

**A Petition to Amend the Australia New Zealand Food Standards Code with an  
Alpha-amylase Enzyme Preparation produced by *Bacillus subtilis***

**EXECUTIVE SUMMARY**

The present application seeks to schedule 18 - Processing Aids of the Australia New Zealand Food Standards Code (the Code) to approve an enzyme preparation from *Bacillus subtilis* (*B. subtilis*) host strain genetically modified to produce a ***Bacillus subtilis* production strain (AR-651) containing an alpha-amylase enzyme** from *Thermoactinomyces vulgaris*. Based upon the food code, the enzyme is to be used in:

- bakery products such as, but not limited to bread, steamed bread, bread buns, tortillas, cakes, pancakes, and waffles.

**Proposed change to Standard 1.3.3 - Processing Aids**

The table schedule 18—4(5), **Permitted enzymes (section 1.3.3—6)—Enzymes of microbial origin**, is proposed to be amended to include a genetically modified strain of *Bacillus subtilis* as permitted source for  $\alpha$ -Amylase (EC 3.2.1.1).

This application is submitted under a general assessment procedure.

The food enzyme is a biological isolate of variable composition, containing the enzyme protein, as well as organic and inorganic material derived from the microorganism and fermentation process.

The main activity of the food enzyme is alpha-amylase.

## **Use of the Enzyme and Benefits**

Like any other enzyme, the alpha amylase acts as a biocatalyst: with the help of the enzyme, a certain substrate is converted into a certain reaction product. It is not the food enzyme itself, but the result of this conversion that determines the effect in the food or food ingredient. After the conversion has taken place, the enzyme no longer performs a technological function.

The **substrates** for the enzyme are starch, glycogen and related polysaccharides and oligosaccharides which can be found in various grain products and therefore occur naturally in nature and are a natural part of the human diet.

The function of the alpha-amylase is to catalyse the hydrolysis of the  $\alpha$ -(1,4) glycosidic linkages of the mentioned substrates in a random manner

**Reaction products:** as a result of the catalytic activity of alpha-amylase, low levels of oligosaccharides are formed. These compounds are already present in the human diet.

Like most of the enzymes, the alpha amylase performs its technological function during food processing. The alpha amylase from *Bacillus subtilis* AR-651, object of this dossier, can theoretically be used as processing aid for bakery products. Alpha-amylase is naturally present in cereal grains. The natural enzymatic conversion of starch in foods containing cereal grains (or derivatives such as flour) is of technological benefit in several industrial food manufacturing processes. But the levels of endogenous cereal alpha-amylases are often too low (measured as a high falling number) and varies from batch to batch of raw material and the specificity of the enzyme may not be optimal to give the desired process advantages. Therefore, their content needs to be standardized.

## Safety Evaluation

The production organism fulfils the specific qualifications for the QPS<sup>1</sup> status, the genetic modifications do not give rise to safety concerns and the manufacturing does not give any risks, therefore the production strain *Bacillus subtilis* AR-651 qualifies for QPS status. The production strain was also tested for cytotoxicity and was found not to be cytotoxic to vero cells.

The *Bacillus subtilis* AR-651 production strain is constructed as part of the same strain lineage of *Bacillus subtilis* AR-475. *Bacillus subtilis* AR-475 alpha-amylase has undergone rigorous toxicological testing. The toxicological studies for AR-475 are presented in this submission to substantiate the safety of AR-651 for consumers. The mutagenicity studies showed that the food enzyme does not have the potential to damage the genetic material of living organisms, including mammals. The oral toxicity study showed that the food enzyme does not exhibit signs of toxicity, up to doses that are several thousand times higher than those which are consumed via food.

The product complies with the recommended purity specifications (microbiological and chemical requirements) of the FAO/WHO's Joint Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) for food-grade enzymes.

The product is free of production strain and recombinant DNA.

The safety of the alpha amylase preparation was confirmed by external expert groups, as follows:

- The *Bacillus subtilis* AR-651 alpha-amylase production strain has been approved in Denmark (Danish Veterinary and Food Administration) and in France (Direction générale de la concurrence, de la consommation et de la répression des fraudes).

AB Enzymes is in the process of registering the *Bacillus subtilis* AR-651 alpha-amylase production strain in other countries such as the USA (FDA), Canada (Health Canada) and the EU (EFSA).

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<sup>1</sup> The European Food Safety Agency (EFSA) maintains a list of the biological agents to which the Qualified Presumption of Safety (QPS) assessment can be applied. In 2007, the Scientific Committee set out the overall approach to be followed and established the first list of the biological agents.

## Conclusion

To conclude, the use of the food enzyme alpha-amylase from *Bacillus subtilis* AR-651 in the production of food is safe based on the following aspects presented in this dossier:

- Safety data and information the production strain
- Allergenicity and toxin analysis assessment on amino acid sequence of food enzyme
- TDMI value based on Budget Method

The *Bacillus subtilis* has been used in the food industry for many years. Strains from the *Bacillus subtilis* microorganism are generally recognized as safe and are recognized to produce a variety of enzymes. *Bacillus subtilis* is listed as a permitted producer of enzymes in multiple global food enzyme positive lists, including in Australia where alpha-amylase from *Bacillus subtilis* is permitted. The *Bacillus subtilis* AR-651 alpha-amylase production strain meets the QPS exception status and the results of a cytotoxicity study conducted on the strain is provided in the dossier. We have demonstrated that the enzyme batches containing the alpha-amylase from *Bacillus subtilis* AR-651 to meet the following criteria:

- Absence of Antibiotic and Toxic Compounds & Analysis of Purity and Identity Specifications of the Enzyme Preparation
- Absence of Production strain
- No Detection of recDNA