



Return address: P.O. Box 360, 3700 AJ ZEIST, THE NETHERLANDS

EBI Food Safety
Attn. Mr. M.L. Offerhaus
Nieuwe Kanaal 7P
6709 PA WAGENINGEN
THE NETHERLANDS



Food and Nutrition
Utrechtseweg 48
P.O. Box 360
3700 AJ Zeist
The Netherlands

www.tno.nl

T +31 30 694 41 44
F +31 30 694 47 77
wegwijzer@tno.nl

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E-mail
Jos.vandervossen@tno.nl

Direct dialling
+31 30 694 47 20

Direct fax
+31 30 69 444 66

Subject
Expert opinion

Dear Mr Offerhaus,

Attached you will find our expert opinion about "Evaluation of the safety of the bacteriophage P100 present in the product Listex P100, used for the eradication of *Listeria monocytogenes* contamination during food processing".

Please feel free to contact me in case you have any questions.

Yours faithfully,

Jos M.B.M. van der Vossen, Ph.D.
Food and Biotechnology Innovations

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Not for Press release

EXPERT OPINION

Evaluation of the safety of the bacteriophage P100 present in the product Listex P100, used for the eradication of *Listeria monocytogenes* contamination during food processing

This safety statement will cover the use of the Listex-P100 product as a food processing aid, taking the use and activity of the bacteriophage P100 during food processing into account.

TNO staff including a microbiologist, toxicologist and risk assessor, has evaluated the documentation provided by EBI Food Safety, legal publications (1), GRAS FDA documentations (2), EFSA opinion (3,4, 5, 6) and literature related to bacteriophage P100 as well as documents regarding the potential safety implications of phages in general. For this statement an independent literature search related to the safety of bacteriophages and more specifically bacteriophage P100 for food applications was performed.

The following considerations are the basis for the conclusion of the present statement:

1. Bacteriophage P100 displays a specific host range covering the species belonging to the genus *Listeria* including the *Listeria monocytogenes* (7, 8, 9). Such implies that the bacteriophage P100 would not affect the microbiota composition of the gastrointestinal tract (GIT) upon ingestion of Listex P100 treated food products by humans, as *Listeria* cannot be considered part of the normal human intestinal flora.
2. Bacteriophage P100 is a strictly lytic phage and does not integrate into the *Listeria* host genome (7,10,11). The latter study by Carlton et al. includes a very thorough bioinformatical analysis of the phage genome which proves the absence of any lysogeny associated genes. Therefore no changes in its virulence character of the bacterial strain are expected. EFSA underlines the importance for the application of strictly lytic bacteriophages for food and feed application.
3. Bacteriophage P100 shows no transducing activity (12). This absence of transductional activity prevents changes in the genetic repertoire by insertion of new genetic information in the bacterial genome by the bacteriophage and therefore adds to the safety of this type of bacteriophage. The study by Hodgson shows experimental evidence that a marker which can positively be selected for (antibiotic resistance) is not transduced by P100-like phages. The procedure allows even the lowest transduction frequencies to be detected. In a later study (13) the structure of the genome ends of this type of phages was



elucidated. It shows that the genome ends are fixed, i.e. always identical in every phage particle. Having fixed genome ends allows for the packaging mechanism to recognize a specific DNA sequence. The phage can thus distinguish between self and cellular DNA and avoid packaging host DNA.

4. The bacteriophage P100 genome is sequenced (11). Putative gene products were subjected to bioinformatic analysis. The putative bacteriophage proteins do neither display homologies to proteins of *Listeria* or other pathogenic bacteria nor do they display homologies to suspected toxins, virulence factors, antibiotic resistance determinants or any known allergens (11).
5. Toxicity testing of bacteriophage P100 by applying repeated oral doses in rats for 5 days did not yield any abnormal histological changes, morbidity or mortality (11).
6. Bacteriophage P100 displays a short-lived period of activity (6 to 24 hours) (14, 15, 16) and could therefore be considered as a processing aid rather than a food additive.
7. From a microbiological point of view, changes in shelf life of a product treated with Listex P100, are not expected, since Listex P100 only affects *Listeria* strains and spoilage organisms are not affected.
8. As bacteriophages are chemically inert the foodstuff is not affected in terms other than *Listeria*.
9. Bacteriophages are ubiquitous in our environment and regularly introduced via our food into the human GIT (17). The intake of a specific bacteriophage P100 via food consumption adds to only a limited extent to the bacteriophage load in the GIT.
10. The environmental impact of Listex P100 application is negligible. Bacteriophages are rapidly degraded in the environment by a number of factors such as UV light, proteolytic degradation of the virion by chemicals and enzymes, temperature and salts (18,19,20,21). For a large phage population to develop, a large host population is required. While listeriae are ubiquitous they are not found in large numbers in the environment. Therefore, there is only a negligible chance that a phage will meet listeriae in the environment; consequently daughter phages are hardly produced before the phage population is entirely degraded.
11. Bacteriophage P100 production is conducted by using a non-pathogenic *Listeria innocua* strain as a host. Additionally, downstream processing prevents the presence of bacterial cells in the final product Listex P100.
12. Resistance to a bacteriophage, including Listex P100 of a *Listeria* spp. target strain, is caused by the acquisition of bacteriophage resistance mechanisms. Bacteriophage resistance mechanisms are completely different from

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mechanisms of resistance to antibiotics (22). Therefore, resistance to bacteriophages will not result in cross resistance to other antimicrobial activities such as antibiotics.

In conclusion, based on the documented information, the bacteriophage P100, present in the Listex P100 product, can be regarded as safe for the claimed use and could be recommended for use as processing aid in food production.



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Signature

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J.M.B.M. van der Vossen, Ph.D.
Microbiologist

A handwritten signature in black ink, appearing to be 'b.a. N.G.M. keestra', written in a cursive style.

b.a. N.G.M. keestra

W.R. Leeman, B.Sc.
Toxicologist

A handwritten signature in black ink, appearing to be 'C. van den Berg', written in a cursive style.

b.a. Marco Jaspers
C. van den Berg, Ph.D.
Project Leader