NSW Food Authority

Application A1045
BACTERIOPHAGE PREPARATION AS A PROCESSING AID

Major Procedure

Summary

The NSW Food Authority supports the use of bacteriophage preparation P100 to provide an additional hurdle to control *Listeria monocytogenes* in ready-to-eat foods, when used in conjunction with a food safety program.

The Authority is satisfied that the application of the P100 phage preparation is technologically justified and does not pose a risk to human health.

However, NSW believes there are still several questions that need resolving before this application can proceed, regarding:

- **the question of ongoing technological function in the final food is still not clear and NSW considers that the addition of P100 should be considered as a food additive under the category of preservative within Schedule 5 of Standard 1.3.1, and**
- **whether the presence of bacteriophage preparation P100 could potentially interfere with detection methods for *L. monocytogenes* leading to false negatives in food samples**

Technologically justified

NSW would generally support the use of the bacteriophage preparation P100 and agrees that there would be significant benefits to its use in certain RTE foods to assist the food industry in the control of *L. monocytogenes*.

NSW also agrees that there are no health effects associated with its use and note the approvals to use in overseas countries and GRAS status granted in the USA.

However, NSW still has concerns over the categorisation of the bacteriophage as a processing aid by the applicant and by FSANZ in the submissions reports. NSW considers that the practical determination of which foods the phage would be permitted in would lack clarity and potentially cause confusion. NSW suggests that the use of the phage may be more suitably categorised as a food additive under Standard 1.3.1.

Ongoing technological function

To this point, the justification by the applicant for categorising bacteriophage P100 as a processing aid centres mainly around the question on whether the bacteriophage performs a technological function in the final food. This appears to be the primary reason for limiting the application to ‘solid foods’ despite the fact that it may be even more efficacious in liquid foods as a food additive.

NSW has examined the data supplied by the applicant as well as peer reviewed journal articles to consider the issue of ongoing technological function of bacteriophages, including P100. The conclusion of NSW is that the data regarding ongoing technological function of phage P100 does not appear to be completely definitive and not as clear cut as the broad conclusions presented by the applicant.

Ongoing presence of active phage particles

The applicant states that the number of phage that can be retrieved from a food surface declines over time due to structural decay and/or irreversible adsorption. The relative persistence of phage particles has been reported in several papers, such as Guenther et al (2009), who demonstrated that the '…phage was not inactivated by these foods…'. The authors noted the stability of the phage within the food – examined after 6°C for six days – such as:

- 'the effect of phage was not neutralized by prolonged storage periods', and
- 'found that phage particles to be quite stable in foods of animal origin'

In addition, Carlton et al (2005) also found that the P100 phage titre remained constant over six days on surface-ripened soft cheese and Leverentz et al (2004) found that the titre of a phage mixture stayed at the level of inoculation on honeydew melons for seven days. Soni and Nannapaneni (2010) found the phage titre of P100 on raw salmon fillets remained relatively stable over ten days at 4°C. Of the initial 8 log pfu/g, there was only a marginal decrease of 0.6 log pfu/g. The paper of Leverentz et al (2003) actually found the phage titre increased by about 1 log unit over a period of seven days.

Therefore, there appears to be persistence of the phage particles in the final food, at least in the initial stages of the food's shelf life.

Are the persistent phage particles 'active'?

The paper by Guenther et al (2009) stated the following 'we conclude that limited diffusion and thus limited contact of bacteria and phage particles was responsible for the lower efficacy'. This was the hypothesis of the authors to explain their experimental results, and was also picked up in the paper by Holck and Berg (2009).

NSW is not aware of any additional research that clearly demonstrates whether this hypothesis is correct. The European Food Safety Authority (EFSA) scientific opinion on the use and mode of action of bacteriophages in food production examined the data of Guenther et al (2009) and concluded that while bacteriophages may become adsorbed to the food matrix soon after application, this does not necessarily mean inactivation, because they can be washed from it and still produce plaques of lysis. EFSA also stated that 'whether these immobilized, but still active, bacteriophages could lyse target bacteria re-inoculated on the foods was not tested' (EFSA, 2009).

Previously, a different hypothesis to that of Guenther et al (2009) was presented by Leverentz et al (2004), where the authors stated 'it appears that in our system it is important to reduce the bacterial levels initially as much as possible with a high phage concentration. However, there is an additional effect of the timing of the application that influences the reduction level over several days of storage and there may be second phage generations'. Together with their earlier observations of an increased phage titre over seven days (Leverentz et al, 2003), this would tend to indicate some level of ongoing function.

EFSA (2009) also stated in its scientific opinion '…the documents provided by industry show that the methods used to measure the persistence of the bacteriophage (either persistence of the activity of the bacteriophage on the food or stability of the bacteriophage on the food) may give different results.…based on data currently available in peer-reviewed literature, it cannot be concluded whether bacteriophages are able or unable to protect against recontamination of food with bacterial pathogens. This is likely to vary with each bacteriophage, each food matrix, and with conditions of application including environmental factors.' Given this different behaviour of the bacteriophage in similar foods, EFSA concluded that this 'impedes the definition of bacteriophages as processing aids or as additives, when applied to foods for decontamination'.

Monk et al (2010) state that there is uncertainty regarding the exact kinetics of the bacteriophage infection process, while Soni and Nannapaneni (2010) stated 'the usefulness of a phage in preventing the proliferation of a host bacterium during longer product storage time depends on the stability of phage particles in any particular food matrices and its surface water content for phage mobilization.' Similarly, Bigot et al (2011) concluded that the 'actual concentration (of phage) needed to achieve a given reduction may well change with foods of different characteristics as the ability of phages to "roam" in liquid films may differ, as may any inactivation or irreversible adsorption that may occur to food or packaging material'.

EFSA also published a more recent scientific opinion on the efficacy of P100 for the removal of *L. monocytogenes* surface contamination of raw fish (EFSA, 2012). The panel concluded that the data were not adequate to allow firm conclusions on persistence or activity of P100 in stored fish (over 10 days stored at 4°C and 10°C).

In this opinion, EFSA examined several peer reviewed publication testing the efficacy and persistence of P100 on two types of raw fish. Different results were observed in that there was an increase in numbers of *L. monocytogenes* on catfish fillets treated with P100, but no growth in treated salmon fillets. EFSA concluded it was unclear whether these counts were due to total prevention of bacterial growth or to the balance between growth and death of bacterial cells. The bacteriophage titre remained stable on raw salmon fillet samples for 4-7 days and showed slight decreases during days 7-10 at 4°C. In contrast, P100 titres decreased during the first 4 days of the 10-day storage period of treated catfish samples at 4°C and 10°C, and remained stable during days 4-10.

In light of the EFSA opinion, NSW does not agree with the FSANZ risk assessment report which maintains that evidence of efficacy and ongoing technological function can only be represented by a continuously declining treatment line (shown as D in Figure 1 on page 10 of the risk assessment report). A more likely scenario with reduced ongoing function is, after the initial reduction, there is a combination where some *L. monocytogenes* cells are actively multiplying while others are being inactivated by phage (described above by EFSA as the balance between growth and death of bacterial cells). This situation would be represented by a positive, but not parallel, growth line when compared to the control. This situation was observed in some of the data examined by FSANZ in the risk assessment report - cabbage, mixed seafood and smoked salmon - but explained away as differences in bacteria strain behaviour or as an artefact of the experimental method.

In the opinion of NSW, these findings and conclusions presented above appear to lack the definitive outcome required to categorise the phage preparation as a processing aid. NSW considers that while the initial inactivation of *L. monocytogenes*

cells immediately after the addition of the phage P100 preparation forms the majority of the technological function, it is not possible to definitively assess whether there is some ongoing (but reduced) technological function performed in the final food. This may vary from food to food and be dependent on the amount of surface liquid present that could allow passive diffusion of the phage particles, especially for the first several days of the shelf life.

Proposed draft variations to the Code

Determining processing aid vs food additive

In the absence of definitive evidence demonstrating there is no ongoing technological function in all foods in which the phage preparation may be used, NSW considers the use of bacteriophage P100 should be considered as a food additive.

Schedule 5 of Standard 1.3.1 lists preservative as one of the technological functions performed by food additives and even lists 'bacteriophage control agent' as one of the functional sub-classes. It is unclear then why P100 would be considered differently in this respect, as the technological function that it performs correlates with the definition of preservative in Schedule 5 that it 'retards or prevents the deterioration of a food by micro organisms'.

Aside from the question of ongoing technological function, NSW contends that, as currently proposed in the draft variations to the Code, to try and differentiate between solid and non-solid foods, and food that may be partially or completely covered in liquid is not going to be practical. Solid foods may have some moisture on the surface which may be ample for the diffusion of persistent active phage particles to perform some ongoing technological function in the food. While the applicant states that the presence of liquid in the final food does not abolish adsorption in any way, NSW does not feel they have not presented evidence to adequately demonstrate this, inline with conclusions of EFSA (2009).

Definition of solid food

The problem of making the differentiation between foods where P100 can or cannot be used is even illustrated in the proposed editorial note drafted by FSANZ to explain the term 'solid foods'.

'Foods that are solid hold their shape and do not flow when placed on a flat surface such as a table. An example of a solid food is a cut melon. Fruit puree, on the other hand, would not be considered a solid food.'

The example given of a solid food is cut melon, which it would be assumed would have a moist surface. From a practical sense would this moisture be enough to allow diffusion of persisting phage particles to allow ongoing technological function? The work of Leverentz et al (2003) appears to demonstrate an increase in phage numbers over seven days, indicating ongoing function.

Range of foods in which P100 is permitted

Assuming bacteriophage P100 is approved for use as a food additive, then it is not clear why FSANZ has drafted provisions to limit the applicability of bacteriophage P100 to specific categories of RTE foods.

There are listed in the proposed variation to Standard 1.3.3 as:

- Meat and meat products
- Fish and fish products
- Fruit and fruit products
- Vegetables and vegetable products
- Cheese

From a practical perspective, the technological function for phage P100 should be effective in any food, and therefore it is unclear why these limitations would be put in place. By limiting the applicability to this list, any other solid (or semi-solid) dairy product, or mixed food would not be applicable.

Issues with analysis of food samples containing bacteriophage P100

In the first round of submissions, Queensland Health raised the issue about handling of food samples that may contain the bacteriophage. It is unclear at this point whether there could be any potential inactivation of any *L. monocytogenes* cells present in the food during the analysis (eg during the enrichment stage when a liquid is added to the food and the samples may be stomached). It is also unclear whether this could lead to the potential for false negative results (ie the *L. monocytogenes* cells are present in food but become inactivated during the addition of liquid to the food sample and any persisting phage particles are able to lyse the *L. monocytogenes* cells).

Although this may not actually be an issue for concern, it has not been addressed at this point and should be before the application is approved. FSANZ has indicated that this issue should be addressed by the proposed new Expert Advisory Group for analytical methods to be set up by ISC. A timeline for this consideration has not been given.

If there are any differences with how samples containing phage should be handled and analysed, without labelling present to indicate whether the phage treatment is present or not, then any food samples would need to be handled in such a way as to presume it is present.

Definition of ready-to-eat

NSW supports the inclusion of a definition of ready-to-eat in Standard 1.1.1, but notes there are currently definitions in both Standard 3.2.2 and 3.3.1 of the Code as follows:

- **3.2.2 - 'ready-to-eat food** means food that is ordinarily consumed in the same state as that in which it is sold and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer.'
- **3.3.1 -** '**ready to eat** in relation to food means food that is ready for consumption, but includes food that may be re-heated, portioned or garnished or food that undergoes similar finishing prior to service.'

Consideration may be given to creating one single definition of ready-to-eat that is applicable throughout the Code to avoid creating inconsistencies

Issues from the 1st call for submissions report

Composition of the phage preparation P100

The FSANZ 2nd call for submissions paper has clarified the Authority's questions regarding the composition of the phage preparation and the allowance of 'similar preparations' (wording from 1st call for submissions report). The Authority now

understands that any changes to the phage would require a new application to FSANZ, in line with approvals granted overseas.

Adherence to policy guidelines

NSW does not agree that the application of bacteriophage P100 be considered as a processing aid when the question regarding ongoing technological function has not been clearly established, or may be variable across different foods, or over the course of a food's shelf life.

While there is certainly evidence that there is decreased technological function after the first 24 hours after application of the bacteriophage, the persisting phage particles do remain infective and the presence of liquid on the surface of a food may potentially facilitate further lysis of *L. monocytogenes* cells. As observed by EFSA, the behaviour of the phage may be dependent upon the factors of the individual food.

In all other aspects, NSW believes that the application has met the requirements of the policy guidelines for the addition to food of substances other than vitamins and minerals in that:

- The purpose for adding the substance has been articulated clearly (reduce the number of *L. monocytogenes* in RTE food products)
- The addition of the substance is safe for human consumption
- The amounts added are consistent with achieving the technological function; and
- The substance will be added in a quantity and a form which is consistent with delivering the stated purpose
- No nutrition, health or related claims will be made on the use of the phage preparation.

References

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ENDS

The views expressed in this submission may or may not accord with those of other NSW Government agencies. The NSW Food Authority has a policy which encourages the full range of NSW agency views to be submitted during the standards development stages before final assessment. Other relevant NSW Government agencies are aware of and agree with this policy.