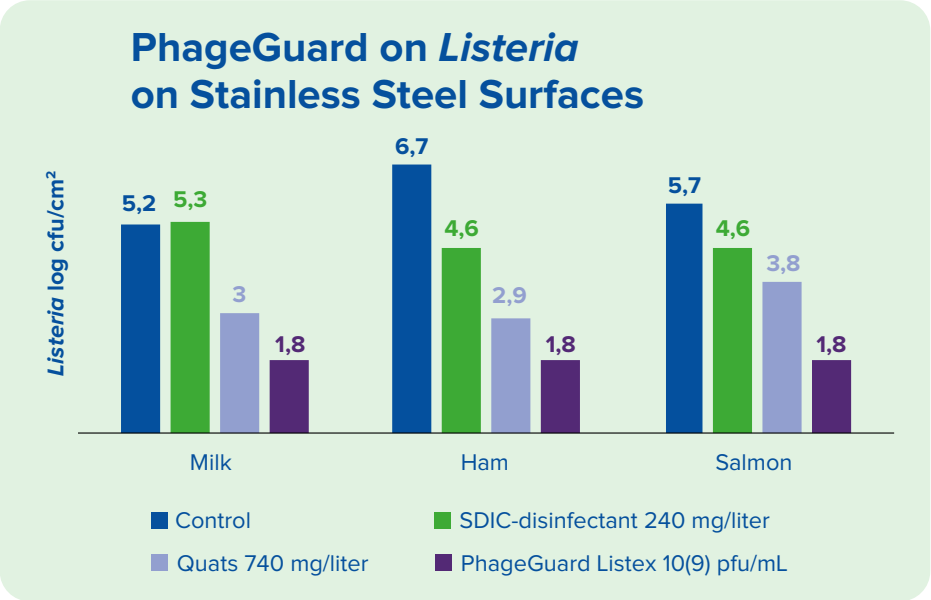
7.9 PhageGuard combats *Listeria monocytogenes* in biofilms

In this experiment, L.m. biofilm was prepared with 6 log cfu/ml of *Listeria* strain mixture with a food matrix of 10% UHT milk. After 14 days of biofilm formation, the SS plates were treated with 5% PhageGuard Listex ($1x10^8$ pfu/cm^2) by spraying and left for 30 min reaction time. And thereafter samples were taken from each groove. This work showed 4 – to 5 log reduction on artificially inoculated stainless steel with grooves 0.2 mm to 2 mm deep in the presence of food debris [36].



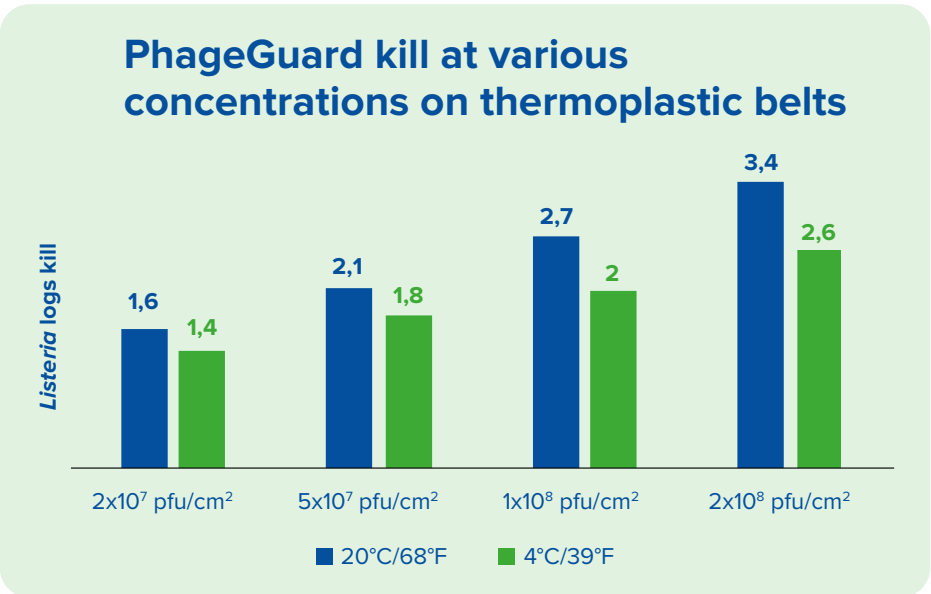
7.10 PhageGuard is more effective on *Listeria* biofilms than sanitation chemicals

PhageGuard Listex outperforms common chemicals in the presence of food debris [40]. This makes it sensible to use PhageGuard Listex as an additional and final spray on critical process equipment AFTER chemical cleaning and a water rinse.



7.11 PhageGuard eliminates *Listeria* on food contact surfaces

Transporting belts are potential risk factors in harboring *Listeria*. Treating a conveyor belt with PhageGuard Listex is effective both on the belt showing 1.5-3 log reductions as well as on the product that is in contact with the belt with a 1.4 log reduction [42].



7.12 Where and how to use PhageGuard Listex

PhageGuard Listex can be applied successfully on different types of cheeses.

PhageGuard Listex can be applied as follows:

- Add Listex to the the brine, smear or wash to prevent bacterial transfer between cheeses.
- Spray or dip the outside of the cheese to kill *Listeria*.
- Dilute PhageGuard Listex with clean water free form chlorine.
- Avoid contact with chemicals since PhageGuard Listex is rapidly inactivated at free chlorine levels higher than 1 ppm.
- PhageGuard Listex is effective in solution between +32F (0°C) and 95 F (35°C).
- PhageGuard solution can be sprayed on food contact surfaces during production to prevent cross contamination.
- PhageGuard can be applied bi-weekly as final step of the sanitation process. Properly rinse the equipment with water before applying PhageGuard Listex.
- Treatment of hotspots in the environment with chemicals alone is not sufficient. Unlike chemicals phages penetrate into the biofilm and kill *Listeria* inside the biofilm. Continue to apply a 5% PhageGuard Listex on hotspot until it tests negative.
- PhageGuard does not replace chemical disinfectants but is an additional tool to eradicate *Listeria*.
- We work closely with major spray equipment suppliers to offer an optimal application solution.

Cheese variety	Application	Dilution
Soft/Semi-hard	On rind at pH 4,5 - 5,5	1%
White Mold	Prior to mold growth	1%
Queso Fresco	In pasteurized milk during cheese making	0,05%
Washed Rind	In wash liquid	0,05%

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