ANSES Opinion
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The Director General

Maisons-Alfort, 23 June 2014

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the use of bacteriophages in foods of animal origin to control *Listeria*

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are made public.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated, 23 June 2014 *shall prevail.*

ANSES received a request on 20 June 2013 from the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) and the Directorate General for Food (DGAL) to carry out the following expert appraisal: Request for an opinion on the use of bacteriophages in foods of animal origin to control *Listeria.*

1. BACKGROUND AND PURPOSE OF THE REQUEST

The regulatory status of bacteriophages is not defined at the European level. Since 2006, several authorisations for the use of bacteriophages as control agents in the agro-food industry have been granted, specifically in the United States, Canada and New Zealand.

In 2009, EFSA issued an opinion on the mode of action of bacteriophages. This opinion indicated that it is impossible to conclude whether bacteriophages protect against recontamination of foods with pathogenic bacteria. EFSA also recommended drafting a guidance document regarding submission of data for the assessment of this type of treatment. In 2012, EFSA evaluated the use of anti-*Listeria* bacteriophages for the decontamination of fish. The data submitted for evaluation did not enable a conclusion to be reached about the efficacy of this treatment.

ANSES has received a request on the benefit and limitations related to use of bacteriophages in foods of animal origin. The request covers two groups of issues:

Part 1:

- Efficacy of the LISTEX P100 bacteriophage (on the basis of the technical dossier and the literature) on the reduction of *L. monocytogenes* in cheeses and other tested foods of animal origin;
- Does the use of this type of bacteriophage in the conditions recommended by the manufacturer, and in addition to good hygiene practices, provide a supplemental method to control *Listeria*?
- What factors restrict the action of bacteriophages in the agro-food sector?

- Could bacteriophages be a vector of genetic material through non-controlled mechanisms, e.g. acquisition of pathogenicity of bacteria?
- Do we have and can we have guarantees on maintaining the specificity and the absence of pathogenicity of bacteriophages?
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Part 2:

What possible interactions are there between bacteriophages used in foods of animal origin and the intestinal flora in consumers, particularly in terms of microbial ecology and the risk of transfer of unsuitable genetic material (e.g. plasmids)? What are the known and/or possible impacts on environmental flora?

This opinion concerns the first part of the request.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in compliance with Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The collective expert appraisal was carried out by the Expert Committee (CES) on Assessment of the biological risks in foods (BIORISK) on the basis of a preliminary report drafted by four rapporteurs. The expert appraisal was based on the technical dossier of the applicant and on the referenced scientific publications.

ANSES analyses the various interests declared by the experts before their nomination and throughout the assessment, in order to avoid any risk of conflicts of interest concerning the issues handled as part of the expert assessment. The declarations of interest of the experts are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

3.1. General presentation of bacteriophage P100

- *Classification and phage structure*

Bacteriophage P100 belongs to the *Myoviridae* family in the *Caudovirales* order. This family of phages has the general characteristic of having a contractile tail and head. The tail, made up of a central core surrounded by a contractile helicoidal sheath, is separated from the head by a collar. Generally, this family of phages has a larger head, more DNA, and a higher molecular weight compared to other families of phages. These phages are often sensitive to freezing-thawing processes and to osmotic shock. The various genera of the *Myoviridae* family are differentiated by genetic organisation, mechanism of DNA replication, and presence of unusual bases or DNA polymerase.

Klumpp *et al*. (2008) placed bacteriophage P100 in the group of SPO1-like myoviruses (*Bacillus subtilis* phage SPO1) on the basis of morphology, host bacteria (Gram+ with low GC%), a broad host range, their strictly virulent character, and the similarity of their DNA sequences. The most similar phage in terms of morphology and genetic organisation is phage A511.

Bacteriophage P100 has a long tail (198 nm) that is contractile and non-flexible and an icosahedral head (90 nm). Klumpp *et al.* (2008) showed for the first time under the electron microscope long fibres linked to the end portion of the tail in addition to short fibres that are more easily observed. Bacteriophage P100 is made up of double-stranded DNA with 131384 bp (137619 bp for A511) with redundant terminal sequences that are invariable and non-cohesive of 6 kbp (3.1 kbp for A511). Based on all the criteria, bacteriophages P100 and A511 could be classified under the *Spounavirinae* subfamily.

- *Specific hosts*

P100, like A511, has a broad host range in the *Listeria* genus, although that of P100 is wider than that of A511. More than 95% of the 250 strains of *Listeria* tested (*Listeria monocytogenes* serogroups 1/2 and 4, *Listeria ivanovii* serogroup 5, and probably *L. innocua* serogroup 6 (data not published)) are susceptible to this phage (Loessner *et al.* cited by Carlton *et al.* 2005). An OFIMER study in 2011, using an *in vitro* test, showed that 78% of the 42 strains of *L. monocytogenes* isolated from smoked salmon and trout are

susceptible to bacteriophage P100, while 12% are classified as intermediate, and 10% as resistant. No other bacterial genus appears to be affected, which ensures controlled treatment (Carlton *et al*., 2005).

However, there is no study on the sensitivity to bacteriophage P100 of the main clonal complexes obtained by MLST (multi-locus sequence typing) and the main epidemic clones that constitute the structure of the population of *L. monocytogenes*. 95% of human strains of *L. monocytogenes* belong to serogroup 1/2 and serotype 4b (Doumith *et al*., 2004) and most epidemic strains belong to serotypes 1/2a and 4b. Nonetheless, no link has been established between serotype and virulence in *L. monocytogenes*, with the exception of serotypes 3a, 3b, 3c, 4c, 4d and 4e which are rarely involved in human cases (Liu *et al*., 2007).

- *Cycle of infection*

Most known phages (>400 phages) of the *Listeria* genus invade the DNA of the host cell (temperate phages) (Carlton *et al*., 2005). For these phages, a DNA cassette controls lysogeny. This cassette is however absent in the genome of bacteriophages P100 and A511; their cycle of infection is therefore purely lytic.

The long and short tail fibres recognise specific bacterial receptors (peptidoglycan, teichoic acids) that enable adsorption to the cell wall. The contractile tail enables injection of DNA into the bacterium. The phage genome can then be transcribed into mRNA and phage proteins expressed. Early genes code specifically for DNA replication and repair functions. Among later genes, there are regions dedicated to structural proteins that are expressed, and then lysis genes.

Phage proteins thus enable the production of new phage particles and cell lysis. To this end, a specific enzyme that degrades the bacterial cell wall is synthesised to lyse the bacterium at the end of the cycle. Like the receptors, this autolysin is specific to the *Listeria* genus. This enzyme is not observed to have an effect on 20 species from genera as different as *Lactococcus*, *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Bacillus*, *Staphylococcus* and *Brochothrix (*Schmelcher *et al*. 2010)*.*

The cycle of phage infection culminates in bacterial lysis and production of new bacteriophages.

- *Mode of action and conditions of application in food substances*

The presence of free water appears to be necessary for the phage to find its direction and reach its target. The physiological state of the bacterium is also a key factor. Infection may depend on the number of phage receptors expressed at the surface, which may or may not promote adsorption and thus phage penetration. The metabolic activity of the bacterium therefore also directly affects phage replication kinetics. The ideal phase for phage replication is therefore the exponential phase but some phages may infect bacteria in stationary phases. Bacteriophage P100 has been isolated from the agro-food environment (Loessner M.J., unpublished data) and studies carried out with this phage show that it is able to infect *Listeria* in food matrices irrespective of the metabolic activity of the bacterium. Phage production kinetics are simply slower.

Once the phage is adsorbed to the food matrix, it becomes incapable of infecting the host bacterium. This could be described as “inactivation by adsorption”. Limited distribution and inactivation by adsorption mean that phages must be applied in great excess in terms of the target bacterium: multiplicity of infection (MOI) of about 10^4 for example is equivalent to 10^7 phages/cm^2 for 10^3 CFU/cm^2 of *Listeria*. Concentrations of up to 10^9 PFU/g could be used according to the technical dossier.

Bacteriophage P100 is sensitive to freezing, to proteolytic enzymes, to acidity, to salt, to UV, and to temperatures > 50°C (Greer, 2005; Hudson *et al*., 2005).

3.2. Efficacy of bacteriophage Listex P100

3.2.1. Efficacy data for cheeses and other products of animal origin

Studies carried out to date all show that the activity of bacteriophages on *Listeria* is highly dependent on the type of matrix, the phage dose, and the initial bacterial concentration (Sulakvelidze, 2013). According to Sillankorva *et al*. (2012), each phage/matrix/bacteria association is a unique case in terms of efficacy and experimental conditions.

As an example, the efficacy of treatment was demonstrated on hot dogs (*L. monocytogenes* inoculated at 10^3 CFU/g was no longer detectable 6h after treatment with bacteriophages P100 and A511 at a concentration of $3x10^8$ PFU/g) (Guenther *et al*., 2009) and on minced meat (annex to the dossier). On smoked salmon or cooked ham, treatment resulted in 1 $\log_{10}$ reduction in *L. monocytogenes* concentrations irrespective of the chosen duration for selected samples between 6h and 6 days of storage. An intermediate result was found for seafood. These authors indicate that the differences in composition, pH and ionic strength of these products could explain the inconsistencies they found. In fact, it appears that the products

with the largest exposed surfaces, i.e. those that are most difficult to treat uniformly, show poor or limited results with this process, irrespective of whether the product is of animal origin (seafood, ham, roast beef, turkey slices) or plant origin (lettuce or cabbage).

The effect of bacteriophage P100 is clearly dose-dependent. Since phages do not readily propagate in solid food matrices, the efficacy of treatment will depend on the initial quantity of phage added and not on the quantity of phages produced by the bacteria in the food substance. Application of $1.5x10^8$ PFU/mL of phage to Munster cheese ($2x10^6/cm^2$) led to 2 to 3 log_{10} reductions in *Listeria,* while with 10^9 PFU/mL ($6x10^7/cm^2$), *Listeria* was no longer detected after treatment. Contamination by *Listeria* was 20 CFU/cm^2 before treatment and 10^5 CFU/cm^2 after 10-16 days with a phage dose of 10^8 PFU/mL. This shows that once the phage is adsorbed, bacteria that evaded treatment are able to grow in certain cases. Importantly, the extent of the initial *L. monocytogenes* population is a promoting factor for treatment efficacy since the phage multiplies within infected bacteria (Greer, 2005; Hudson *et al*., 2005). This is particularly true in liquid matrices. High concentrations of annex flora in the treated product may also interfere with phage activity (Greer, 2005; Hudson *et al*., 2005).

The conditions of use of phages, including initial concentrations and application methods, the type and physico-chemical composition of the target matrices, and efficacy data reported in the literature, are extremely diverse. For each application, it is important to adjust and validate the phage dose to apply, which can vary from one to several log PFU/mL depending on the food.

3.2.2. Bacterial resistance

Given the wide range of resistance mechanisms described in *Listeria* (CRISPR-Cas, restriction-modification, abortive multiplication, etc.), bacterial resistance development could occur for this phage.

In the dossier, it is indicated that among the 5% of strains considered resistant to the phage, the mechanism of resistance was mainly related to a process of abortive infection. In this case, the bacterium infected by a phage programmes its own lysis, thus preventing replication of the specific phage. Whether the cycle is productive or abortive, the intended objective of bacterial lysis is obtained.

A type II restriction-modification system has been described for a *Listeria monocytogenes* clone II epidemic strain. This system is expressed at temperatures lower than 25°C and renders the strain insensitive to phage infection. It is not expressed at temperatures above 25°C and the bacterium is therefore sensitive to phages at 37°C for example (Denes & Wiedmann, 2014).

A CRISPR-Cas system has also been described in *Listeria*. It provides the bacterium with adaptive immunity that protects it against invasion by phages and plasmids. By studying the sequenced strains of *L. monocytogenes*, including those in serogroup 1/2 and serotype 4b, Sesto *et al*. (2014) identified a CRISPR (Rlib) component. This component contained spacers[1] which are directed, among others, against phages of *Listeria,* particularly virulent phages A115, P35, P70, and P100 (Sesto *et al*., 2014).

Resistance mechanisms in the bacterial hosts of bacteriophage P100 have thus been documented and studies are needed on their rate of development under selection pressure. Although the mechanism of resistance to phages for serotypes 1/2c and 3 remains unknown, teichoic acids of the bacterial cell wall and glucosamine in particular have been shown to be receptors of *Listeria* phages, and absence of or changes to these teichoic acids can lead to phage resistance (Wendlinger *et al*., 1996).

No bacteria that were initially sensitive and then became resistant to infection with bacteriophage P100 have been described so far in artificially contaminated food matrices. The *Listeria* concentration in cheeses is often low and therefore the probability of development of occasional phage-resistant mutants is reduced. In line with multiple reports in the literature, particularly in phages infecting lactic acid bacteria, it is also probable that the bacteriophage would adapt to the new resistant strain (Labrie *et al*., 2010; Samson *et al*., 2013). The probability that *Listeria* variants resistant to the bacteriophage will develop increases in line with the number of *Listeria* cells. As a result, the phage must be applied only to contaminated food and must under no circumstances be found elsewhere in the agro-food environment where it could infect biofilms of *Listeria* that would favour development of these variants. A study to assess the development of phage-resistant variants could have been performed in the laboratory by culturing *Listeria* strains with bacteriophage P100 rather than only using foods where the phage is in fact inactive, through adsorption, when the bacteria proliferate. As a result, no resistant bacteria were observed since growth took place without selection pressure from the bacteriophage.

[1] Non-transcribed DNA sequence, separating genes within repeated units

3.3. Safety of bacteriophage P100 and host bacteria

- Oral toxicity and allergenicity

Carlton *et al.* (2005) demonstrated the safety of bacteriophage P100 in the rat. Administration of the phage in this model at doses 10,000 higher than those that humans would ingest showed no effects on weight or on intestinal sections. Other studies have shown the safety of phages through the oral route. For example, administration to humans of phage T4 showed no serum antibodies against the phage (Bruttin & Brüssow, 2005). They therefore do not appear to be absorbed in the intestine or to induce a detectable immune response. Phage T4 is detected in stools the first day after administration in humans. In this case, *E. coli*, the phage host, was present in the intestinal tract. It is therefore not possible to determine whether the phage was excreted passively following ingestion or whether it is actually able to replicate in the intestinal microbiota.

Alignment of the sequences of the 174 proteins of bacteriophage P100 in the database of food allergens yielded only one result for protein gp71 (ORF71). Homology involved a small part of the Ct portion of a wheat protein. The observed similarities do not appear to result in cross immunological reactions.

- *Lysogeny*

Lysogeny is common in the *Listeria* genus but to date no correlation has been made between the presence of prophages and the occurrence of epidemic clones (Klumpp & Loessner, 2013). Temperate phages of *Listeria* code for an integrase that controls insertion/excision of phage DNA in the bacterial chromosome. These are mainly serine and tyrosine recombinases. According to scientific studies and the applicant's dossier, the genetic structure of the bacteriophage P100 genome does not suggest the possible presence of a lysogenic module, with the gene coding integrase.

- *Transduction capacity*

During phage infection, bacterial genetic material potentially including virulence or resistance genes to antibiotics can be transferred to the bacterium to which the phage is adsorbed through transduction. This process can be specialised (the prophage carries a segment of bacterial DNA during excision) or generalised (encapsidation of bacterial DNA by phage particles).

Only temperate phages are capable of specialised transduction but generalised transduction remains possible during the lytic cycle. In this second case, the mechanism of genome packaging in the head of the phage is the key factor. Phages that are able to circularise their genome when entering the cell through cohesive ends can perform this type of transduction because the phage terminase controlling packaging expresses only very low specificity for the DNA and can thus accidentally package fragments of bacterial DNA. Conversely, phages with DNA that has redundant invariable non-cohesive ends are incompatible with transduction because the terminase specifically recognises phage DNA. Since bacteriophage P100 belongs to this latter category, transfer of bacterial genes by transduction appears unlikely (Klumpp & Loessner, 2013).

- *Virulence factors*

For the numerous phages of *Listeria* that have been sequenced, no link with virulence factors of *Listeria* has been clearly identified to date (Klumpp & Loessner, 2013). Nonetheless, a recent study showed the role of the A118-like *L. monocytogenes* prophage integrated into the comK gene in the regulation of bacterial escape from macrophage phagosomes during infection (Rabinovich *et al*., 2012). Another study has suggested the involvement of a prophage integrated into the comK gene in the persistence of *Listeria monocytogenes* in production facilities (Verghese *et al*., 2011). This prophage is also present in epidemic strains (Knabel *et al*., 2012).

The genome of bacteriophage P100 was deposited in Genbank under reference DQ004.855. In addition, 174 open reading frames (ORF) have been identified with 18 tRNAs. Only 25 genes have been associated with known functions while the others are new entries in the database. No function was associated with known pathogenicity or virulence factors for *Listeria monocytogenes* or any other known pathogen (Carlton *et al*., 2005). As databases have grown in size considerably since 2005, this research work on homologous sequences in pathogens, including *Listeria,* should now be repeated.

- *Possibility of* L. monocytogenes *endotoxin release during bacterial lysis*

Until now, bacterial lysis of *L. monocytogenes* has not been found to lead to release of bacterial endotoxins. However, it is thought that the mechanism underlying *L. monocytogenes* gastro-enteritis involves one or more toxins not described to date.

- *Safety of the propagation strain (L. innocua)*

For propagation of bacteriophage P100, the technical dossier refers to use of a strain of *L. innocua* that has not been completely sequenced to date. It is essential that this strain be sequenced to evaluate the presence of prophages and the related risks.

The CLIP 11262 strain of *Listeria innocua*, which has been completely sequenced and annotated, can contain up to six prophages, while the EGD-e strain can sometimes contain two, and the strain of serogroup 3 none (WSLC1001) (Klumpp & Loessner, 2013). Use of bacterial strains for propagation that contain one or more prophages leads to a risk of simultaneous production of bacteriophage P100 and another phage that is this time temperate. Likewise, recombination is possible between P100 and one or more prophages, leading to the development of new variants.

3.4. Limitations and issues concerning the consequences of using bacteriophages

- *Based on the dose used, potential effects on the intestinal microbiota and the ecosystem of* Listeria

Vongkamjan *et al*., (2012) studied 134 silage samples from dairy farms, of which 47.8% were positive for *Listeria* phages (140 phages identified) with concentrations of more than $1.5x10^4$ PFU/g. *Listeria* phages are therefore abundant in silage and act naturally against serogroups 4 of *L. monocytogenes*. Strains of *L. monocytogenes* that contaminate milk are therefore already naturally in contact with high concentrations of a range of phages, each with diverse hosts.

Given the very high MOI used in foods, it is unlikely that resistant *Listeria* strains will develop during treatment. Nonetheless, since the concentration used is very high (10^9 PFU/mL), appreciable quantities of phages may be found in the environment near production sites, in the stools of people who consume the food, and then in the environment at large. It is therefore in these secondary non-controlled environments that resistant strains could emerge. As a result, it is warranted to consider the issue of interactions with intestinal flora and the fate of phages once they are excreted in the environment.

Chibeu *et al*. (2013) showed that phages desorbed from the matrix retain their ability to infect bacteria. The authors point out that the phage is stable for about 28 days in meat at 4°C or 10°C. The available data in the literature and phage manufacturer's data do not enable satisfactory evaluation of all the conditions that allow desorption of phages. It is possible that on ingestion of foods containing large quantities of adsorbed phages, these phages may be released from the matrix and regain their ability to infect their host. Phages from the same family (phage T4) resist gastric acidity and are found in stools of people treated orally with this phage.

Consequences on intestinal microbiota seem limited since *Listeria* is not a bacterial genus known to be part of commensal flora in the gut. There are few studies on asymptomatic carriage of *Listeria* and this rather involves transient carriage related to ingestion of contaminated food.

In the agro-food environment, the presence of phages may affect most strains of *Listeria*, whether pathogenic or not, as a selection pressure, and could release ecological niches for strains of *Listeria* that are pathogenic and resistant to the phage. The ecology between the serogroups and serotypes is not well understood. It is therefore not possible to predict the consequences of a decrease in certain serogroups or serotypes that are sensitive to phages on the behaviour of other groups that are more pathogenic or resistant to phages.

- *Possibility of lysogenic conversion of bacteriophage P100*

Most phages of *Listeria* are temperate. It is not known whether bacteriophages P100 and A511, which are lytic, evolved from a temperate ancestor after losing the ability to integrate their genome into that of the bacterium or whether they are lytic phages that are very different from a genetic point of view compared to lysogenic phages. In other words, is it possible that these phages may retrieve their lysogenic ability by integration, via mobile genetic elements, of a cassette that controls lysogeny (lysogenic module)? Answers may be found based on phylogenetic trees of *Listeria* phages if they are available.

In view of these questions, it is necessary to obtain more information from the applicant:

- Efficacy data concerning use of bacteriophage P100 in industrial conditions on naturally contaminated products (treatment has been authorised since 2006 in certain countries). Information on the selection of resistant bacteria in industrial conditions in the food or in the production environment. Were P100-resistant *Listeria* variants screened for in production environments where this phage is used o? If so, what were the results?

- Experimental data on the conditions enabling selection of resistant bacteria (e.g. phage dose, bacterial concentration): is it possible, and at what frequency, to isolate P100-resistant *Listeria* variants by culturing the bacteria in a liquid medium in contact with the phage? What are the characteristics of these variants?
- Information on the production methods of new mixtures of phages in the event of decreased efficacy of bacteriophages.

  Are there other phages that have an adsorption spectrum as wide as that of P100? Is there a wide diversity of phages that can be used in cocktails to lyse all strains of *L. monocytogenes*? Can these phages be produced from non-lysogenic strains or strains cured of their prophages?
- Experimental data to assess the sensitivity of phages to gastric acidity (studies on faecal excretion of bacteriophage P100 in subjects who consumed treated products).

CONCLUSIONS OF THE CES BIORISK

- **Efficacy of the LISTEX P100 bacteriophage (on the basis of the technical dossier and the literature) on the reduction of *L. monocytogenes* in cheeses and other tested foods of animal origin**

  The results of the studies presented show the efficacy of Listex P100 for the reduction of *L. monocytogenes* in the tested food products. The efficacy of bacteriophage LISTEX P100 appears to be dependent on the concentration used and the choice of the process step when it is inoculated. Each category of food appears to have a specific application dose, which requires validation of treatment efficacy for a given production process. Depending on the type of product tested, single or multiple applications may be needed to achieve the intended antimicrobial effect.

- **Does use of this type of bacteriophage in the conditions recommended by the manufacturer, and in addition to good hygiene practices, provide a supplemental method to control *Listeria*?**

  Use of P100 may be an additional tool that can be used to control the *Listeria* hazard in foods but not in the agro-food environment or in the event of recontamination of the product. It can supplement good hygiene practices and HACCP but cannot be considered a means of extending the shelf-life of products or of obtaining a product that is fully decontaminated in the event of contamination of a production facility with *Listeria*. It is also possible that this treatment may have no real effect, particularly in the context of contamination with *Listeria* occurring subsequent to treatment with phages or in certain specific matrices.

- **What factors restrict the action of bacteriophages in the agro-food sector?**

  The free water content in a food substance and the free or adsorbed state of the phage are key points for its activity. Bacteriophage P100 has no effect once adsorbed on the matrix in the event of recontamination with *Listeria* post-treatment. Once desorbed from the food matrix, the phage may become active again in the host or in the environment.
  Given the concentrations used, the emergence of bacteria resistant to bacteriophage P100 in the agro-food environment is highly likely and could in the long term lead to reduced efficacy of treatment. Use of phage cocktails or rotation of phages are strategies that may help to limit the development of phage-resistant strains.

- **Could bacteriophages be a vector of genetic material through non-controlled mechanisms, e.g. acquisition of pathogenicity of bacteria?**

  In food matrices, this seems to be unlikely given the available data. However, in the environment near industrial sites, in the host, or in the environment after release via waste water, this cannot be ruled out.

- **Do we have and can we have guarantees on maintaining the specificity and absence of pathogenicity of bacteriophages?**

  Bacteriophage P100, like all *Listeria* phages, appears to be genus specific. Furthermore, phages are the most abundant organisms on the planet (Sulakvelidze, 2013). Despite this, no phage has been shown to cause infection in humans and no phage sequence has been identified in the human genome. Phages have already been administered to humans as part of viral phage therapy (oral or rectal route, or topically) with no adverse effects on health. Possible adverse effects linked to phage use in the agro-

food sector can therefore only be secondary, through changes in the intestinal flora, in the bacterial host, or in the balance of the ecosystem inhabited by *Listeria monocytogenes* and *Listeria innocua*.

4. CONCLUSIONS AND RECOMMENDATIONS OF THE AGENCY

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions of the CES BIORISK.

Marc Mortureux

KEY WORDS

Bacteriophage; *Listeria*; decontamination procedures

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