

**Memorandum**

Date June 1, 2005

From Negash Belay, Ph.D., Division of Biotechnology and GRAS Notice Review, Office of Food Additive Safety, HFS-255

Subject Revised FAP 2A4738

To Raphael A. Davy, Division of Petition Review, HFS-265

The microbiological aspects of FAP 2A4738, submitted by Intralytix, Inc. (Intralytix), were reviewed. The concerns raised in previous microbiology memos have now been adequately addressed by the petitioner. The petitioner seeks approval for the safe use of a mixture of bacteriophages on ready-to-eat (RTE) meat and poultry products as an antimicrobial agent to control the pathogenic microorganism *Listeria monocytogenes* (LM). The trade name for the bacteriophage cocktail formulation is LMP-102™. LMP-102™ is to be used in accordance with current good manufacturing practice to supplement existing methods to control LM. LMP-102™ is to be applied by spray to the RTE meat and poultry products just prior to packaging. The additive is to be applied at levels of approximately 1 ml per 500 cm² of food surface area.

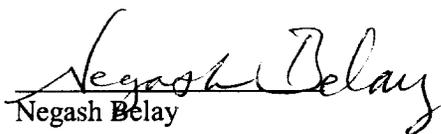
LMP-102™ is a mixture of equal proportions of six individually purified LM-specific bacteriophages in phosphate-buffered saline. The phages are lytic double-stranded DNA phages that belong to the family *Siphoviridae*. The individual phages have been deposited with the American Type Culture Collection (ATTC) and were assigned patent depository ATTC numbers. The lytic character of the bacteriophages was demonstrated using plaque morphology and host range criteria, i.e., lytic phages have a broad host range and produce clear plaques. Moreover, the petitioner has fully sequenced each phage and intends to ensure that the phages in LMP-102™ carry no undesirable genes and no sequences encoding any portion of 16S bacterial ribosomal RNA (presence of 16S sequences would be indicative of a non-lytic phage because the phage would have acquired these sequences from prior integration into bacterial host genomes). Undesirable genes include genes encoding bacterial toxins or genes associated with drug resistance. The petitioner has screened for a wide variety of toxin genes from bacterial sources that are listed under 40 CFR § 725.421 and shown their absence in the LMP-102™ phages. The petitioner has also shown that the phages do not carry bacterial 16S ribosomal RNA genes.

The phages in LMP-102™ are produced using their appropriate LM host strains. The manufacturing process includes measures to remove intact cells of the production organism (LM) and the toxin Listeriolysin O (LLO). The petitioner intends to analyze every batch of LMP-102™ to monitor toxin residues and potential presence of live LM in the product. The petitioner has done LLO testing and the toxin was not detected in LMP-102™ with a hemolysis assay that is sensitive to 5 hemolytic units (HU) LLO/ml

[1 HU = 1ng LLO].^{1,2} Based on this, the petitioner has established a <5 HU LLO/ml specification for LLO content. The petitioner has also tested LMP-102™ batches for LM presence and overall bacterial contamination. No LM or other bacterial contamination was observed using a method based on USDA's method (detection limit better than 1 CFU/25g of sample), "Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg and Environmental Samples." This limit is now included in the petitioner's specifications for LMP-102™.

The petitioner has conducted studies, carried out under good laboratory practices (GLP), to evaluate the effectiveness LMP-102™ in reducing LM populations on RTE meat and poultry products. Samples representing twelve categories of RTE meat and poultry products were used in these studies. The samples were inoculated with approximately 2×10^3 CFU per cm² of a 1:1:1 mixture of three LM strains (representing serogroups 4b, 1/2a, and 1/2b) and then spray treated with LMP-102™ at the proposed use levels. Water treated controls and untreated controls were included for the respective samples. Analysis of the samples after storage at $5 \pm 2^\circ\text{C}$ for 24 ± 4 , 72 ± 4 , or 168 ± 4 hours showed a 1.0 - 2.75 logs reduction in LM populations on LMP-102™ treated samples compared to controls. Based on this information, it can be concluded that LMP-102™ is likely to achieve its intended technical effect when used under the conditions stated in the petition.

We have no further questions at this time.


Negash Belay

E-mail cc: HFS-255 (RHarris); HFS-255 (AMattia); HFS-255 (RMartin)

R/D:HFS-255:NBelay:5/31/05
Review:RMerker:HFS-255:5/31/05
Review and Int.:RHarris:6/01/05
F/T: HFS-255:NBelay:6/01/05

¹Geoffroy et al., Infection and Immunity 55:1641-1646; 1987.

²Alouf et al.,ann. Inst. Pasteur 108:476-500;1965.