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To whom it may concern

Regulatory status Listex P100, based on the Scientific Opinion of the EFSA Panel on Biological Hazards (BIOHAZ), January 2010.

As stated by EFSA in the EFSA Journal 2009; 7(12)1431 [1], phages should - in regard to their regulatory status in the EU - be evaluated on a case by case basis, and two criteria need to be considered:

1) mode of action
2) safety

This document (the following 3 pages) substantiates that under these EFSA criteria:

a. Listex P100 is a processing aid in solid food stuffs
b. Listex P100 is safe

Zurich, 11. January 2010

Yours sincerely,

Prof. Dr. Martin J. Loessner

1. Mode of Action as stated by EFSA in the EFSA Journal 2009; 7(12)1431 [1]

"The European Commission, through its Directorate General on Health and Consumer protection, has recently asked EFSA to provide technical assistance on an assessment on the mode of action of phages when added to foods of animal origin (animal carcasses, meat products, dairy products and others) and on their persistence i.e.: whether their antibacterial effect would be short-lived or whether they would protect against recontamination at subsequent stages of the processing or even in the final product. In the first case, phages would be considered as processing aids and then not a subject for European regulation, although they might fall under Regulation (EC) No 853/2004 (Anonymous, 2004d), when applied to remove surface contamination, while in the second, they would be considered as food additives, and so be affected by the Council Directive 89/107/EEC and Regulation (EC) No 1333/2008 (Anonymous, 1989; Anonymous 2008). The document delivered by EFSA (2009c) concludes that persistence of the antibacterial effect depends on the particular phage/bacteria combination, on the food matrix being treated, on the conditions of application and on environmental factors, so that a general answer cannot be provided."

The EFSA expert panel on the mode of action of phages concludes in its report nr. Q 2008 400, [2] that Listex P100 appears to have limited activity based on an extensive phage efficacy study by Guenther et al. [3] on a large variety of food stuffs such as cheese, meat, fish and vegetables. In that study phage activity on solid food stuffs stopped after 6 to 24 hours. All other phage biocontrol experiments on solid foodstuffs show the same pattern[4,5,6,7]. Additional studies using Listex P100 have since been published and further confirm this[8,9]. With such a short-lived period of activity Listex P100 phages should be considered a processing aid. This is in conformity with the viewpoint expressed by the Ministry of Health, Welfare and Sports of The Netherlands (letter Mr. Drs. A.M.P. van Bolhuis, director Food, Health Protection and Prevention, July 14, 2009)

2. Safety as stated by EFSA in the EFSA Journal 2009; 7(12)1431 [1]

"The conclusion [by the EFSA Biohazard Panel] is that phages do not cause any safety concerns by themselves but through the transmission and expression of viral and bacterial genes, which may enhance the virulence and spoilage abilities of their hosts".

The EFSA Biohazard Panel elaborates on this in the same report:

"Possibility to exclude safety concerns
Phage conversion of the bacterial host is dependent on the ability of the incoming virus to lysogenize and on the presence in its genome of harmful genes such as those encoding virulence determinants. Lysogenization requires a genetic switch that may repress the expression of the genes that lead phage development towards the lytic cycle and, in most cases, of a site-directed recombinase that catalyzes the incorporation of the phage genome to that of the bacterial host through a phage-bearing attP sequence. For the moment, the only safe way to ascertain whether these elements are part of the gene pool of a bacterial virus is the analysis of its genome sequence, complemented with physiological experiments to exclude the generation of lysogenic cells upon infection. Similarly, the DNA packaging mechanism and transduction ability of any phage can only be assessed with ad hoc experiments. These characteristics could be used in a case by case safety assessment of phages."

Therefore, safety is the second EFSA criterion that needs to be considered in the use of bacteriophages. EFSA states above that:

1) **the phage must be strictly lytic** (i.e., virulent in their nature and unable to integrate)

2) **the phage must be unable to transduce bacterial DNA** (i.e., not being able to package non-phage DNA into their capsids)

For Listex P100 both points have been clearly qualified:

The host range of P100 indicates that this phage is strictly lytic[10]. Bioinformatic analysis of its genetic code unequivocally proves this. The phage lacks the typical lysogeny control region found in temperate phages and no gene corresponding to genes with integrase activity are detectable in the genome, nor does the genome reveal any virulence or toxin genes[11].

Transduction experiments with *Listeria* phages have shown conclusively that P100-like phages show no transducing activity[12]. Recently, the exact nature of genome ends of P100 phage DNA in phage particles has been elucidated. This shows that the phage is likely to have a packaging mechanism which is able to differentiate the own phage DNA from the foreign bacterial DNA[13].

Conclusion

Based on the EFSA criteria quoted above, Listex is safe and can be used as a processing aid in solid food stuffs.

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