Ministry for Primary Industries
Manatū Ahu Matua

November 2015

Project Officer Application A1111
Food Standards Australia New Zealand
PO Box 10559
The Terrace
WELLINGTON 6036

[redacted]

Dear Sir/Madam

Application A1111 – Bacteriophage S16 and FO1a as a Processing Aid – Call for Submissions Report

Thank you for the opportunity to comment on this application. The Ministry for Primary Industries (MPI) has the following comments to make.

Clarification about intended use
The draft variation, states "reduce *Salmonella* species on or in raw meat during processing". However the application states: " the intended use of *Salmonelex* is on carcasses, fresh pork cuts, fresh beef cuts and on fresh poultry". We note that Standard 2.2.1 of the Code includes poultry in the definition of meat, but in Schedule 22, "foods and classes of foods", there is a distinction between meat (mammalian origin) and poultry meat, which may cause confusion. Furthermore, we question whether the phage should be permitted to be applied "on" and not "on or in" raw meat meaning that the words "or in" should be deleted in the draft variation.

MPI expects that directions for use of the phage would be clearly stated when sold (eg spray, dipping) and when used, this should be as part of a food safety management system.

Lack of evidence of no ongoing technological function: The applicant states that the phage "is present in the finished food at insignificant levels". In section 3.1.5.5.4 the applicant states that "*Salmonelex* is inactivated within 24 hours after addition to the food". However, there is no proof of this. For example, by enumeration of the phage levels at the end of incubation times. No ongoing technological function, is based on there being no further *Salmonella* reduction after 8 hours, which is in contradiction with some results demonstrated in several publications for similar products and conditions noted below.

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The applicant also states that *Salmonella* cells grow in the treated samples at the same growth rate than in the controls when samples are incubated at room temperature. However, this could be the result of a balance between bacterial growth and bacterial death (due to bacteriophage activity).

- Bigwood et al. (2008), using raw beef cuts artificially contaminated with overnight *Salmonella* cultures, show a clear bacterial reduction after *Salmonella* phage application until 6 days of incubation at 5°C. Sharma et al. (2015) demonstrate similar results with turkey breast cutlets contaminated by *Salmonella*, treated with *Salmonella* phages and incubated at 4°C for 7 days.

  Spricigo et al. (2013) demonstrate that the *Salmonella* phage concentration remained constant after application on artificially contaminated pig skin, chicken breast, fresh eggs and lettuce. Guenther et al. (2012) showed that the phage remained stable with no significant loss of infectivity for different artificially contaminated food products (hot dogs, mixed seafood, chocolate milk and egg yolk) for a period of 6 days. These authors also underline that in the current state, they may not kill further bacteria as they are immobilised on the surface of the food matrix (which is also one argument of the applicant to justify the absence of ongoing technological function).
- We note that fresh cuts of meat or poultry may be further processed (grinding) or mixed with other food ingredients, in this case, the complete food product structure may change and the phage particles may come into contact with new *Salmonella* cells. This outcome is not a bad thing as such, but it contradicts the claims from the applicant that "there is no ongoing technological function": if the product is not consumed directly in the same state it was after the *Salmonella* phage application (and it is unlikely for poultry products), there could be an ongoing function. In our view, this is a significant difference with the *Listeria* phage preparation which is intended to be used for RTE products.

Problems of interferences with *Salmonella* detection: The persistence of bacteriophages in the final product could be a problem for any subsequent detection and enumeration analyses.

In a publication, Muniesa et al. (2005) note when the stomaching is performed to produce the enrichment in the first step of the *Salmonella* detection, phages present on the surface of the food sample, could kill any *Salmonella* cells present in the food product, leading to no colony growth on the selective media used for the isolation steps. This could therefore give an underestimation of the real number of *Salmonella* in the food product and lead to false-negative results.

EPA considerations

New Zealand's Environmental Protection Authority (EPA), regulates new organisms (plants, animals, genetically modified organisms) and hazardous substances and chemicals. Importers need to consult the EPA to determine if any phage material may be considered to be a new organism. Similarly there may be biosecurity implications that require consultation with MPI.

Some errors or inconsistencies in results

Several minor errors or inconsistencies in the application by *Micreos* have been noted that could to some extent affect the confidence in the applicant results.

- Figure 8 p.52 of the application: the control points at 1, 2 and 6 hours in the graph do not correspond to the values in Table 13 p.81 for beef.
- There are also some errors in the calculation of the averages in the raw data (for beef for example), however the FSANZ risk assessment has corrected these (eg in Table 3 p.13).

Yours sincerely

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Manager Food Science and Risk Assessment

References:

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Guenther, S., Herzig, O., Fieseler, L., Klumpp, J., Loessner, M.J. (2012). Biocontrol of Salmonella Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. International Journal of Food Microbiology 154 (66-72).

Muniesa, M., Blanch, A.R., Lucena, F., Jofre, J. (2005). Bacteriophages may bias outcome of bacterial enrichment cultures. Applied and Environmental Microbiology 71 (4269-4275).

Sharma, C.S., Dhakal, J., Nannapaneni, R. (2015). Efficacy of lytic bacteriophage preparation in reducing Salmonella in vitro, on Turkey breast cutlets, and on ground Turkey. Journal of Food Protection 78 (1357-1362).

Spricigo, D.A., Bardina, C., Cortés, P., Llagostera, M. (2013). Use of a bacteriophage cocktail to control Salmonella in food and the food industry. International Journal of Food Microbiology 165 (169-174).