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

21 June 2018
[50-18]

Supporting document 1

Risk and technical assessment report – Application A1151

ß-Galactosidase from *Papiliotrema terrestris* as a Processing Aid (Enzyme)

Executive summary

The purpose of this Application is to seek amendment of Schedule 18 – Processing Aids, of the *Australia New Zealand Food Standards Code* (the Code), to include the food enzyme β-galactosidase (EC 3.2.1.23) sourced from *Papiliotrema terrestris* strain AE-BLC as a processing aid in the production of galacto-oligosaccharide (GOS) from lactose. The β-galactosidase sourced from this microorganism has enhanced acid resistance and temperature resistance, when compared with β-galactosidases derived from other sources.

FSANZ has assessed the technical information and safety evidence of β-galactosidase provided with the Application and other technical information including scientific literature, and determined that the data provided are adequate for this assessment.

The stated purpose of this enzyme, namely for use as a processing aid in the production of GOS from lactose, is clearly articulated in the Application. The evidence presented to support the proposed uses provides adequate assurance that the enzyme, in the form and prescribed amounts, is technologically justified and has been demonstrated to be effective in achieving its stated purpose as a processing aid in the production of GOS from lactose. Inactivation of the enzyme can be accomplished either by temperature or pH changes and the enzyme does not perform any technological function in the final food. It is therefore appropriately categorised as a processing aid and not a food additive. The enzyme meets international purity specifications

There are no public health and safety issues associated with the use of β-galactosidase from *P. terrestris* strain AE-BLC as a food processing aid in the production of GOS from lactose. *P. terrestris* strain AE-BLC was not pathogenic *in vivo* and not toxigenic *in vitro*. β-Galactosidase from *P. terrestris* was not genotoxic and did not cause adverse effects in a subchronic toxicity study in rats. In the absence of any identifiable hazard an Acceptable Daily Intake 'not specified' is appropriate. A dietary exposure assessment was therefore not required.

The enzyme does not have the characteristics of a potential food allergen and ingestion of any residual β-galactosidase in food products is unlikely to pose an allergenicity concern.

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1 Introduction

1.1 Objectives of the assessment

Currently, there are no permissions for the enzyme β-galactosidase sourced from *Papiliotrema terrestris* strain AE-BLC in the Code. Therefore, 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 were 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; i.e specifically for the enzyme that is produced from strain AE-BLC, and its role in GOS production

- evaluate any potential public health and safety concerns that may arise from the use of the β-galactosidase sourced from *P. terrestris* strain AE-BLC as a processing aid.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity of the enzyme

Information regarding the identity of the enzyme that was taken from the Application has been verified using an appropriate enzyme nomenclature reference (IUBMB 2017). Additional information has also been included from this reference.

Generic common name:	β-Galactosidase
Accepted IUBMB[1] name:	β-Galactosidase
Systematic name:	β-D-galactoside galactohydrolase
IUBMB enzyme nomenclature:	EC 3.2.1.23
CAS[2] number:	9031-11-2
EINECS[3] number:	232-864-1
Other names:	lactase; β-lactosidase; maxilact; hydrolact; β-D-lactosidase; S 2107; lactozym; trilactase; β-D-galactanase; oryzatym; sumiklat
Reaction:	Hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides

[1] International Union of Biochemistry and Molecular Biology
[2] Chemical Abstracts Service
[3] European INventory of Existing Commercial chemical Substances

Transgalactosylation of galactose where the glycosyl group of one or more D-galactosyl units is transferred onto the D-galactose moiety of lactose

The source microorganism is *P. terrestris,* production strain AE-BLC, which has been sourced from the parent microorganism through a conventional chemical mutation process. Strain AE-BLC is not genetically modified and has been selected as the production strain because the β-galactosidase derived from this strain has certain enhanced functional properties, compared with β-galactosidases derived from other sources.

2.1.2 Technological purpose

β-Galactosidase sourced from *P. terrestris* strain AE-BLC is intended to be used in the commercial production of galacto-oligosaccharide (GOS) from lactose.

Once prepared, the pure β-galactosidase enzyme powder is blended with lactose (the main substrate for GOS synthesis) to form an enzyme preparation; there is a range of different grade products available. The Applicant states that, typically, the β-galactosidase is added to lactose at levels of up to 0.03%. However, it is the β-galactosidase enzyme powder itself (and not the blended enzyme preparations) that is being assessed under this Application.

The β-galactosidase enzyme catalyses the hydrolysis of β-1,4 glycosidic bonds in β-galactosides and releases galactose and a residual organic molecule, such as glucose. When the substrate is lactose, it releases galactose and glucose, as shown in Figure 1.

The enzyme is also responsible for the transgalactosylation of galactose. Where lactose is the primary substrate, the galactose released from lactose is transferred to another lactose to form GOS, as shown in Figure 2. This reaction is of primary commercial importance, with respect to this Application. The GOS mixture obtained is expected to contain lactose as well as GOS and monosaccharides.

Lactose hydrolysis and transgalactosylation are concomitant reactions resulting in monomeric products as well as newly formed β-glycosides (Otieno 2015).

Figure 1 *Representation of hydrolysis reaction catalysed by β-galactosidase.*

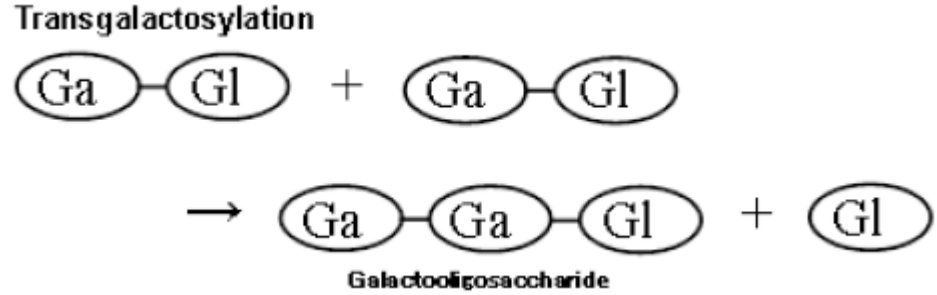

Figure 2 *Representation of transgalactosylation reaction catalysed by β-galactosidase.*

2.1.3 Technological justification

As described above, when lactose is the substrate, the enzyme β-galactosidase catalyses the hydrolysis of β-galactosides and formation of GOS.

The technological justification for using this particular β-galactosidase enzyme derived from *P. terrestris* strain AE-BLC, is that it has enhanced acid resistance and temperature resistance, compared with β-galactosidases derived from other sources, and these properties confer several distinct advantages in the production of GOS.

The transgalactosylation reaction occurs more effectively in higher concentrations of the substrate (in this case, lactose); higher concentrations can be achieved by dissolving the substrate at a higher temperature. The β-galactosidase enzyme that is the subject of this Application remains functional at higher temperatures and, thus, is more useful than other β-Galactosidases. Amano Enzyme Inc. states that based on the assays conducted, the enzyme exhibits activity from 40-75°C, with the optimum temperature being 70°C.

Furthermore, in the production of GOS in high pH environments, isomerisation and browning of the product is more liable to occur. The Applicant states that for the production of high quality products, it is necessary to use the enzyme at lower pHs. The β-galactosidase enzyme of this Application exhibits activity from pH 3.0. Thus, this particular β-galactosidase is again more useful than other β-Galactosidases. Assays indicated that the pH range for enzyme activity is pH 3.0-8.0, with the optimum being pH 5.0.

The enzyme may be inactivated by either changes in the processing temperatures or pH. Amano Enzyme Inc. recommends that to inactivate the enzyme, the processing temperature be increased to over 70°C. Manufacturers following these conditions of use will ensure that the enzyme is inactivated and, as such, will not interact with other constituents present in the final food.

2.2 Manufacturing process

2.2.1 Production of the enzyme

The enzyme powder is produced by the fermentation of the production strain *P. terrestris* strain AE-BLC. The production steps can be summarised as fermentation, filtration, concentration, and spray drying, to produce a concentrated enzyme powder.

Production begins with the fermentation of *P. terrestris* strain AE-BLC under standard culturing conditions, to produce the enzyme, which is secreted into the culture medium. Once the fermentation is complete, the fermentation broth undergoes a series of separation and concentration steps, in which the fermentation media and production strain is removed by filtration. In particular, micro-filtration with a 0.45µm filter ensures the removal of the production strain. The resultant solution is free of the production strain and other fermentation constituents. The concentrated liquid enzyme is then spray dried to produce an off-white to brown enzyme powder. This is then be blended with lactose to form the final enzyme preparation.

The main steps of the manufacturing process are shown in Figure 3.

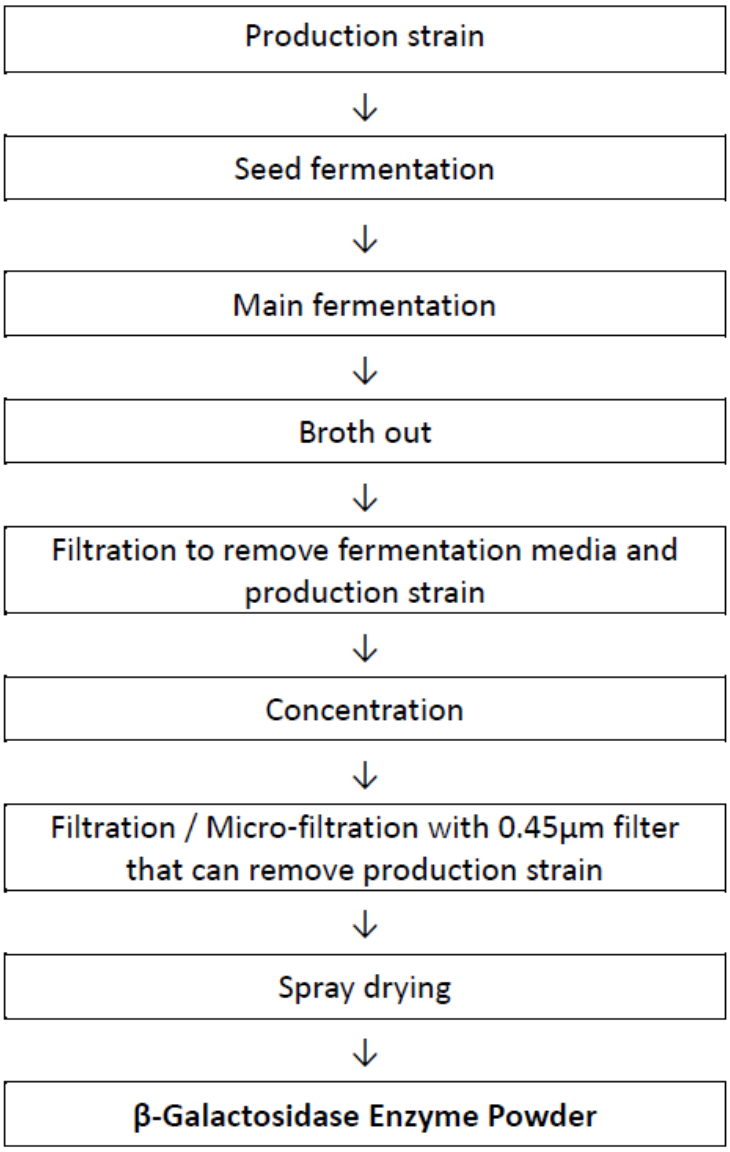

Figure 3 *Production process for β-galactosidase.*

The raw materials used in the production process are food grade and meet the requirements of the Code, where relevant. The enzyme powder is produced according to the FSSC22000 quality control system (International quality control system), and production is in accordance with Good Manufacturing Practices (GMP) and the international guidelines for the safe handling of microbial enzyme preparations published by the Association of Manufacturers of Fermentation Enzyme Products (AMFEP). Relevant documentation indicating conformity with FSSC22000 and GMP was provided with the Application.

2.2.2 Specifications

There are international specifications for enzymes used in the production of food. These have been detailed in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Compendium of Food Additive Specifications (FAO/WHO 2016) and the Food Chemicals Codex specifications for enzymes (Food Chemicals Codex 10 2016). These primary sources of specifications are listed in Schedule S3—2 of the Code. Enzymes need to meet these enzyme specifications. Schedule 3 also includes specifications for heavy metals (section S3—4) if they are not specified within specifications in sections S3—2 or S3—3.

Table 1 provides a comparison of batch analysis of β-galactosidase with the international specifications established by JECFA and Food Chemicals Codex, as well as those detailed in the Code (as applicable).

Table 1 *Batch analysis of β-galactosidase compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes*

Analysis	**Enzyme batch analysis (3 different batches)**	**Specifications**		
		JECFA	**Food Chemicals Codex**	**Australia New Zealand Food Standards Code (section S3—4)**
Lead (mg/kg)	0.031 0.015 0.036	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	0.45 0.25 0.65	-	-	≤1
Mercury (mg/kg)	<0.005 <0.005 <0.005	-	-	≤1
Cadmium (mg/kg)	0.019 0.010 0.007	-	-	≤1
Coliforms (cfu/g)	<10 <10 <10	≤30	≤30	-
Salmonella (in 25 g)	ND	Absent	Negative	-
E. coli (in 25 g)	ND	Absent	-	--
Antimicrobial activity	Negative	Absent	-	-
Mycotoxins	No significant amount of major mycotoxins	No toxicologically significant amounts	-	-

Based on the above results, the enzyme powder meets international and Code specifications for enzymes used in the production of food.

2.2.3 Stability

Results of stability analyses provided by the Applicant indicate that β-galactosidase activity after a storage period of six months remained at over 90% of that measured initially, for samples stored at both 25°C and 5°C.

2.3 Food technology conclusion

The stated purpose of this β-galactosidase enzyme powder, namely, for use as a processing aid in the production of GOS from lactose is clearly articulated in the Application. The Applicant states that this enzyme will be used in the manufacture of GOS through the hydrolysis of lactose and transgalactosylation of galactose. The β-galactosidase that is the subject of this Application is sourced from a chemically mutated strain of *P. terrestris* (strain

AE-BLC), and has certain enhanced functional properties, compared with β-galactosidases derived from other sources. The evidence presented to support the proposed use of the enzyme provides adequate assurance that the enzyme, in the form and prescribed amounts, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme meets international purity specifications.

3 Hazard assessment

3.1 Background

The aims of the current hazard assessment were to:

- review the available data on the toxicology of β-galactosidase from *P. terrestris* strain AE-BLC to determine its safety as a food processing aid
- if appropriate, establish a health-based guidance value.

3.2 Hazard of the production organism

The applicant has provided information that the parent strain of the production organism is *P. terrestris*. The parent strain was isolated from soil and was originally identified as *Cryptococcus terrestris*. In 2015 *Cryptococcus terrestris* was reclassified as *P. terrestris* (Liu et al 2015). The parent strain underwent several rounds of chemical mutagenesis using N-methyl-N'-nitro-N-nitrosoguanidine (NTG) to generate the intermediate M12-22-196 strain. This intermediate strain underwent several additional rounds of mutagenesis with NTG to derive the production strain AE-BLC. The production strain *P. terrestris* strain AE-BLC is not genetically modified.

Neither the parent strain nor the production strain has been deposited in a culture collection. The applicant provided information to establish the identity of the production organism AE-BLC. Sequence analysis of the D1/D2 region of the 26S rDNA subunit identified strain AE-BLC as *P. terrestris* (100% similarity to *P. terrestris* CDS10813, ATCC MYA-4954, NT07, C107DX4-Y11 and CJDX4-Y23).

Neither *P. terrestris* (nor *C. terrestris*) have been previously assessed by FSANZ.

The applicant submitted mycotoxin analysis of β-galactosidase produced from the production strain *P. terrestris* strain AE-BLC. The results showed that the β-galactosidase did not contain a significant amount of major mycotoxins, implying the production strain is non-toxigenic. The Applicant supplied a pathogenicity study that demonstrated that *P. terrestris* strain AE-BLC was not infectious or pathogenic. The Applicant did not identify any reports indicating concern with regard to the safety of *P. terrestris* strain AE-BLC as a source of enzyme used in food.

The Applicant has indicated that in order to avoid genetic drift a two-tiered cell banking system is used. This consists of a master cell bank and a working cell bank. When the working cell bank is prepared, the growth characteristics of the production strain are checked and enzyme activity is measured. During fermentation, the genetic stability of the production organism is monitored through changes in pH and growth rates. To confirm the production organism has not undergone strain drift and ensure the culture conditions can be applied consistently between batches, the enzyme activity and pH of the broth obtained after completing the fermentation are confirmed. If a deviation is detected in either of these parameters the fermentation media is removed from production and discarded. The strain is then checked to ensure that no genetic drift has occurred.

3.3 Hazard of the enzyme

3.3.1 Use of the enzyme as a food processing aid in other countries

β-Galactosidase from *P. terrestris* strain AE-BLC is a newly developed product and therefore has not yet been approved for use in other countries. An application has been lodged in Denmark, and the Applicant has indicated that it intends to lodge an application in France and apply for Generally Recognised as Safe (GRAS) status in the USA.

3.3.2 Evaluation of toxicity studies of the enzyme

The test item used in the toxicity studies submitted with this application was a β-galactosidase concentrate (Lot no. GFE68-001@K) with an activity of 2550 u/g. This is lower than the activity of the food enzyme β-Galactosidase, which is around 5800 u/g.

The β-galactosidase used in the toxicity studies was produced by the *P. terrestris* strain M12-22-196, which is obtained by chemical mutation of the parent strain. The production strain used to produce the food enzyme (*P. terrestris* strain AE-BLC) is obtained from M12-22-196 by several further rounds of mutation. The Applicant provided bioinformatics analysis indicating that the open reading frame gene sequence of β-galactosidase from M12-22-196 is identical to that of β-galactosidase from *P. terrestris* strain AE-BLC. This indicates that the β-galactosidase obtained from the current production strain is equivalent to that from M12-22-196.

Genotoxicity

Reports of two *in vitro* genotoxicity studies have been submitted by the Applicant: a bacterial reverse mutation test (Ames test) and a chromosomal aberration test in mammalian cells.

Bacterial reverse mutation assay – BoZo Research Center study report No. T-2095 (2016)

The study was conducted in compliance with OECD principles of Good Laboratory Practice (GLP) and in accordance with OECD Test Guideline (TG) 471, the Bacterial Reverse Mutation Test (adopted 21 July 1997). The test item was β-galactosidase concentrate produced by *P. terrestris* (referred to as *Cryptococcus terrestris* in the study report) and the vehicle was distilled water.

Tester strains used in the study were *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 plus *Escherichia coli* WP2 *uvrA*. The test was conducted by the pre-incubation method in the presence and absence of metabolic activation. Based on the results of a dose range-finding test, six concentrations ranging from 156 – 5000 µg β-galactosidase/plate were tested without metabolic activation, and five concentrations of 313 – 5000 µg/plate were tested with metabolic activation. Growth inhibition had been observed at 5000 µg/plate in the dose-finding test in the absence of metabolic activation, but not when metabolic activation was present. The main test was performed with triplicate plating, and conducted twice. Criteria for a positive response were a two-fold or more increase in the number of revertant colonies compared with the negative control, together with consideration of dose-response and reproducibility. Positive controls in the absence of metabolic activation were 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for strains TA98, TA100 and WP2 *uvrA*, sodium azide for strain TA1535 and ICR-191 for strain TA1537. In the presence of metabolic activation positive controls were benzo[a]pyrene (strains TA98, TA100 and TA1537) and 2-aminoanthracene (strains TA1535 and WP2 *uvrA*).

Precipitation of the test article was not observed. Growth inhibition was observed at ≥ 2500 µg/plate for all strains in the absence of metabolic activation. The test article did not induce a

two-fold increase in the number of revertant colonies in comparison with the negative control in any strain, irrespective of the presence/absence of metabolic activation. A dose-response was also not observed. All positive controls produced the expected mutagenic response, and negative and positive control values were within the range of the historical control values for the test facility, confirming the validity of the test system.

β-galactosidase from *P. terrestris* was not mutagenic under the conditions of this study.

Chromosomal aberration assay in mammalian cells – BoZo Research Center study report No. T-G212 (2016)

The study was conducted in compliance with OECD principles of GLP and with OECD TG 473, the *In Vitro* Chromosomal Aberration Test (2014 version). The test item was β-galactosidase concentrate produced by *P. terrestris* (referred to as *C. terrestris* in the study report).

The test material was examined for its potential to induce structural chromosome aberrations in Chinese hamster lung fibroblast (CHL/IU) cells in both the absence and presence of metabolic activation (S9 mix). The solvent and negative control was water for injection. The positive control substances were mitomycin C in the absence of S9 and cyclophosphamide in the presence of S9. Both short-term treatment and continuous exposure assays were conducted. Short-term treatment was conducted in the presence and absence of metabolic activation, while the continuous exposure assay was conducted only in the absence of metabolic activation. The exposure period in the short-term treatment assay was 6 hours, and cells were harvested 18 hours following removal of the test article. In the continuous exposure assay a 24 hour culture period was initially employed, although follow-up studies were conducted using a 48 hour culture time. Colcemid® was added to cultures approximately 2 hours before cells were harvested.

Based on the results of cell-growth inhibition tests, concentrations of 625, 1250, 2500 and 5000 µg/mL were used in the short-term treatment without S9, concentrations of 1250, 2500 and 5000 µg/mL in the short-term treatment with S9, and concentrations of 400, 800, 1200, 1600 and 2000 µg/mL in the 24 hour continuous treatment assay. The test was conducted in duplicate with 150 metaphases counted per plate, 300 metaphases per treatment group in total.

Criteria for a positive response were statistically significant increases in comparison with the negative control group that are outside the 95% probability distribution of historical negative control data.

The incidence of structural chromosomal aberrations excluding gaps and the incidence of polyploid cells did not show statistically significant increases in any test article treatment group as compared to the negative control. However, in the 24 hour continuous treatment group the incidence of cells forming a ski-pair (a pair of sister chromatids lined side by side) was noted to be significantly increased compared with controls. This finding was reported to be dose-dependent, although the incidence at each concentration is not specified in the study report. As this finding could suggest a delay in the cell cycle or inhibition of cell division, a further continuous exposure test was conducted using a 48 hour exposure period and concentrations of 14.8, 22.2, 33.3 and 50.0 µg/mL. These concentrations were selected on the basis of 48-hour cell-growth inhibition tests which showed a 50% reduction in relative population doubling compared with controls at a concentration of 38 µg/mL. In the follow up study there was no increase in the incidence of chromosomal aberrations excluding gaps or polyploid cells in the treated groups compared with the negative controls. A slight increase in the incidence of a ski-pair was observed, but it was not a significant increase as was seen in the 24 hour continuous exposure group. Furthermore, cell division was not considered to

have been inhibited as the population doubling count was greater than 1 in all groups.

Significant increases in the incidence of cells with structural chromosomal aberrations were observed in the positive controls compared with negative controls, demonstrating the validity of the test system.

β-Galactosidase from *P. terrestris* did not induce structural chromosomal aberrations under the conditions of this study.

Animal studies

13-week repeated-dose oral toxicity study in rats – BoZo Research Center study report No. TT-160003

The study was conducted in compliance with Japanese regulations for GLP, the Ordnance on Standard for Conduct of Non-Clinical Studies on Safety of Drugs, and following Japanese toxicity study guidelines. The test item in this study was the same as that used in the genotoxicity studies, β-galactosidase concentrate (Lot no. GFE68-001@K) with an activity of 2550 u/g. The total organic solid (TOS) content was 90%.

The study was conducted using Sprague-Dawley (Crl:CD[SD]) rats, 12/sex/group. Rats were acclimatised for 8 (males) or 9 (females) days prior to the first day of treatment, at which point they were approximately 6 weeks old. Animals were pair-housed in plastic solid floor cages with *ad libitum* access to food and water, and maintained under standard laboratory husbandry conditions.

The test item was administered by oral gavage once daily at dose levels of 0, 500, 1000 or 2000 mg/kg bw/day for 90 days. The vehicle and negative control article was water. Clinical signs, body weight and food consumption were monitored for the duration of the study. Ophthalmology was assessed before the start of the administration period and after dosing in Week 13 (Day 90 for males and Day 89 for females). Urine and blood were collected prior to terminal necropsy, where animals were killed by exsanguination. Organs were examined macroscopically and weighed, and a range of organs and tissues were collected for histopathological evaluation.

No deaths occurred over the course of the study and no clinical signs of toxicity were observed. No treatment related effects were observed on body weight, food consumption, ophthalmology, urinalysis, haematology, blood chemistry, gross necropsy, organ weight and histopathology. A statistically significant increase in urinary chloride was observed in males at 2000 mg/kg bw/day and a significant increase in urinary potassium was recorded in males at 500 and 2000 mg/kg bw/day, however these changes were minimal and not considered to be of toxicological significance. In clinical blood chemistry analyses, a significant decrease in chloride and increase in phosphate was recorded in males at 2000 mg/kg bw/day, however these changes were within the range of the historical control data and were not considered adverse. A statistically significant increase in relative liver weight was observed in females at 2000 mg/kg bw/day, however the increase was minimal and not accompanied by any histopathological or clinical chemistry changes and therefore was not considered to be of toxicological significance.

The no observed adverse effect level (NOAEL) for β-galactosidase from *P. terrestris* in this study was 2000 mg/kg bw/day, the highest dose tested. This corresponds to 1800 mg TOS/kg bw/day.

3.3.3 Bioinformatic analysis for potential allergenicity

The Applicant submitted the result of an *in silico* comparison of the amino acid sequence of β-galactosidase from *P. terrestris* strain AE-BLC with that of known allergens using the Structural Database of Allergenic Proteins.

Searches were conducted to investigate whether there are matches for:

- more than 35% identity in the amino acid sequence of the expressed protein, using a sliding window of 80 amino acids and a suitable gap penalty, or

- a match of eight contiguous amino acids.

These homology assessments are consistent with the recommendations of international organisations for screening of new food enzymes or newly expressed proteins in genetically modified plants for potential allergenicity (Codex 2003, 2009; WHO 2016).

No matches with known allergens were identified.

The database used by the applicant has not been updated since February 2013, and therefore FSANZ staff conducted a further *in silico* analysis using a more up to date database. The Food Allergy Research and Resource Program's Allergen Online Database, which was last updated in January 2017, was used. The same matching criteria as described above were used.

The searches did not identify any matches with known allergens.

It is concluded that β-galactosidase from *P. terrestris* strain AE-BLC does not have the characteristics of a potential food allergen and ingestion of any residual enzyme in food products is unlikely to pose an allergenicity concern.

Residual allergens from the culture medium

Lactose is used in the fermentation media, however the fermentation media are removed during the enzyme purification process. Residual milk allergen in β-galactosidase enzyme powder has been analysed and found to be less than 1 µg/g. The maximum concentration of β-galactosidase expected to be added to substrate (lactose) is 0.03%. Exposure to lactose or residual milk allergen from the enzyme powder in final food products is expected to be very low. However, the GOS mixture obtained from treating lactose with β-galactosidase is expected to contain lactose as well as GOS and monosaccharides such as glucose and galactose.

Milk allergy is related to immune responses to protein components present in milk, whereas lactose is a sugar (Rangel et al 2016). While concerns have been raised about the possibility of children with cow milk allergy reacting to lactose following incidents after inhalation of lactose-containing drugs, EFSA (2014) have noted that no single case of an adverse reaction to lactose ingestion has been reported among children with cow milk allergy. A prospective study on the allergenicity of whey-derived lactose did not document any allergic reactions, and some products intended for use by milk-allergic children may contain lactose (reviewed by EFSA 2014).

3.4 Hazard assessment conclusions

There are no public health and safety concerns associated with the use of β-galactosidase from *P. terrestris* strain AE-BLC as a food processing aid in the production of GOS from lactose, based on the following considerations:

- *P. terrestris* strain AE-BLC was not pathogenic *in vivo* and not toxigenic *in vitro.*

- β-Galactosidase from *P. terrestris* was not genotoxic *in vitro*.

- The NOAEL in a 13-week repeated dose oral toxicity study in rats was the highest dose tested and corresponds to 2000 mg/kg bw/day, or 1800 mg TOS/kg bw/day. This is more than 370-fold higher than the Applicant's estimate of an individual's theoretical maximal daily intake (4.75 mg TOS/kg bw/day) based on the proposed uses, as stated in the Application.

- β-Galactosidase from *P. terrestris* strain AE-BLC does not have the characteristics of a potential food allergen and ingestion of any residual β-galactosidase in food products is unlikely to pose an allergenicity concern.

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

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