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Supporting document 1

Risk and technical assessment report – Application A1255

Alpha-amylase from GM *Bacillus subtilis* as a processing aid

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Executive summary

AB Enzymes GmbH (AB Enzymes) has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme alpha-amylase as a processing aid in the manufacture of bakery products such as bread, steamed bread, bread buns, tortillas, cakes, pancakes, and waffles. This alpha-amylase is produced from a genetically modified (GM) strain of *Bacillus subtilis*, containing the alpha-amylase gene from *Thermoactinomyces vulgaris*.

FSANZ has undertaken an assessment to determine whether the enzyme achieves its technological purpose in the quantity and form proposed, and to evaluate public health and safety concerns that may arise from the use of this enzyme.

FSANZ concludes that the proposed use of alpha-amylase produced by GM *B. subtilis* as a processing aid in the manufacture of bakery products is technologically justified. Analysis of the evidence supplied by the applicant provides adequate assurance that the enzyme achieves its technological function in the quantity and form proposed.

No public health and safety concerns were identified in the assessment of alpha-amylase produced by this GM *Bacillus subtilis* strain AR-651 under the proposed use conditions. *B. subtilis* has a long history of safe use as a production microorganism of enzyme processing aids, including several that are already permitted in the Code. The production organism is neither pathogenic nor toxigenic. Analysis of the modified production strain confirmed the presence and stability of the inserted DNA. The *B. subtilis* strain AR-651 produces low levels of a *Bacillus* sp. hydrolase. Bioinformatics analysis indicated that neither the produced alpha-

amylase, nor a co-expressed hydrolase important for the manufacturing of alpha-amylase, shows significant homology with any known toxins or food allergens.

Toxicity testing of the alpha-amylase enzyme showed no evidence of genotoxicity *in vitro* and the no observed adverse effect level (NOAEL) in a 90-day oral gavage study in rats was the highest dose tested, 1000 mg total organic solids (TOS)/kg body weight (bw)/day. The theoretical maximum daily intake (TMDI) from solid food is 1.78 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of around 600.

Based on the reviewed data it is concluded that in the absence of any identifiable hazard an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate.

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1 Introduction

AB Enzymes GmbH (AB Enzymes) has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme alpha-amylase (EC 3.2.1.1) as a processing aid in the manufacture of bakery products such as bread, steamed bread, bread buns, tortillas, cakes, pancakes, and waffles. This alpha-amylase is produced from a genetically-modified (GM) strain of *Bacillus subtilis*, containing the alpha-amylase gene from *Thermoactinomyces vulgaris*.

There are permissions for alpha-amylase from GM and non-GM microbial sources in the Code. However, this particular source is not specified as permitted. If a pre-market assessment leads to permission being granted, this alpha-amylase will provide an additional option for manufacturers of bakery products.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is a solely technological purpose (function) and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used
- evaluate potential public health and safety concerns that may arise from the use of this enzyme, produced by a GM microorganism, as a processing aid. Specifically by considering the:
  - history of use of the gene donor and production microorganisms
  - characterisation of the genetic modification(s), and
  - safety of the enzyme.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity of the enzyme

The enzyme is produced by a GM strain of *B. subtilis*, with the gene for alpha-amylase provided from *T. vulgaris* (see Section 3 for more information). AB Enzymes provided relevant information regarding the identity of the alpha-amylase enzyme. FSANZ verified this information using an appropriate enzyme nomenclature reference (IUBMB 2018). Details of the identity of the enzyme are available in Table 1.

Table 1: Identity

Generic name	alpha-amylase
IUBMB nomenclature	α-amylase

Synonyms	glycogenase; α-amylase; endoamylase; Taka-amylase A; 1,4-α-D-glucan glucanohydrolase
IUBMB No.	EC 3.2.1.1
CAS No.	9000-90-2

IUBMB: International Union of Biochemistry and Molecular Biology; CAS: Chemical Abstracts Service

For a graphical representation of the hydrolysis reaction catalysed by alpha-amylase, refer to its record in the enzyme database BRENDA[1] (Chang et al 2021).

2.2 Manufacturing process

2.2.1 Production of the enzyme

AB Enzymes' alpha-amylase is produced by submerged fermentation of GM *B. subtilis*. The main fermentation steps are inoculum, seed fermentation, and main fermentation. This is followed by the recovery stage which involves primary and liquid separation, concentration to achieve the desired enzyme activity and/or to increase the ratio of enzyme activity to total organic solids (TOS) before formulation, and germ filtration. The resulting product is a concentrated enzyme solution that the applicant states is free of the production strain and insoluble substances.

The enzyme is manufactured in accordance with current Good Manufacturing Practices for Food and the principals of Hazard Analysis of Critical Control Point. The applicant states that their quality management system adheres to the European Food Hygiene Regulation (852/2004). Details of the manufacturing process, raw materials and ingredients used in the production of the alpha-amylase enzyme preparation were provided in the application, some as Confidential Commercial Information.

The applicant formulates the enzyme into a final concentrated enzyme preparation in the form of a free-flowing brown powder marketed as VERON® 1000. It can also be added to bread improvers.

The typical composition of the applicant's enzyme preparation is:

Enzyme concentrate	20–25 %
Sunflower oil	0.4 %
Wheat flour	remainder

[1] https://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.1

2.2.2 Specifications

Internationally recognised specifications are available for enzyme preparations used in food production. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2017) and the Food Chemicals Codex (FCC) (USPC, 2018). These specifications are included in the primary sources listed in section S3—2 of Schedule 3 of the Code and enzymes used as a processing aid must meet either of these specifications. Schedule 3 of the Code also includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

Table 2: Analysis of alpha-amylase compared to specifications of enzymes

			Specifications		
Analysis	**Unit**	**AB Enzymes analysis**	**JECFA**	**Food Chemicals Codex**	**Australia New Zealand Food Standards Code**
Lead	mg/kg	< 2	≤ 5	≤ 5	≤ 2
Arsenic	mg/kg	< 1	-	-	≤ 1
Cadmium	mg/kg	< 1			≤ 1
Mercury	mg/kg	< 1			≤ 1
Total coliforms	CFU/g	< 30	≤ 30	≤ 30	-
Enteropathogenic *Escherichia coli*	CFU/25 g	ND	Absent	-	-
Salmonella spp.	CFU/25 g	ND	Absent	Negative	-
Antimicrobial activity	—	ND	Absent	-	-

ND: Not detected; LOD: Limit of detection; CFU: Colony-forming unit

Note: Analysis was performed on three batches of enzyme preparation.

2.3 Technological purpose of the enzyme

Alpha-amylase is a glycosidase, belonging to the hydrolase enzyme class. It catalyses endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units (IUBMB 2018). These polysaccharides include starch, glycogen, and related poly- and oligosaccharides. These substrates are commonly found in cereal grains such as wheat and cereal grain products such as wheat flour.

Alpha-amylase is an endo-hydrolase as it acts on the interior of a polysaccharide substrate. This breaks the long molecule into smaller oligosaccharides called dextrins which consist of six to eight glucose molecules (Sundarram and Murthy 2014). Adding alpha-amylase to bread dough increases the yield of dextrins from the hydrolysis of starch. These dextrins are fermented by yeast; the increased fermentation improves the volume and texture of the baked product. Dextrins can also be broken down further into mono- and disaccharides such as glucose and fructose. The extra sugar produced improves other qualities of bakery products such as taste, crust colour, and toasting characteristics (de Souza and de Oliveira Magalhães 2010). The addition of alpha-amylase can also reduce the rate of staling in bakery products (van der Maarel 2002).

AB Enzymes has modified the production organism to co-express a hydrolase. This minimises thickening of the fermentation broth during manufacturing of the enzyme preparation. The applicant indicated that the amount of hydrolase in the enzyme preparation is insignificant, and similar to that produced by unmodified *B. subtilis*.

Use of commercial enzyme preparations should follow Good Manufacturing Practice (GMP), where use is at a level that is not higher than that necessary to achieve the desired enzymatic reaction. The conditions of use of the enzyme in the manufacture of bakery products will depend on a number of factors including the nature of the application and the individual food manufacturers' production processes. The optimum use level should be assessed and adjusted using trials that reflect their particular processes.

The applicant provided a description of the method used for determining enzyme activity, which is CCI.

2.4 Technological justification for the enzyme

As outlined above, alpha-amylase is used to catalyse the breakdown of starch and other polysaccharides. The small dextrins released can be fermented. In the manufacture of bakery products, a higher fermentation rate increases the volume and improves the texture of the final product. Its use as requested by the applicant is therefore technologically justified.

2.5 Food technology conclusion

FSANZ concludes that the proposed use of this alpha-amylase in the manufacture of bakery products is consistent with its typical function of catalysing the hydrolysis of starch.

Analysis of the evidence provides adequate assurance that the proposed use of the enzyme, at a level not higher than necessary to achieve the desired enzyme reaction under GMP usage levels, is technologically justified.

Alpha-amylase performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid as defined in the Code.

There are relevant identity and purity specifications in the Code that enzyme preparations containing this enzyme would need to meet if its use is approved.

3 Safety assessment

The objectives of this safety assessment are to evaluate any potential public health and safety concerns that may arise from the use of this enzyme, produced by this microorganism, as a processing aid.

Some information relevant to this section is Confidential Commercial Information (CCI), so full details cannot be provided in this public report.

3.1 History of use

3.1.1 Host organism

Bacillus subtilis

There is a long history of safe industrial use of *B. subtilis* as a microorganism to produce enzymes for food processing in many countries including Australia. JECFA (FAO/WHO, 2006) has conducted a technical review of *B. subtilis* as a safe strain for enzyme production which concludes its safety.

Schedule 18 to Standard 1.3.3 of the Code currently permits the following enzymes derived from *B. subtilis:* alpha-acetolactate decarboxylase, alpha-amylase, beta-amylase, asparaginase, endo-1,4-beta-xylanase, beta-glucanase, hemicellulase multicomponent enzyme, Maltogenic alpha-amylase, metalloproteinase, pullulanase, and serine proteinase.

EFSA has accorded *B. subtilis* Qualified Presumption of Safety (QPS) status since 2007 (EFSA, 2007). Data provided with the application (CCI information provided) confirmed the identity of the production strain as *B. subtilis*. Analysis of cytotoxicity was provided with the application, following the current guidelines for the assessment of toxigenic potential of *Bacillus* species (EFSA, 2020). The report shows the absence of toxigenic activity in Vero cells of the production strain. Data was also provided with the application that demonstrates that the *B. subtilis* production strain AR-651 was not detectable in the final enzyme preparation to be used as a food processing aid.

The manufacturing process involves appropriate controls to prevent microbial contamination. The microbial quality of the final enzyme preparation meets the specifications required by JECFA (2006) and no antibiotic or toxic compounds were detected.

To prepare the host organism for the expression of alpha-amylase, a series of genetic modification steps were undertaken to transform the recipient strain *B. subtilis* into the production strain *B. subtilis* AR-651 with the alpha-amylase gene derived from *Thermoactinomyces vulgaris*.

3.1.2 Gene donor organisms

Thermoactinomyces vulgaris

The application dossier includes reference to one donor species, namely *Thermoactinomyces vulgaris* 94-2A. *T. vulgaris* is a Biosafety level 1 organism, that are generally regarded as safe to humans and the environment.

3.2 Characterisation of the genetic modification(s)

3.2.1 Description of DNA to be introduced and method of transformation

The alpha-amylase enzyme is encoded by a gene derived from an isolate of *T. vulgaris.* Data provided by the applicant and analysed by FSANZ confirmed the identity of the alpha-amylase enzyme.

The alpha-amylase gene was cloned into a plasmid vector constructed using select sequence elements from pBC16-1 and pUB110 (Kreft et al., 1978; Gryczan et al., 1978), to create the pAA-A002 expression plasmid. Expression of the alpha-amylase gene is regulated by a *Bacillus* sp. promoter and transcription termination sequence from *T. vulgaris*.

Transformation of the pAA-A002 plasmid into a *B. subtilis* was achieved through protoplast fusion, based on the methods described in Chang & Cohen (1979). Transformants were separated using an antibiotic-independent mechanism, utilising the presence of a metabolic gene on pAA-A002 to select for recovery from the parental strain's auxotrophic phenotype.

In addition to the alpha-amylase gene, a native hydrolase derived from *Bacillus* sp. is co-expressed from pAA-A002, which aids in the manufacturing process post-fermentation. Data provided by the applicant and analysed by FSANZ confirmed the identity of this hydrolase.

3.2.2 Characterisation of the inserted DNA

The applicant supplied whole genome sequencing data demonstrating that the pAA-A002 plasmid has not been incorporated into the host chromosome and is maintained extra-chromosomally.

3.2.3 Stability of the production organism and inheritance of the introduced DNA

The applicant supplied data to demonstrate stability of pAA-A002 in the *B. subtilis* AR-651 production strain.

3.3 Safety of alpha-amylase

3.3.1 History of safe use

There are multiple alpha-amylase enzymes from microbial sources or from malted cereals that are currently permitted as processing aids in Schedule 18 of the Code. However, *T. vulgaris* alpha-amylase is not permitted and does not have a history of safe use in Australia or New Zealand. The applicant stated that the enzyme is approved for use in Denmark and France.

There are no known reports of adverse effects arising from the consumption of *T. vulgaris* alpha-amylase used as a processing aid internationally.

3.3.2 Bioinformatic assessment of enzyme toxicity

A BLAST-P search was performed by the applicant using the alpha-amylase protein sequence against all sequences in the NCBI Identical Protein Groups database[2] that

[2] NCBI IPG database: https://www.ncbi.nlm.nih.gov/ipg/

contained the search word ‘toxin’. Only two sequences were identified above a conservative E-value[3] threshold of 0.1, of which neither was a toxin sequence.

FSANZ conducted a BLAST-P search against the UniProt[4] database using the mature sequence of the expressed hydrolase. No similar sequences identified above a conservative E-value threshold of 0.1 contained the keyword ‘toxin’.

Hence, the bioinformatics analysis indicated that neither the alpha-amylase enzyme nor the expressed hydrolase shows significant homology with known protein toxins.

3.3.3 Evaluation of toxicity studies

The *T. vulgaris* alpha-amylase test item used in the supplied toxicity studies was produced using *B. subtilis* AR-475, a research and development strain in the same safe-strain lineage as *B. subtilis* AR-651. FSANZ compared the specifications supplied by the applicant and reviewed the genetic differences between both strains, and is satisfied that the test item used in the toxicity studies is suitably equivalent to the alpha-amylase produced by *B. subtilis* AR-651.

Animal studies

90-day repeated dose oral toxicity study in rats ([Redacted], 2018). Regulatory Status: GLP; conducted according to OECD Test Guideline (TG) 408.

The alpha-amylase test item was administered to Wistar crl: WI(Han) rats (10/sex /group) at doses of 0, 100, 300 and 1000 mg total organic solids (TOS)/kg body weight (bw)/day by oral gavage for 13 weeks. The vehicle control was water.

Animals were observed daily. Body weight, food consumption and detailed clinical examinations for signs of toxicity were recorded weekly. Ophthalmological examination was conducted on all test animals prior to treatment and at study termination. Functional performance and sensory reactivity tests were performed in week 13. Gross pathology, histopathological examination, haematology, clinical chemistry and measurement of organ weights was conducted on all animals at termination.

No treatment-related effects were observed on mortality, feed consumption, body weights, haematology, clinical chemistry, ophthalmology, or functional observations (functional performance or sensory reactivity). No treatment-related macroscopic abnormalities or histopathological findings were observed in any of the test animals at necropsy.

The No Observed Adverse Effect Level (NOAEL) was set at 1000 mg TOS/kg bw/day, which was the highest dose tested.

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[3] The E value (or Expect value) indicates the significance of a match found when searching a sequence database. The closer an E value gets to zero, the less likely an alignment could have been produced by chance.

[4] UniProt database: https://www.uniprot.org

Genotoxicity

Bacterial reverse mutation test ([Redacted], 2017). Regulatory Status: GLP; conducted according to OECD TG 471.

The potential mutagenicity of alpha-amylase was evaluated in *Salmonella enterica* ser. Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102, with and without metabolic activation using rat liver homogenate (S9). The alpha-amylase maximum dose and dose range evaluated was consistent with the OECD TG recommendations. Bacterial cultures were treated for 1 hour with the test item, before treatment cultures were plated using the plate incorporation method.

Positive controls in the absence metabolic activation were sodium azide (TA100 and TA1535), 4-nitro-o-phenylene (TA98 and TA1537) and methylmethane sulphonate (TA102). The positive control in the presence of metabolic activation was 2-aminoanthracene (all strains). Distilled water was used as the vehicle control.

No concentration-related increases in revertant colonies were observed in cultures treated with the test item, relative to vehicle controls, with or without metabolic activation. All positive control treatments showed the anticipated increases in mutagenic activity demonstrating the validity of the assay.

It was concluded that alpha-amylase test item was not mutagenic under the conditions of this test.

In vitro mammalian micronucleus test in human lymphocytes ([Redacted], 2018). Regulatory status: GLP; conducted according to OECD TG 487.

The potential of alpha-amylase to induce micronuclei formation in mammalian cells was tested using human lymphocytes isolated from peripheral blood, collected from a single healthy volunteer. Treatment with the alpha-amylase test item was either a 4-hour pulse exposure with or without S9, followed by a 42-hour recovery; or 44 hours of continuous exposure without S9. Positive control assays were conducted in parallel using methylmethane sulphonate as the clastogen positive control without S9, cyclophosphamide as the clastogen positive control with S9, and colchicine as the aneugen positive control. Cell culture medium was used as the vehicle control.

As a result of dose-selection experiments, the dose range for the 4-hour treatments were adjusted to 100 – 500 µg/mL TOS, and the dose range used for the 44-hour treatment was adjusted to 1 - 50 µg/mL TOS.

There were no increases in the incidence of micronucleated human lymphocytes following exposure to the test item, relative to the vehicle controls, under the conditions tested. The positive controls demonstrated a statistically significant increase in micronuclei formation, validating the sensitivity of the experimental methodology. It was concluded that the test alpha-amylase was not clastogenic or aneugenic in human lymphocytes, under the conditions of this study.

Cytotoxicity

In vitro cytotoxicity assessment. ([Redacted], 2021). Regulatory status: Non-guideline.

The cytotoxicity of bacterial supernatant collected from *B. subtilis* AR-651 was tested on Vero primate cells *in vitro*. The cytotoxic positive control was supernatant from *Bacillus cereus* DSM 31, and the negative control was supernatant from a non-cytotoxic *Bacillus licheniformis* strain.

Vero cells were exposed to bacterial supernatants for 3 hours. Cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) activity present in the cell culture medium.

Under these test conditions, neither the *B. subtilis* AR-651 supernatant or *B. licheniformis* negative control exhibited cytotoxic activity in Vero cells, while the *B. cereus* positive control did exhibit a substantial increase in cytotoxicity.

3.3.5 Potential for allergenicity

A FASTA search was performed using the amino acid sequence of alpha-amylase using the AllergenOnline[5] database (queried February 2021) using two sequence alignments: the full-length protein (more than 35% identity) and an 80 mer sliding window (more than 35% identity). Three allergen sequences were identified using these search parameters. None were food allergens.

An equivalent FASTA search for potential allergenicity was performed using the mature amino acid sequence of the hydrolase co-expressed by AR-651. No sequence matches to known allergens were identified using these parameters.

Respiratory sensitisation of occupationally exposed individuals to some food enzyme processing aids, such as alpha-amylase and other glycoside hydrolase enzymes has been reported (Baur & Posch, 1998). However, food enzyme processing aids that are respiratory allergens are not usually food allergens (Poulsen 2004, Bindslev-Jensen et al. 2006) and there are no reports of sensitisation to *T. vulgaris* alpha-amylase in the scientific literature.

The final product contains wheat products as part of the formulation. Soy-based ingredients are used in the manufacturing process, but do not pose an allergen risk to consumers.

3.3.6 Assessments by other regulatory agencies

The *T. vulgaris* alpha-amylase produced using GM *B. subtilis* is approved for use in Denmark and France. The written assessments undertaken by these countries were not provided to FSANZ.

No written assessments by other regulatory agencies were provided.

[5] AllergenOnline: http://www.allergenonline.org/

3.4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a 'worse-case scenario' approach to estimating likely levels of dietary exposure, assuming that all of the TOS from the alpha-amylase enzyme preparation remained in the food.

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass et al 1997). The calculation is based on physiological food and liquid requirements, the food additive concentration in foods and beverages, and the proportion of foods and beverages that may contain the food additive. The TMDI can then be compared to an ADI or a NOAEL to estimate a margin of exposure for risk characterisation purposes. Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020). The method is used by international regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids.

In their budget method calculation, the applicant made the following assumptions:

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- 50% of solid food is processed
- the maximum physiological requirement for liquid is 100 mL/kg body weight/day (the standard level used in a budget method calculation for non-milk beverages)
- 25% of non-milk beverages are processed
- 1 kg bakery products contain 0.71 kg flour
- all of the TOS from the enzyme preparation remains in the final food
- all solid foods contain the highest use level of 100 mg TOS/kg raw material (flour)
- there is no use of the enzyme preparation in non-milk beverages, therefore a zero concentration of TOS is assigned.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.8875 mg TOS/kg body weight/day.

As assumptions made by the applicant differ from those that FSANZ would have made in applying the budget method, FSANZ independently calculated the TMDI using the following assumptions that are conservative and reflective of a first tier in estimating dietary exposure:

- The maximum physiological requirement for solid food (including milk) is 50 g/kg body weight/day (the standard level used in a budget method calculation where there is potential for the enzyme to be in baby foods or general purpose foods that would be consumed by infants).
- FSANZ would generally assume 12.5% of solid foods contain the enzyme based on commonly used default proportions noted in the FAO/WHO Environmental Health Criteria (EHC) 240 Chapter 6 on dietary exposure assessment (FAO/WHO 2009). However, the applicant has assumed a higher proportion of 50% based on the nature and extent of use of the enzyme and therefore FSANZ has also used this proportion for solid foods as a worst case scenario.

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI of the TOS from the enzyme preparation based on FSANZ's calculations from solid food only is 1.78 mg TOS/kg body weight/day.

Both the FSANZ and applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes that it was assumed that all of the TOS from the enzyme preparation remains in the final foods whereas the applicant has stated that the enzyme would be inactivated and perform no function in the final food to which the ingredient is added.

4 Discussion

No public health and safety concerns were identified in the assessment of alpha-amylase produced by this GM *B. subtilis* strain AR-651 under the proposed use conditions. The host organism *B. subtilis* from which *B. subtilis* strain AR-651 was derived, is neither pathogenic nor toxigenic and has a long history of safe use. Analysis of the modified production strain confirmed the presence and stability of the inserted DNA. The *B. subtilis* strain AR-651 produces low levels of a *Bacillus* sp. hydrolase. Bioinformatics analysis indicated that neither the produced alpha-amylase nor hydrolase shows significant homology with any known toxins or food allergens.

Toxicity testing of the enzyme showed no evidence of genotoxicity *in vitro*, and the NOAEL in a 90-day oral gavage study in rats was the highest dose tested, 1000 mg TOS/kg bw/day. The TMDI based on FSANZ's calculations from solid food is 1.78 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of around 600.

5 Conclusion

Based on the reviewed data it is concluded that in the absence of any identifiable hazard an Acceptable Daily Intake (ADI) 'not specified' is appropriate.

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