FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting document 1

Risk and technical assessment report (approval)

Application A1061

Amylomaltase as a Processing Aid (Enzyme)

Executive Summary

Application A1061 seeks approval for the use of amylomaltase, derived from a genetically modified (GM) strain of *Bacillus amyloliquefaciens*, as a food processing aid. Amylomaltase is proposed to be used to produce modified potato starch. The Applicant claims that the modified potato starch can be used as a replacement for fat and casein and other fat and casein substitutes in foods such as yoghurt, yoghurt drinks, ice cream and low-fat spreads.

This risk assessment has considered the technological suitability of amylomaltase as a food processing aid and the potential hazards of the production microorganism and amylomaltase protein.

Based on the information supplied by the Applicant, including publicly available scientific literature, FSANZ concludes that amylomaltase fulfils its intended technological function. It is effective as a processing aid in producing modified potato starch at the level of proposed use. The amylomaltase preparation meets international specifications for enzyme preparations used in the production of food.

No food safety concerns were identified by FSANZ with the use of amylomaltase as a food processing aid on the basis of the following considerations:

- *B. amyloliquefaciens* has a history of safe use in the production of enzyme processing aids.
- The source microorganism, including any residues, is removed from the final enzyme preparation, so it is not likely to be present in the final food.
- Any residual enzyme that may be present in the final food would be at very low levels, inactive and as susceptible to digestion as the vast majority of dietary proteins.
- Bioinformatic analysis indicated that amylomaltase has no biologically relevant homology to known protein allergens or toxins.
- There was no evidence of toxicity of the enzyme preparation at the highest doses tested in 14- and 90-day toxicity studies in rats. The No-Observed-Adverse-Effect-Level (NOAEL) in both studies was 1000 mg total organic solids (TOS)/kg bw per day, the highest dose tested.
- The enzyme preparation was not genotoxic *in vitro*.

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

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1. Introduction

On the 21st April 2011, FSANZ received an application from DSM Food Specialties (DSM) seeking approval for the enzyme, amylomaltase, to be used as a food processing aid. The enzyme is produced from a genetically modified (GM) strain of *Bacillus amyloliquefaciens* (strain MAS-3) expressing a modified form of the amylomaltase gene (*malQ*, designated *masQ*), from *Thermus thermophilus*. The enzyme encoded by the *masQ* gene is identical to the wild type enzyme produced by the donor organism.

The Applicant proposes to use amylomaltase to produce modified potato starch. The Applicant claims that the modified potato starch can be used as a replacement for fat and casein and other fat and casein substitutes in foods such as yoghurt, yoghurt drinks, ice cream and low-fat spreads.

1.1 Objectives of the Assessment

As there are no permissions for amylomaltase currently in the Code, any application to amend the Code to permit the use of this enzyme as a food processing aid requires a pre-market assessment.

The objectives of this risk assessment are to:

- determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function in the quantity and form proposed to be used as a food processing aid; and
- evaluate any potential public health and safety concerns that may arise from the use of amylomaltase as a processing aid

1.2 Risk Assessment Questions

The following risk assessment questions were developed to address the objectives of the assessment:

- Does the enzyme achieve its stated technological purpose?
- Does the enzyme preparation present any food safety concerns?

This report is structured to address these questions in order.

2. Food Technology Assessment

2.1 Characterisation of amylomaltase

2.1.1 Identity of the enzyme

The following information regarding the identity of the enzyme has been taken from the Application and verified from enzyme nomenclature references.

Systematic name: (1→4)-α-D-glucan:(1→4)-α-D-glucan 4- α –D glycosyltransferase

IUBMB Enzyme nomenclature: EC 2.4.1.25

C.A.S. number: 9032-09-1

Common name: Amylomaltase

Other names: Disproportionating enzyme; dextrin glycosyltransferase; D-enzyme; debranching enzyme maltodextrin glycosyltransferase; amylomaltase; dextrin transglycosylase

Marketing name: Meltamase™

Molecular weight: 57.2 KDa (deduced from the amino acid sequence)

2.1.2 Enzymatic properties

Amylomaltase catalyses the cleavage of α-1,4 linkages between glucose molecules in starch, and in a second step, catalyses the formation of another α -1,4 linkage (Tafazoli et al 2009). According to the Applicant, this results in the breakdown of amylose and changes in the length and distribution of the amylopectin side chains.

The Applicant notes that although the production microorganism produces amylomaltase in excess, the enzyme preparation will also contain minor, non-standardised enzymes needed for nutrient breakdown and synthesis of cell material of the production microorganism. The Applicant reports that these minor enzymes will not have an effect on the substrate and the resulting modified potato starch.

2.1.3 Chemical and physical properties

The enzyme preparation is a light yellow to brown liquid with a pH range of 6.5-7.5 and typically has an enzyme activity of 1000 +/-5% amylomaltase units (ATU)/g. One ATU is defined by the Applicant as the amount of enzyme which produces 1 µmol of glucose per minute under the assay conditions of their in-house test (pH 6.50 and 70°C). The enzyme preparation is formulated with glycerol, an approved food additive, to ensure the desired and standardised activity concentration is achieved.

2.2 Production of the enzyme

The amylomaltase enzyme preparation is produced by a controlled submerged fermentation of a selected, pure culture of *B. amyloliquefaciens*. The fermentation process is performed in

accordance with Good Food Manufacturing Practice. The nature and production of the source organism is discussed in later sections.

The production steps can be summarised as including a fermentation process, recovery steps to extract the enzyme from the fermentation broth, purification steps and formulation of the final commercial enzyme preparation.

An initial inoculum fermentation is employed to produce enough of the microorganism for the main production fermentation. After the main fermentation, the production strain (source microorganism) is killed off using sodium diacetate. It is important for the Applicant, for proprietary commercial reasons, that the final commercial enzyme preparation does not contain any viable production organisms.

The enzyme is released by mechanically disrupting the cells in a homogeniser. Ultrafiltration and sterile filtration are performed during the final clean up and purification steps to remove any residual organisms and to concentrate the enzyme. The enzyme is then stabilised and standardised using glycerol.

Thus, the final enzyme preparation should not contain the source organism or its residues.

2.3 Specifications

There are international specifications for enzyme preparations used in the production of food which have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) (monograph 3, 2006) and the Food Chemicals Codex (7th edition, 2008). The Applicant considers that the specifications for amylomaltase meet these specifications based on the results in Table 2.1. Both of these specifications are primary reference sources for specifications listed in clause 2 of Standard 1.3.4 – Identity and Purity, of the Code.

Table 2.1: Specifications for three representative samples of commercial amylomaltase preparations compared to JECFA specifications for enzymes

Analysis	Sample 1	Sample 2	Sample 3	JECFA spec
Lead (mg/kg)	< 0.2	<0.5	<0.5	≤ 5
Arsenic (mg/kg)	<0.02	ND	-	-
Mercury (mg/kg)	<0.02	ND	-	-
Cadmium (mg/kg)	<0.01	0.02	<0.02	-
Standard plate count (cfu/ml)	<10	<1	<1	-
Coliforms (cfu/ml)	<10	-	<10	≤30
Salmonella (absent in 25 ml)	Absent	-	Absent	Absent
E. coli (absent in 25 ml)	<10[1]	-	Absent	Absent

The Application states that the amylomaltase preparation contains no antimicrobial activity, as also required by the JECFA specifications for enzymes used in food processing. The Applicant confirmed that there are no mycotoxins found in the enzyme preparations.

The final enzyme preparation meets international specifications for enzyme preparations used in the production of food.

The enzyme preparation does not contain any allergenic substances that would require mandatory labelling declarations.

[1] This sample was taken before the JECFA specification was published in 2006. Sample 3 which was taken in 2006 was compliant with the JECFA specification for E. coli. In any case, the enzyme preparation will be required to comply with JECFA specifications regardless of the results obtained during earlier periods OR during the development

2.4 Methods of analysis

A method of analysis for the presence of the enzyme or source organism in food containing modified potato starch is unnecessary because:

- The production microorganism is killed off at the end of the fermentation stage by the addition of sodium diacetate so that the commercial enzyme preparation does not contain viable *B. amyloliquefaciens*.
- During the production of modified potato starch, the reaction mixture is processed by a jet-cooker at 120^0C to inactivate the enzyme once the desired viscosity has been reached.

However, the Applicant has an in-house method of analysis for determining the activity of the enzyme. More details about the enzyme activity assay are provided in the Application.

2.5 Technological function of the enzyme

The technological function proposed by the Applicant is to use amylomaltase for the production of modified potato starch for use as a food ingredient. The Applicant states that modified potato starch has excellent thermoreversible gelling properties which enable it to mimic fat. The Applicant states that at ambient temperature, the modified potato starch is a gel, and at higher temperatures it behaves more like a liquid. The Applicant therefore argues that modified potato starch can be used as a replacement for fat and casein and other fat and casein substitutes in foods such as yoghurts, curds, mousses, ice creams, cheese analogues and low fat spreads.

Native starch is a combination of amylose and amylopectin, which are polymers of glucose molecules joined via α-1,4 and/or α -1,6 glycosidic bonds. Amylose has mainly α-1,4 glycosidic bonds which results in linear molecules of about 1000-6000 glucose units. Amylopectin has additional α-1,6 bonds which results in branching of the molecules. The branches consist of about 10-60 glucose units.

Amylomaltase breaks down α-1,4 linkages and, in a second step, catalyses the formation of another α-1,4 linkage (Tafazoli et al 2009). The Applicant states that when amylomaltase acts on native starch, amylose will be broken down and the length of the branches of the amylopectin will effectively increase. The Applicant concludes that the modified potato starch is a special kind of potato starch which differs from native potato starch only in chain length distribution and not in the primary structure.

To produce this modified potato starch, amylomaltase is added to a suspension of potato starch. After the desired viscosity of the starch solution has been achieved, the solution is processed by a jet-cooker at 120°C to inactivate the enzyme. The starch solution is then spray-dried. Therefore no active enzyme will be present in the final food.

It is noted that Standard 1.3.1 of the Code allows the use of enzyme-treated starches (INS 1405) in food (see Schedule 2 - Miscellaneous additives permitted in accordance with GMP in processed foods specified in Schedule 1).

2.6 Conclusion

Based on the information supplied by the Applicant including literature in the public domain, amylomaltase fulfils its intended technological function. It is effective as a processing aid in the production of modified potato starch at the rate of proposed use. The enzyme preparation meets international specifications for enzyme preparations used in the production of food.

3. Hazard Assessment

3.1 Background

3.1.1 Chemistry

Details of the chemistry of amylomaltase, including relevant physicochemical and enzymatic properties, and product specifications, are provided in the Food Technology Assessment (section 2).

3.1.2 Description of the genetic modification

Amylomaltase is produced by a GM strain of *B. amyloliquefaciens* (production strain MAS-3), which expresses the *masQ* gene. The *masQ* gene is based on the *malQ* gene from *Thermus thermophilus* HB8 (ATTC27634) and has been codon optimised for expression in Bacillus. The Applicant stated that the introduced gene encodes the same primary amino acid sequence as that encoded by wild-type *T. thermophilus*. The *masQ* gene was synthesised as two separate fragments covering the 5' and 3' ends of the gene which were then sequentially cloned into a plasmid vector, thus restoring the full coding sequence of the gene into one open reading frame (ORF). This plasmid vector was then introduced into the parental strain (EBA127) by electroporation. *B. amyloliquefaciens* strain MAS-3 containing the desired level of amylomaltase activity was selected from kanamycin or neomycin resistant clones. FSANZ notes that consumers will not be exposed to the kanamycin or neomycin resistant production organism – it is not present in the final enzyme preparation and therefore not present in the final food.

The Applicant has indicated that *B. amyloliquefaciens* strain MAS-3 stably overproduces amylomaltase for >60 generations.

3.1.3 Scope of the hazard assessment

The hazard of amylomaltase derived from *B. amyloliquefaciens* strain MAS-3 was evaluated by considering the:

- hazard of the production organism, including any history of safe use in food production processes;
- hazard of the encoded protein, including potential allergenicity; and
- toxicity studies on the enzyme preparation intended for commercial use.

3.2 Hazard of the production organism - *B. amyloliquefaciens strain MAS-3*

The parental lineage of the production organism is European *B. amyloliquefaciens* (EBA-1), which has been previously modified by a combination of UV mutagenesis and recombinant DNA technology to generate *B. amyloliquefaciens* strain EBA-127 (the parental strain), which is deficient in the genes for α-amylase and alkaline serine protease. This parental strain is neither pathogenic nor toxigenic. The production organism, *B. amyloliquefaciens strain MAS-3* differs from the parental strain only by the presence of additional amylomaltase genes.

FSANZ has previously assessed *B. amyloliquefaciens* as a safe production organism for a number of food-grade enzymes. Standard 1.3.3 of the Code permits the use of the following enzymes derived from *B. amyloliquefaciens* as food processing aids: α-acetalactate, α-amylase, β-amylase, β-glucanase, hemicellulose endo-1,4-xylanase, hemicellulose multicomponent enzyme, metalloproteinase, pullulanase and serine proteinase.

Additionally, the European Food Safety Authority (EFSA 2008) has granted *B. amyloliquefaciens* Qualified Presumption of Safety (QPS) status because of the absence of emetic food poisoning toxins, surfactant activity and enterotoxic activity.

Based on the nature of the genetic modification, which results in the overproduction of amylomaltase, it is highly unlikely that *B. amyloliquefaciens* strain MAS-3 would be less safe than parental *B. amyloliquefaciens* strain EBA-127. Further, *B. amyloliquefaciens* strain MAS-3 is asporogenic and is killed at the end of the fermentation process by the addition of sodium di-acetate (i.e. the sodium salt of acetic acid) and subsequent homogenisation of the cells to release amylomaltase; cell material is removed by filtration and activated carbon treatment.

3.3 Hazard of the encoded protein - amylomaltase

Amylomaltase is a 500 amino acid protein with a molecular weight of ~50 kDa. It is a protein that humans would not normally encounter in the diet because it occurs in a microorganism that lives in a niche environment (geothermal pools). On this basis there is no history of dietary exposure to this protein although homologues of the protein occur in many bacterial species[2].

The applicant stated that following the use of amylomaltase in the production of modified potato starch, the enzyme is inactivated via processing at 120ºC. This inactivated enzyme remains in the modified potato starch at a concentration of 3.8 amylomaltase units (ATU)/g (equivalent to 3.8 mg protein) and is further diluted when the modified potato starch is used to make the final food. Based on food consumption data from the Netherlands, Jansen et al (2008) estimated the potential mean intake of the modified potato starch ingredient to be 2.8 g/day. This would equate to a mean intake of residual amylomaltase of 10.6 mg/day (0.15 mg/kg bw/day for a 70 kg adult or 0.53 mg/kg bw/day for a 20 kg child). Relatively speaking, this level of intake of residual enzyme is very low compared to the total dietary intake of protein. It is likely that any residual enzyme would be present as denatured protein and undergo normal proteolytic digestion in the gastrointestinal tract. To confirm the digestibility of amylomaltase, potential cleavage sites were investigated by FSANZ using the amino acid sequence of amylomaltase and the PeptideCutter tool in the ExPASy Proteomics Site[3]. Amylomaltase has multiple cleavage sites for pepsin (174 sites at pH 1.3 and 120 sites at pH >2), trypsin (54 sites), chymotrypsin (64 high-specificity sites, 129 low-specificity sites) and endopeptidases (90 sites). On this basis, amylomaltase is considered likely to be as susceptible to digestion as the vast majority of dietary proteins.

Bioinformatic analyses were undertaken by FSANZ on the degree of homology between the amino acid sequence of amylomaltase and other proteins. A Basic Local Alignment Search Tool (BLAST) search was conducted on the amylomaltase amino acid sequence, which found significant homology ($p<0.01$) with 250 protein sequences[1]. These homologies were shared entirely with 4-α-glucanotransferases from other species of bacteria, as would be expected.

The FASTA algorithm was used to determine the degree of sequence alignment between amylomaltase and known allergens contained in the Structural Database of Allergic Proteins (SDAP)[4] and Allermatch[5] database.

[2] http://www.uniprot.org/blast/uniprot/2011090860NI5HR0B6
[3] http://expasy.org/tools/peptidecutter/
[4] http://fermi.utmb.edu/SDAP/
[5] http://www.allermatch.org/

The FASTA alignment threshold for potential allergenicity was 35% homology over 80 amino acids, which is consistent with the criterion established by the Codex Alimentarius (2003). This threshold aims to detect potential conformational IgE-epitopes. No significant homology with any known allergens was determined. The potential allergenicity of amylomaltase was further evaluated using a sliding window search for the presence of immunologically-relevant sequences of six contiguous and identical amino acids (i.e. linear IgE epitopes and possible T-cell epitopes). More frequently, stretches of 8 amino acids are used in this analysis in order to preclude false positives. No potential epitopes were detected.

The conclusion from these bioinformatic analyses is that amylomaltase does not show biologically relevant homology to any known allergen and on this basis is unlikely to be allergenic.

3.4 Evaluation of unpublished toxicity studies

Unpublished toxicity studies on the preparation of amylomaltase were submitted by the Applicant and independently evaluated by FSANZ. These studies included 14- and 90-day toxicity studies in rats, and *in vitro* genotoxicity assays. The test material used in these studies was equivalent to that intended for commercial use (i.e. consistent with the product specifications). All studies were performed according to Good Laboratory Practice (GLP).

3.4.1 Short-term repeat-dose toxicity study

Sathish PM (2010a) Repeat dose (14-day) oral toxicity study by gavage with enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity in Wistar rats. Study No. G6596. Lab: Toxicology Department of Safety Assessment, Advinus Therapeutics Private Ltd, Bangalore, India. Sponsor: DSM Food Specialities, Delft, The Netherlands. **GLP**: OECD. **QA Statement**: Yes. **Test Guidelines**: OECD Test Guideline 407.

In a range-finding study, a preparation of *B. amyloliquefaciens*, containing amylomaltase activity (Batch No. MEG.GRZ.0905; 5.5% purity; sourced from DSM Nutritional Products, The Netherlands), was administered by gavage to groups of 6 Wistar rats/sex at doses of 0, 100, 300 or 1000 mg Total Organic Solids (TOS)/kg bw/d for 14 days (water vehicle). Rats were sourced from Advinus Therapeutics Private Ltd (Bangalore, India), and were 5-weeks old and weighed ~100 g (males) or ~92 g (females) at the commencement of dosing. Rats were housed under standard conditions, with food and water available *ad libitum*. Standard gross toxicological endpoints were recorded during the treatment period (deaths, clinical signs, bodyweight and food consumption). Blood was collected on day 15 for the analysis of standard haematology and clinical chemistry parameters. Following sacrifice, rats were necropsied, organ weights recorded and standard tissues prepared for histopathology. Stomachs from the control and high-dose groups were examined for histopathology.

There were no deaths or clinical signs. Bodyweight and food consumption was comparable across all groups. There was no treatment-related effect on any haematology or clinical chemistry parameters. There were no treatment-related macroscopic abnormalities, differences in organ weights (absolute and relative) or histopathological changes in the stomach. The No-Observed-Adverse-Effect-Level (NOAEL) was 1000 mg TOS/kg bw/d, the highest dose tested.

3.4.2 Subchronic toxicity study

Sathish PM (2010b) Repeat dose (90-day) oral toxicity study by gavage with enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity in Wistar rats. Study No. G6597. Lab: Toxicology Department of Safety Assessment, Advinus Therapeutics Private Ltd, Bangalore, India.

Sponsor: DSM Food Specialities, Delft, The Netherlands. **GLP**: OECD. **QA Statement**: Yes. **Test Guidelines**: OECD Test Guideline 408; Commission Directive 2001/59/EC

A preparation of *B. amyloliquefaciens*, containing amylomaltase activity (Batch No. MEG.GRZ.0905; 5.5% purity; sourced from DSM Nutritional Products, The Netherlands), was administered by gavage to groups of 6 Wistar rats/sex at doses of 0, 100, 300 or 1000 mg Total Organic Solids (TOS)/kg bw/d for 90 days (water vehicle). The dose selection was based on the results of the 14-day range-finding study (see above). Rats were sourced from Advinus Therapeutics Private Ltd (Bangalore, India), and were 5-6 weeks old and weighed ~130 g (males) or ~110 g (females) at the commencement of dosing. Rats were housed under standard conditions, with food and water available *ad libitum*. Standard gross toxicological endpoints were recorded during the treatment period (deaths, clinical signs, bodyweight and food consumption). Ophthalmoscopy was performed prior to treatment and on day 90. A functional observational battery (FOB) was performed on days 88-90. Blood was collected on day 91 for the analysis of standard haematology and clinical chemistry parameters. Urine was collected prior to sacrifice for analysis of standard urinary parameters. Following sacrifice, rats were necropsied, organ weights recorded and standard tissues prepared for histopathology. Rats from the control and high-dose groups were examined for histopathology.

There were no deaths, clinical signs or ophthalmic abnormalities. The FOB was unremarkable. Bodyweight gain and food consumption were comparable across all groups. There was no treatment-related effect on any haematology, clinical chemistry or urinary parameter. There were no treatment-related macroscopic abnormalities, differences in organ weights (absolute and relative) or histopathological findings. The NOAEL was 1000 mg TOS/kg bw/d, the highest dose tested.

3.4.3 Genotoxicity

The results of two unpublished *in vitro* studies are summarised in Table 3.1. Positive and negative (vehicle) controls were tested in each study and gave expected results. The enzyme preparation was not mutagenic or clastogenic in these assays.

Table 3.1: Summary of genotoxicity studies

Test	Test system	Test article	Concentration or dose range	Result	Reference
Bacterial reverse mutation (Ames test)[1]	*Salmonella typhimurium* strains TA 98, 100, 1535 & 1537 *Escherichia coli* strain WP2 *uvrA*	Enzyme preparation of *B. amyloliquefaciens* containing amylomaltase activity (Batch No. MEG.GRZ.0905; 5.5% purity) Water vehicle	62-5000 µg/plate	Negative (±S9)[2]	van den Wijngaard (2010) [GLP; QA]
Chromosomal aberration test[3]	Human lymphocytes	As above	Test 1: 1250, 2500 & 5000 µg/mL Test 2: 1000, 3000 & 5000 µg/mL	Negative (±S9)[4]	de Vogel (2008) [GLP; QA]

S9 = 9000 × *g* supernatant from rat liver; GLP = statement of compliance with principles of GLP; QA = quality assurance statement;
1 = Statement of compliance with OECD Test Guideline 471
2 = Cytotoxicity at ≥1667 µg/plate (-S9) and at 5000 µg/plate (+S9)
3 = Statement of compliance with OECD Test Guideline 473
4 = Cytotoxicity +S9: all concentrations in Test 1, at 3000 and 5000 µg/mL in Test 2

3.5 Conclusions

There are no public health and safety issues associated with the use of amylomaltase produced by a GM strain of *B. amyloliquefaciens* as a food processing aid on the basis of the following considerations:

- The production organism is not toxigenic, pathogenic or sporogenic and is not likely to be present in the final enzyme preparation proposed to be used as a food processing aid. Further, *B. amyloliquefaciens* has a history of safe use as the production organism for a number of enzyme processing aids that are already permitted in the Code.

- Very low levels of residual enzyme are expected to be present in the final food but would be inactive and as susceptible to digestion as the vast majority of dietary proteins.

- Bioinformatic analysis indicated that amylomaltase derived from *T. thermophilus* has no biologically relevant homology to known protein allergens or toxins.

- The enzyme preparation caused no observable effects at the highest tested doses in 14- and 90-day toxicity studies in rats. The No-Observed-Adverse-Effect-Level (NOAEL) in both studies was 1000 mg total organic solids (TOS)/kg bw per day, the highest dose tested.

- The enzyme preparation was not genotoxic *in vitro*.

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

4. Dietary Exposure

Processing aids perform their technological function during the manufacture of food. They are used at levels sufficient to achieve the purpose. Information contained in this application on the use of amylomaltase and subsequent food processing steps, indicated that very small amounts may be present in the final food. Any traces of residual inactive enzyme would undergo normal proteolytic digestion in the gastrointestinal tract.

Given the absence of a health-based guidance value (an ADI) for amylomaltase derived from *B. amyloliquefaciens*, an estimate of the dietary exposure was considered unnecessary.

5. Conclusion

This risk assessment considered the technological suitability, potential hazard of the production microorganism and the potential hazard of amylomaltase.

It was concluded that the proposed use of the enzyme was technologically justified in the form and prescribed amounts, and was demonstrated to be effective. The evidence presented was sufficient to determine that no safety concerns with production microorganisms or the enzyme exist. Thus amylomaltase derived from *B. amyloliquefaciens* is unlikely to pose any health risk when used as a food processing aid.

6. References

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