Dextransucrase from *Bacillus subtilis*

An application to amend the Australia New Zealand Food Standards Code with a Dextransucrase preparation produced by a genetically modified strain of *Bacillus subtilis*

PROCESSING AID APPLICATION

Food Standards Australia New Zealand

Applicant: IFF AUSTRALIA PTY LTD (Trading as Danisco Australia Pty Ltd)

4th November 2025

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3.1 General requirements for applications

A. Executive Summary

An Executive Summary is provided as a separate copy with this application.

B. Applicant details

(a) Applicant:
This application is made by Danisco Australia (IFF)

(b) Company:
Danisco Australia Pty Ltd

(c) Address:
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Chatswood NSW 2067
AUSTRALIA

(d) Contact details:
Regulatory Affairs Manager Australia/NZ
Danisco NZ Ltd
Tel:
Email: iff.com

(e) Email address:
See above

(f) Nature of applicants business:
Danisco Australia Pty Ltd – A subsidiary of International Flavors and Fragrances Inc (IFF), manufacturer/marketer of specialty food ingredients, food additives and food processing aids. Danisco Australia is also an affiliate of Genencor International Ltd, the manufacturer of the product, and another subsidiary of International Flavors and Fragrances Inc (IFF).

(g) Details of other individuals.:
No other individuals, companies or organisations are associated with this application.

C. Purpose of the application

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a genetically modified strain of *Bacillus subtilis* as a permitted source of dextransucrase. The intended use of the processing aid is for the in-situ production of oligosaccharides in foods. The enzyme converts sucrose present in foods into polysaccharides providing sugar reduction and texture formation. The enzyme is designated as "Dextransucrase" throughout the dossier, Appendix A1 (**Confidential Commercial Information**) outlines a summary of internal names that could be referenced in reports throughout this dossier.

This application is made solely on behalf of IFF Health & Biosciences (IFF), the manufacturer/marketer of the *Processing Aid*. When approved, the *Processing Aid* would be available for use by any food manufacturer in Australia and New Zealand.

D. Justification for the application

Currently no Dextransucrase from *Streptococcus salivarius* expressed in *Bacillus subtilis* is permitted as a Processing Aid, however other enzymes including α-Acetolactate decarboxylase, α-Amylase, Asparaginase, Endo-1,4-beta-xylanase, β-Glucanase, Maltogenic α-amylase, Metalloproteinase, Pullulanase, and Serine proteinase from *B. subtilis* are listed in Schedule 18 section S18-4(5) as permitted enzymes. Approval of this application would provide food processors with a new enzyme preparation offering the benefits and advantages as discussed in Section 3.3.2, Part A.

No marketing or promotional activities have been undertaken for Dextransucrase derived from *B. subtilis* containing the gene for Dextransucrase from *S. salivarius* in the Australia/New Zealand market. Hence at this stage, no requests from food manufacturers are provided in support of this application. It is anticipated that support from the food processing industry will be submitted during the Public Notification period of this assessment.

D.1 Regulatory impact information

D.1.1 *Costs and benefits of the application*

The application and approval of the enzyme is not anticipated to impose regulatory restrictions or costs on industry or consumers. As outlined in Section 3.3.2, Part A, the approval of Dextransucrase from *B. subtilis* for the in-situ production of oligosaccharides in foods will deliver numerous production benefits to industry. In turn the government will only incur the burden of the required activities related to variation of Schedule 18.

More information on the benefits of this enzyme can be found in Section 3.3.2, Part A.

Enzyme preparations are widely used as processing aids in the manufacture of food products. Currently no Dextransucrase from *S. salivarius* expressed in *B. subtilis* is permitted as a Processing Aid. Approval of this application would provide food processors with a new enzyme preparation offering the benefits and advantages as discussed previously.

D.1.2 *Impact on international trade*

The inclusion of Dextransucrase from *S. salivarius* expressed in *B. subtilis* in the Australia New Zealand Food Standards Code to Schedule 18 may promote international trade on products produced with this enzyme product and reduce technical barriers to trade.

E. Information to Support the application

Support for the application

No public health and safety issues related to the proposed change are foreseen. In accordance with the Food Standards Australia New Zealand (FSANZ) Application Handbook (FSANZ, 2024), in the following Section 3.3.2 Parts A through F, detailed data has been provided to support the quality, efficacy and safety of the Dextransucrase under the proposed conditions of use. The data pertaining to Dextransucrase derived from *Bacillus subtilis* presented in this application is representative of the commercial product for which approval is being sought.

The information is provided in this application to enable the objectives specified in Section 18 of the FSANZ Act to be addressed as follows:

a. The protection of public health and safety: Information to support objective (a) is provided in Section C of the application, in which the safety of Dextransucrase derived from *B. subtilis*, based on the available safety data, is discussed in detail.

b. The provision of adequate information relating to food to enable consumers to make informed choices: Data to support objective (b) are provided in Section F, in which the impact and purpose of Dextransucrase are described in detail.

c. The prevention of misleading or deceptive conduct: Information supporting objective (c) is provided in Section F, in which the consumer awareness and potential behaviour in response to products manufactured using Dextransucrase are described. This objective can also be further supported by human safety data contained in Section C.

F. Assessment procedure

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a Processing aid that is currently not permitted. Based on guidance in the Application Handbook, IFF considers General Procedure Level 1 (up to 240 hours) to be the appropriate procedure for assessment of the application.

G. Confidential commercial information (CCI)

Certain (identified) technical and manufacturing information included as follows: -

Appendix A1-4,

Appendix B1-6

Appendix C1-4

Appendix D1-5,

Other information including amino acid sequences labelled with Confidential Commercial information is regarded by the applicant as "**Confidential Commercial Information**" and is provided in the application strictly on this basis. This information is the result of a significant research and development effort and investment by the applicant; it is not in the public domain and is considered as either proprietary or commercially sensitive. It would be disadvantageous to the applicant if this information were released into the public domain.

H. Other confidential information

Certain redactions throughout the dossier have also been made to avoid identification or disclosure of information on the proprietary strain, product or other details considered commercially sensitive. Where applicable, both redacted and non-redacted versions of all documentation have been supplied to FSANZ. The applicant requests that only redacted versions are provided for public consultation purposes.

I. Exclusive commercial capturable benefit (ECCB)

According to Section 8 of the FSANZ Act, this application is not expected to confer Exclusive Capturable Commercial Benefit (ECCB).

J. International and other national standards

J.1 International standards

Use of enzymes as processing aids in food is not restricted by any Codex Alimentarius Commission (Codex) Standards or any other known regulations. Additionally, Dextransucrase from *S. salivarius* produced by *B. subtilis* as not been reviewed by JECFA there is no specific Codex Standard relevant to this application.

J.1.1 Supporting evaluations

The Joint Expert Committee on Food Additives (JECFA) of FAO/WHO have evaluated enzymes from *B. subtilis* (α-amylase, mixed microbial carbohydrase and protease) and genetically modified *B. subtilis* (α-acetolactate decarboxylase, α-amylase (2), branching glycosyltransferase, maltogenic amylase, and xylanase (2)). For all these evaluations, an acceptable daily intake (ADI) was indicated as either "not specified" or "not limited".

J.2 Other national standards or regulations

Please find below the status of submissions to other countries for the Dextransucrase from *S. salivarius* expressed in *B. subtilis*, as of the date of this submission.

United States of America

The enzyme

Refer to Appendix C (**Confidential Commercial Information)** for further details.

Supporting USFDA approvals

Carbohydrase and protease enzyme preparations derived from *B. subtilis* were affirmed as GRAS by the U.S. FDA per 21 C.F.R. 184.1148 and 184.1150, respectively. More recently, 20 GRAS Notices (GRNs) for food ingredients manufactured using *B. subtilis* production strains received positive "FDA has no questions" letters (GRNs 1011, 974, 969, 956, 955, 905, 831,861, 751, 746, 714, 649, 592, 579, 476, 406, 274, 205, 114, and 20).

Europe

This Dextransucrase from genetically a modified strain *B. subtilis* has been approved by authorities (Appendix C, **Confidential Commercial Information**).

In Europe, most enzyme preparations used in food processing are classified as processing aids, meaning that they have their technological function in the food-processing stage and not in the final food. They are excluded from the Food Additives Framework Directive. On 16 December 2008 the European Parliament and the Council adopted Regulation 1332/2008 EC on food enzymes which aims to harmonise authorisation and safety assessment procedures of enzymes used in food processing in the EU (EC, 2008), (Annex 9). Several years yet will be needed for the new rules to become fully applicable across the EU. Until then, all national provisions on the use of food enzymes in individual EU Member States remain valid and applicable. Only France and Denmark have legislation covering all food-use enzymes. In Denmark and France, approval is needed prior to use. The information contained in the application dossier necessary for approval should follow the EFSA guidelines laid down in the Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes (EFSA, 2021). France has some additional national requirements specified in the Arrêté du 19 octobre 2006 relatif à l'emploi d'auxiliaires technologiques dans la fabrication de certaines denrées alimentaires as amended. In the other EU countries, enzyme preparation should be proved to be safe for use in food before being sold in EU according to the General EU Food Law. It is the producer's responsibility how to meet this requirement. IFF uses the USA GRAS system as the backbone for this.

Other countries

This Dextransucrase from genetically modified strain of *B. subtilis* has been approved in [redacted] (Appendix C, **Confidential Commercial Information**).

K. Statutory declaration

I,

of Regulatory Affairs Manager/Director

solemnly make the following declaration under the Oaths and Declaration Act 1959:

The information provided in this application fully sets out the matters required; and the information is true to the best of my knowledge and belief; and no information has been withheld which might prejudice this application to the best of my knowledge and belief.

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the Statutory Declarations Act 1959, and I believe that the statements in this declaration are true in every particular.

Signature ______________________

Declared at Auckland on 5 of November 2025

Before me, ______________________

____

Signature

Lawyer
Kemps Weir Lawyers
Auckland

L. Checklist

General Requirements		
Check	**Page No.**	**Mandatory Requirements**
		A. Form of application
☒		Application in English
☒		Executive Summary (separated from main application electronically)
☒		Relevant sections of Part 3 clearly identified
☒		Pages sequentially numbered
☒		Electronic copy (searchable)
☒	32	All references provided
☒	5	B. Applicant Details
☒	6	C. Purpose of the application
☒	6	D. Justification for the application
☒	6	Regulatory impact information
☒	6	Impact on international trade
☒	7	E. Information to support the application
☒		Data requirements
☒	7	F. Assessment procedure
☒		General
☐		Major
☐		Minor
☐		High level health claim variation
☒	7	G. Confidential commercial information
☒		CCI material separated from other application material
☒		Formal request including reasons
☒		Non-confidential summary provided
☒	8	H Other confidential information
		Confidential material separated from other application material
		Formal request including reasons
☒	8	I Exclusive Capturable Commercial Benefit
		Justification provided
☒		J International and other national standards
	8	International standards
	8	Other national standards
☒	10	K Statutory Declaration
☒		L Checklist/s provided with application
☒	11	3.1.1 Checklist
☒		All page number references from application included
☒		Any other relevant checklists for Chapters 3.2–3.7

Processing Aids		
Check	**Page No.**	**Mandatory Requirements**
		A. Technical information on the processing Aid
☒	13	A.1 Type of processing aid
☒	13	A.2 Identification information
☒	14	A.3 Chemical and physical properties
☒	18	A.4 Manufacturing process
☒	20	A.5 Specification information
☒	21	A.6 Analytical method for detection
N/A		B. Information related to the safety of a chemical processing aid
		C. Information related to the safety of an enzyme processing aid
☒	21	C.1 Information on enzyme use on other countries
☒	22	C.2 Toxicity information of enzyme
☒	24	C.3. Allergenicity information of enzyme
☒	25	C.4. Overseas safety Assessment Reports
		D. Additional information related to the safety of an enzyme processing aid derived from a microorganism
☒	26	D.1 Information on source organism
☒	28	D.2 Pathogenicity and toxicity of source microorganism
☒	29	D.3 Genetic stability of source
		E. Additional information related to the safety of an enzyme processing aid derived from a genetically-modified microorganism
☒	29	E.1 Nature of genetic modification of source organism
		F. Information related to the dietary exposure to the processing aid
☒	30	F.1 List of foods likely to contain the processing aid
☒	30	F.2 Anticipated residue levels in foods
☒	30	F.3 Information on likely level of consumption
☒	31	F.4 Percentage of food group to use processing aid
☒	31	F.5 Information on residues in foods in other countries (if available)
☒	31	F.6 Where consumption has changed, information on likely consumption

3.3.2 Processing Aids

A. Technical information on the processing aid

A.1. Information on the type of the processing aid

The Dextransucrase enzyme is a liquid enzyme of microbial origin produced by submerged fermentation of *Bacillus subtilis*, carrying the Dextransucrase gene from *Streptococcus salivarius*.

This Processing Aid belongs to the category "Enzymes of microbial origin" from the Food Standard Code, Standard 1.3.3 Processing Aids, Section 1.3.3-6 Enzymes.

The Dextransucrase enzyme preparation is to be used in the food industry as a processing aid during the processing of raw materials containing sucrose. Dextransucrase converts sugar to oligosaccharides (and polysaccharides).

The highest dosage of the Dextransucrase used during a food manufacturing process is 3 mg active enzyme protein per gram of sucrose in the food.

A.2. Information on the identity of the processing

A.2.1. Enzyme

The systematic name of the principal enzyme activity is 4-β-D-xylan xylanohydrolase.

- IUBMB number: 2.4.1.5
- CAS number: 9032-14-8
- Reaction catalysed: sucrose+ $[(1\rightarrow6)\text{-}\alpha\text{-D-glucosyl}]_n$ = D-fructose + $[(1\rightarrow6)\text{-}\alpha\text{-D-glucosyl}]_{n+1}$
- Other names used:

- Sucrose:(1→6)-α-glucan glucosyltransferase
- Glucosyltransferase, sucrose-1,6-α-glucan
- Sucrose 6-glucosyltransferase
- Glucosyltransferase C
- Gene gtfS glucosyltransferases
- Glucosyltransferase-S
- Oligo-isomaltosaccharide synthase
- Sucrose-1,6-α-glucan glucosyltransferase
- α-1,6-Glucan synthetase, Glucosyltransferase GTF-1
- Glucosyltransferase GTF-SI
- Streptococcal glucosyltransferase
- Sucrose α1→6-glucosyltransferase
- Sucrose: glucan α1→6-glucosyltransferase
- Streptococcal glucosyltransferase GTF-S
- Gene gtfD glucosyltransferase, Gene gtfG glucosyltransferase
- Gene gtfC glucosyltransferase
- Glucosyltransferase GtfU
- Glucosyltransferase C.

<u>Biological source</u>:
The Dextransucrase enzyme is an enzyme produced by submerged fermentation of *Bacillus subtilis*, carrying the Dextransucrase gene from *Streptococcus salivarius*. This Dextransucrase has been protein engineered.

Enzyme Preparation:
The enzyme will be formulated into a liquid a commercial preparation. The commercial preparation may contain only the Dextransucrase enzyme, or it could be formulated with other permitted enzyme concentrates depending on the required end application.

The typical composition of an enzyme concentrate is:

Glycerine	46-67 % w/w
Water	26-51 % w/w
Dextransucrase	1-5 % w/w
Calcium chloride	0-0.1 % w/w

Other enzymes:
Downstream processing concentrates and purifies the enzyme product. The resulting enzyme preparation will not be totally pure and traces of other enzyme activities (e.g. protease) might be found but their level will be very low

A.2.2. Marketing name of processing aid

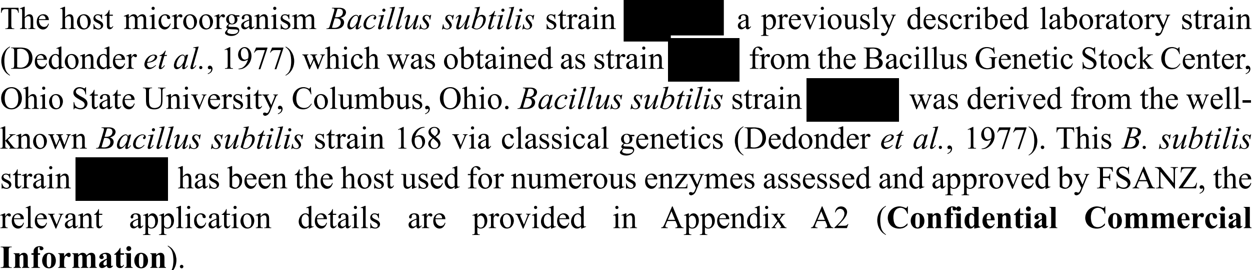
The marketing name of this enzyme preparation will depend on the application. An example marketing name of Dextransucrase is TEXSTAR™ EVO.

A.2.3. Host Organism

The host microorganism *Bacillus subtilis* strain a previously described laboratory strain (Dedonder *et al.*, 1977) which was obtained as strain from the Bacillus Genetic Stock Center, Ohio State University, Columbus, Ohio. *Bacillus subtilis* strain was derived from the well-known *Bacillus subtilis* strain 168 via classical genetics (Dedonder *et al.*, 1977). This *B. subtilis* strain has been the host used for numerous enzymes assessed and approved by FSANZ, the relevant application details are provided in Appendix A2 (**Confidential Commercial Information**).

A.2.4. Donor Organism

The donor strain used as a source for the Dextransucrase sequence is *Streptococcus salivarius*



A.3. Chemical and physical properties of the processing aid

Like any other enzyme, Dextransucrase acts as a biocatalyst: with the help of the enzyme, a certain substrate is converted into a certain reaction product. As the catalytic activity is very specific, it is not expected that the food enzyme will act in another way than described below. After the conversion has taken place, the enzyme no longer performs a technological function in the final food.

A.3.1. Use in food

The **substrate(s)** for Dextransucrase is sucrose.

The **function** of Dextransucrase is to catalyse the hydrolysis of the conversion of sucrose into oligosaccharides, polysaccharides and to a less extent maltose isomers (e.g. nigerose) and sucrose isomers (e.g. leucrose). Sucrose is a disaccharide composed of glucose and fructose subunits which is produce naturally by plants and is the main constituent of sugar. Consequently, the substrate for

Dextransucrase occurs naturally in foods. The food enzyme is to be used in sucrose containing foods for the production of oligosaccharides.

The **benefits** of Dextransucrase are:

- In-situ production of oligosaccharides.
- Improved texture.
- Reduction of sucrose content in food.

The process flow provided in Appendix A4 (**Confidential Commercial Information**) details just one example of a process where Dextransucrase can be applied.

Individual food processors ensure that the pH and temperature conditions are such that the Dextransucrase can perform its technological function during food processing.

A.3.2. Fate in food

The Dextransucrase performs its technological function during the food manufacturing process only. For the enzyme to perform a technological function in the final food, a number of conditions have to be fulfilled at the same time:

- the enzyme protein must be in its 'native' (non-denatured) form, and
- the substrate must still be present, and
- the enzyme must be free to move (able to reach the substrate), and
- conditions like pH, temperature and water content must be favourable.

In production of oligosaccharides (and polysaccharides), the enzyme is inactivated during the UHT pasteurisation process, and/or substrate depletion in food processes that do not include a UHT pasteurisation step. See section A.3.4 for evidence of this.

Thus, the enzyme does not have any enzymatic activity in the final food and does not exert any technological function in the final food.

A.3.3. Activity

The activity of Dextransucrase is defined in GTFU/g (Glucotransferase units). The Dextransucrase hydrolyses a sucrose solution to produce glucose and fructose. The reaction is then stopped with sodium hydroxide.

Dextransucrase preparations' enzyme activity will depend on the final product. An example product has a Dextransucrase activity of min. 85 GFTU/g. A detailed assay method is present in Appendix A3 (**Confidential Commercial Information**).

A.3.4. Temperature, pH and thermal stability

The activity of the food enzyme Dextransucrase from *Bacillus subtilis* was measured under various pH and temperature conditions. The optimal pH and temperature conditions for the activity of the food enzyme are:

- pH 6.5-8.0
- Temperature: 35-50°C

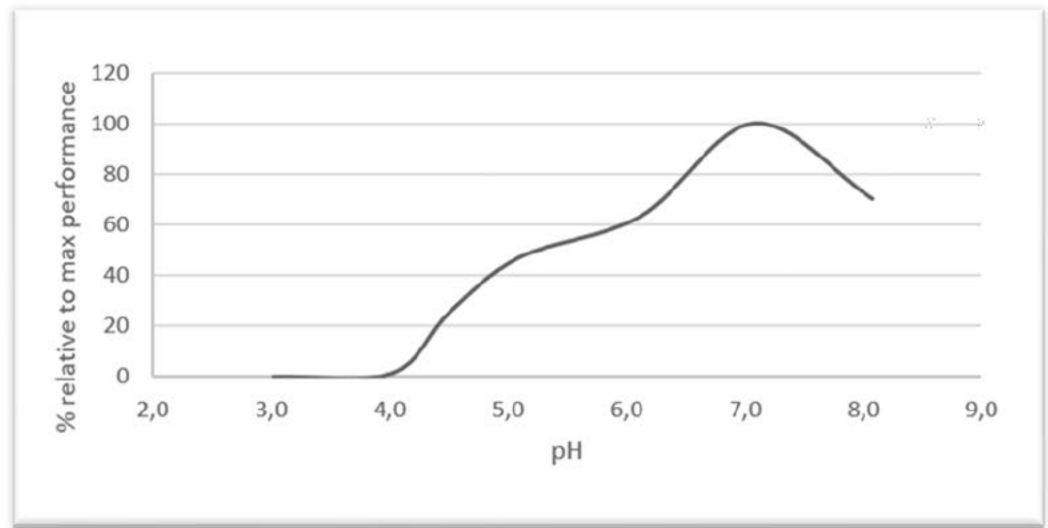


Figure 1. Glycosyltransferase activity across varied pH conditions

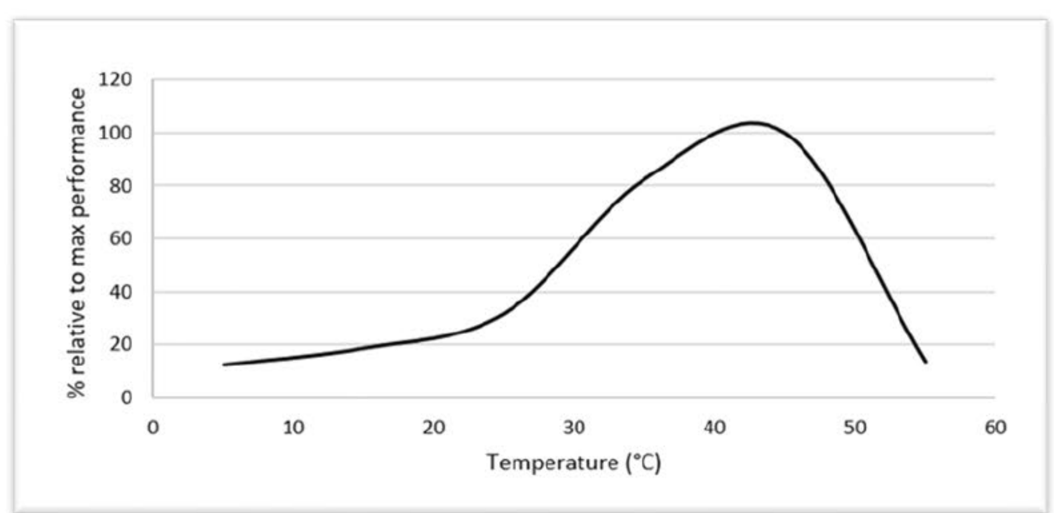


Figure 2. Glycosyltransferase activity across varied temperature conditions

As demonstrated in Figure 1.the Dextransucrase enzyme exhibits activity from pH 4, and from 5 °C. The optimum pH range is pH 6.5 - 8.0, whereas the optimum temperature range lies between 35 - 50°C (Figure 2).

A.3.5. Characterisation of the enzyme: UHT and substrate depletion

An example hereof was documented in a cocoa dairy beverage product (Figure 3). Dextransucrase from *Bacillus subtilis* was added to the product at 0.1% (S1) or 0.2 % (S2) dosage prior to UHT pasteurisation (143°C 4 sec.). Additional sucrose was then added to the pasteurised cocoa product, to ensure presence of available sucrose. Each sample was split in two and one of each was prepared for HPLC analysis directly and the other was incubated 3 hours at 37°C before preparation for HPLC to allow potential residual enzyme to convert sucrose.

The carbohydrate profile of the samples shows no change in sucrose or other carbohydrates between the sample that was incubated at 37°C and the one that was prepared for HPLC directly. Hence, it is documented that UHT pasteurisation can inactivate the added enzyme.

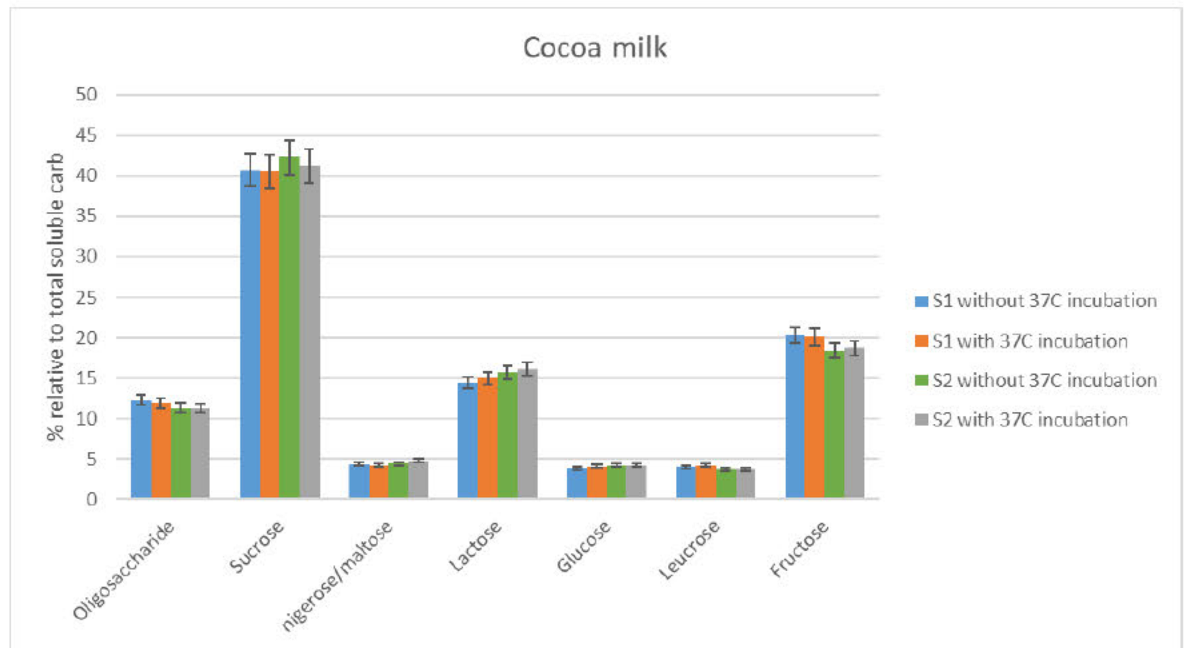


Figure 3. Polysaccharide production in chocolate milk product using Dextransucrase

In any case the enzyme activity is self-limiting and fully dependent on the availability of the substrate sucrose in the food matrix. Due to the nature of the polysaccharides produced by this enzyme, and the fact that impacts the texture of the product, substrate depletion or enzyme inactivation is mandatory to prevent changes in the product texture during storage.

This is shown below (Figure 4.) in a fresh fermented product in which Dextransucrase from *Bacillus subtilis* was used in co-fermentation at 6% sucrose and a dosage of 0.05%. It was clear that the sucrose was still being converted up until day 3 after which all the carbohydrates were stable over the rest of the shelf life. In this example the fresh fermented product should not be released until after day 3.

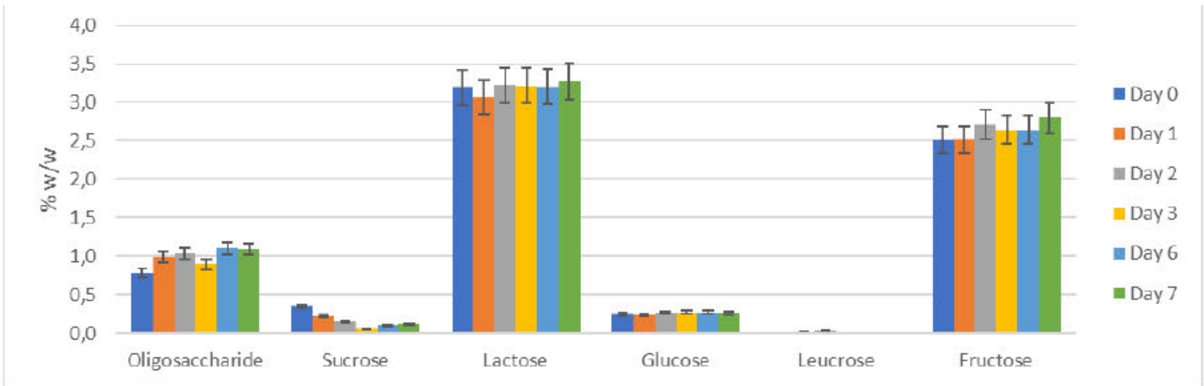


Figure 4. Polysaccharide production in fresh fermented dairy product using Dextransucrase

Alternatively, a higher dosage of the enzyme such as 0.07% for 6% sucrose could be used so that total possible sucrose conversion was achieved during the fermentation after which no change in carbohydrate profile transpired (Figure 5).

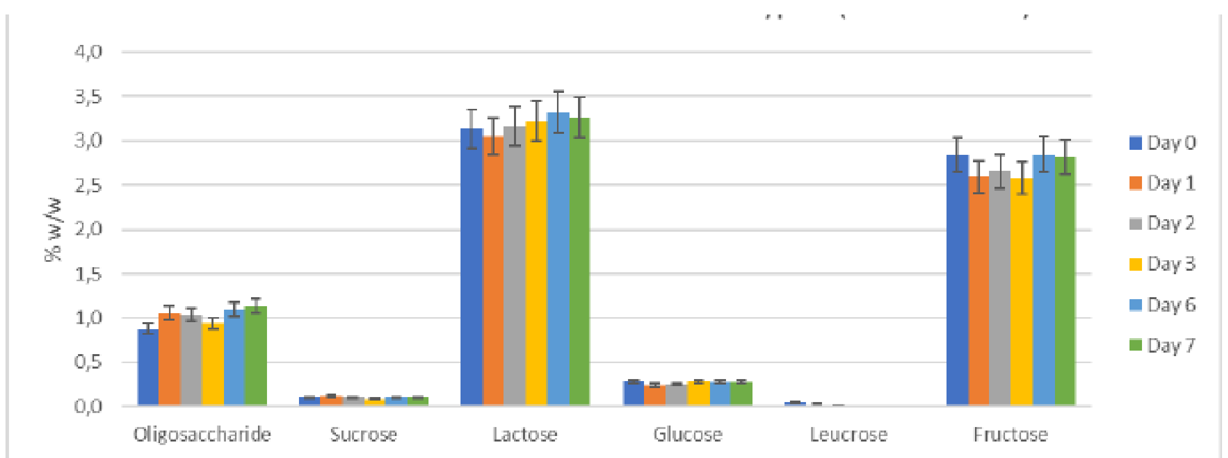


Figure 5. Polysaccharide production in fresh fermented dairy product using Dextransucrase

A.3.6. Storage

Food enzymes are not sold as such but formulated into various enzyme preparations in order to obtain standardised and stable products. The stability thus depends on the type of formulation, not on the food enzyme as such.

According to Standard 1.2.5 of Food Standards Code, the date of minimum durability or use-by-date is indicated on the label of the food enzyme preparation. If necessary, special conditions of storage and/or use will also be mentioned on the label.

Figure 5 below shows storage stability of an example commercial product of Dextransucrase. As seen in the figure, when refrigerated (4°C), the enzyme is stable for at least 12 months with close to 95% remaining activity.

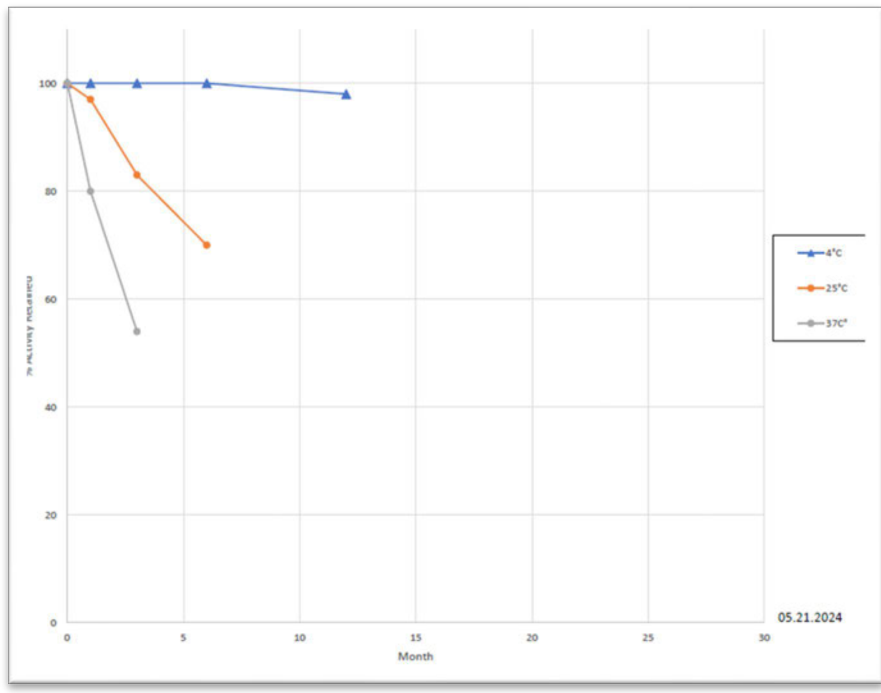


Figure 6: Storage stability of the enzyme product

A.4. Manufacturing process

The enzyme is produced by a submerged fermentation process using appropriate substrate and nutrients. The following sections describe the manufacturing process for the enzyme, which follows standard industry practice.

A manufacturing process flow is provided in Annex 1.

The production of Dextransucrase is monitored and controlled by analytical and quality assurance procedures that ensure that the finished preparation complies with the specifications and is of the appropriate quality for use as a processing aid in food processing applications.

A.4.1. Raw materials

Materials used in the fermentation process (inoculum, seed and main fermentation) are:

- Potable water
- A carbon source
- A nitrogen source
- Vitamins (typically part of one of the used complex fermentation materials)
- Salts and minerals
- An inducer (that encourages the microorganism to produce the intended enzyme protein)
- pH adjustment agents
- Foam control agents

A list of raw materials used for the production of the food enzyme is provided in Appendix D5 (**Confidential Commercial Information**).

A.4.2. Fermentation process

The production of food enzymes from microbial sources follows the process involving fermentation as described below. Fermentation is a well-known process that occurs in food and has been used for the production of food enzymes for decades. This section describes the different fermentation process steps.

A.4.2.1. Inoculum

A suspension of a pure culture of the production strain is aseptically transferred to an inoculum flask containing fermentation medium.

The culture is grown in the flask until a sufficient amount of biomass is obtained, which can subsequently be used as inoculum for the seed fermentation.

A.4.2.2. Seed fermentation

The inoculum is aseptically transferred to the seed fermenter containing fermentation medium. The seed fermentation is run under the specifically defined and controlled process conditions

The fermentation is run under controlled agitation and controlled air supply. When a sufficient amount of biomass has developed, the content of the seed fermenter is used for inoculation of the main fermentation.

A.4.2.3. Main fermentation

Biosynthesis of the enzyme protein by the production organism occurs during the main fermentation.

The fermentation in the main fermenter is operated as a batch or fed-batch fermentation. In both cases, the content of the seed fermenter is aseptically transferred to the main fermenter containing fermentation medium.

In the case of a fed-batch process, additional fermentation medium is added during the fermentation. In order to control the growth of the production organism and the enzyme production, the feed-rate of this medium is based upon a predetermined profile or on deviation from defined set points for pH or dissolved oxygen concentration.

During growth, the fermentation broth can be drawn from the bottom of the main fermenter to storage tanks if the production exceeds the capacity of the main fermenter. The main fermentation is run under the following defined process conditions.

The fermentation process is continued for a predetermined time or until laboratory test data show that the desired enzyme production has been obtained or that the rate of enzyme production has decreased below a predetermined production rate. When these conditions are met, and depending on the batch size, the fermentation is completed.

A.4.3. Recovery process

The purpose of the recovery process is:

- to separate the fermentation broth into biomass and fermentation medium containing the desired enzyme protein,
- to concentrate the desired enzyme protein and to improve the ratio enzyme activity/TOS

During fermentation, the enzyme protein is excreted by the producing microorganism into the fermentation medium. During recovery, the enzyme-containing fermentation medium is separated from the biomass.

During recovery, the enzyme-containing fermentation medium is separated from the cell remnants.

A.4.4. Formulation and standardisation process

Subsequently, the food enzyme is formulated. The resulting product is defined as a ‘food enzyme preparation’.

Food enzymes can be sold as dry or liquid preparations, depending on the final application where the enzyme is intended to be used. For all kinds of food enzyme preparations, the food enzyme is standardised and preserved with food ingredients or food additives regulated according to Annex III of the EU Food Additive Regulation No 1333/2008 and which fulfil specifications for food additives listed in Commission Regulation No 231/2012.

For the manufacture of liquid food enzyme preparations, the food enzyme is standardised with food grade ingredients and adjusted to the desired activity. The preparation is in certain cases stabilised with preservatives.

For the manufacture of dry food enzyme preparations, the food enzyme is typically spray dried using food grade materials. The dried food enzyme is then standardised to the desired/declared activity with food grade ingredients.

A.5. Specification for identity and purity

The specification for impurities and microbial limits for the Dextransucrase product can be found in Annex 2. Certificates of Analysis for three lots of product are provided in Annex 5.

The Dextransucrase meets or exceeds the specifications for enzyme preparations established by the Joint FAO/WHO Expert Committee on Food Additives ("JECFA") in 2006 and FCC, 13th edition. These specifications are provided in Annexes 3 and 4.

Impurity profile:
Appropriate GMP controls and processes are used in the manufacture of Dextransucrase to ensure that the finished preparation does not contain any impurities of a hazardous or toxic nature. The specification for impurities and microbial limits is as follows:

Metals:	
Lead	Less than 2 mg/kg
Arsenic	Less than 1 mg/kg
Microbial:	
Total Viable Count	Less than 100 CFU/g
Total coliforms	Less than 30 CFU/g
E. coli	Absent in 25g
Salmonella	Absent in 25g
Antimicrobial activity	Negative by test
Production strain	Negative by test
Physical properties	
Appearance	Liquid

A.6. Analytical method for detection

According to the FSANZ Application Handbook, July 2024, this is not required for enzymes used as processing aids.

B. Information related to the safety of a chemical processing aid

This Section is not applicable enzymatic processing aids such as Dextransucrase.

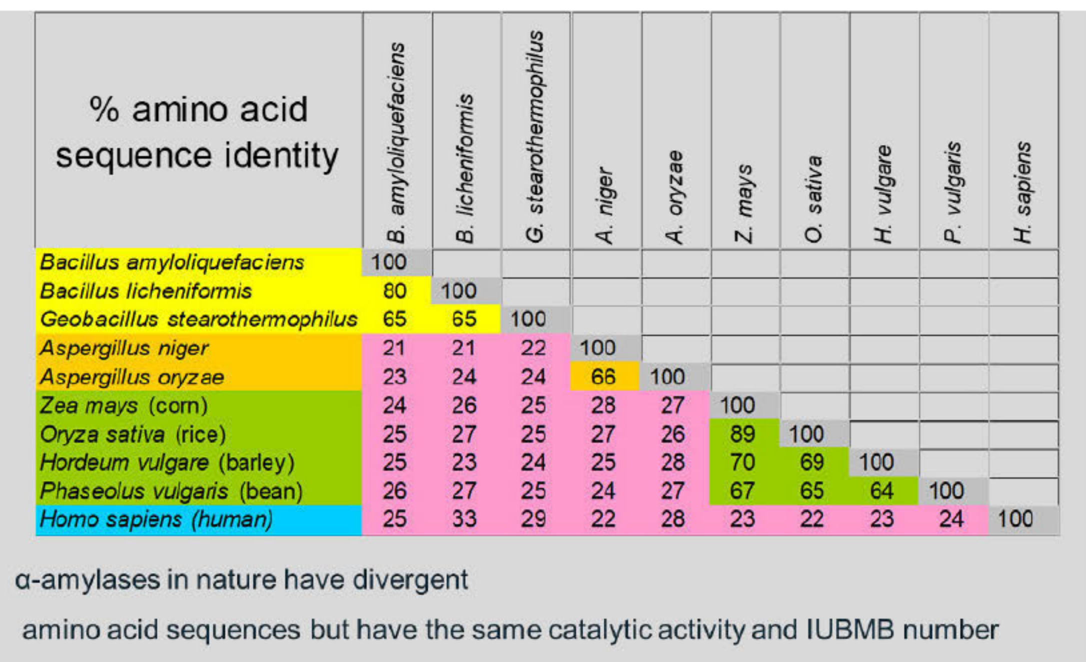
C. Information related to the safety of an enzyme processing aid

C.1 General information on the use of the enzyme as a food processing aid in other countries

Enzyme products are developed for a specific function, i.e. to catalyse a specific chemical reaction. That reaction determines the IUBMB classification. Enzyme variants may be selected to have a better performance of that function under the specific conditions of the application (e.g. temperature or pH). Enzymes of a certain IUBMB classification share conserved structural elements, called domains, which are needed for their specific function. As such the enzymes of our approval procedures do resemble those already permitted by FSANZ both in function and in structure.

Figure 8 below shows an example of natural variation of alpha-amylases. The same holds for any other enzyme type. While significant differences in sequence amongst the various species exist, they all catalyse the same reaction and therefore fit under the same IUBMB entry. There will also

be natural variation within one species. All this also applies to the enzymes under the current approval procedures by FSANZ:



% amino acid sequence identity	B. amyloliquefaciens	B. licheniformis	G. stearothermophilus	A. niger	A. oryzae	Z. mays	O. sativa	H. vulgare	P. vulgaris	H. sapiens
Bacillus amyloliquefaciens	100									
Bacillus licheniformis	80	100								
Geobacillus stearothermophilus	65	65	100							
Aspergillus niger	21	21	22	100						
Aspergillus oryzae	23	24	24	66	100					
Zea mays (corn)	24	26	25	28	27	100				
Oryza sativa (rice)	25	27	25	27	26	89	100			
Hordeum vulgare (barley)	25	23	24	25	28	70	69	100		
Phaseolus vulgaris (bean)	26	27	25	24	27	67	65	64	100	
Homo sapiens (human)	25	33	29	22	28	23	22	23	24	100

α-amylases in nature have divergent
amino acid sequences but have the same catalytic activity and IUBMB number

Figure 7. Variation of enzymes in nature.

The expressed mature enzyme amino acid sequence of Dextransucrase shows a clear conserved glycol-hydro 70 superfamily sequence domain, which includes glucosyltransferase and sucrose 6-glycosyl transferase activities.

Dextransucrase enzyme, the subject of this dossier, belongs to the Hexosyltransferases family. The enzyme under evaluation is not currently listed as a permitted processing aids on Schedule 18 of the ANZ Food Standards Code, i.e. from *Bacillus licheniformis* however other enzymes from the same family are permitted as processing aids on Schedule 18 (e.g. Amylomaltase produced by *Bacillus amyloliquefaciens*; Cyclodextrin *glucanotransferase* produce by *Paenibacillus macerans*; Transglucosidase produced by *Aspergillus niger*, etc)

The dextransucrase enzyme derived from *Bacillus subtilis* carrying the dextransucrase gene from *Streptococcus salivarius* has been determined to be and is has been approved by several countries such as here have not been any adverse events reported since this Dextransucrase has been in commercial use in these countries.

Please refer to Section 3.3.1, J and Appendix C for details of different authorisations and evaluations.

C.2 Information on the potential toxicity of the enzyme

To assess the potential toxicity of the enzyme, a weight-of-evidence approach was applied, as required by JECFA, consisting of the following elements:-

- Toxin homology study

The mature *Streptococcus salivarius* Dextransucrase variant sequence is given in Appendix D4 (**Confidential Commercial Information**).

The UniProt annotated Protein Knowledge database (http://www.uniprot.org), release 2025_01 of February 5, 2025, contains 572,970 reviewed proteins, of which 7,065 sequences are manually annotated as toxins and 7,418 as venom proteins (http://www.uniprot.org/biocuration_project/Toxins/statistics). These toxin and venom sequences are grouped in the animal toxin database subset (http://www.uniprot.org/program/Toxins).

A BLAST search for homology of the Dextransucrase variant sequence against the complete Uniprot database was performed with a threshold *E*-value of 0.1. The majority of the 24 matches were Dextransucrases (referred to as glucotransferase). There were 7 matches to toxins/cytotoxins. However, these matches were not considered significant as the following three criteria as defined by EFSA (2024) were not met: No matches were found combining an E < 0.1, a pairwise identity > 80% and a query coverage > 70%. (see Appendix B1, **Confidential Commercial Information**).

In addition, a specific BLAST search for homology of the Dextransucrase sequence was performed against the Uniprot animal toxin database. This yielded no matches (see Annex B7). Therefore, the *Streptococcus salivarius* Dextransucrase variant sequence does not share homology with a known toxin or venom sequence.

- Safe strain lineage concept

The Safe Strain Lineage concept has been discussed by Pariza and Johnson (2001) in their publication on the safety of food enzymes and is commonly utilised by enzyme companies in the determination of the safety of their products for specific uses, as appropriate.

The primary issue in evaluating the safety of a production strain is its toxigenic potential, specifically the possible synthesis by the production strain of toxins that are active via the oral route. The toxigenic potential of the production organism is confined to the Total Organic Solid (TOS) originating from the fermentation.

As the toxicological evaluation is based on the TOS originating from fermentation of the production organism, studies conducted on strains from the Safe Strain Lineage can support other production strains pertaining to this same Safe Strain Lineage.

The position of the food enzyme in the IFF *B. subtilis* Safe Strain Lineage is presented in Appendix B2 (**Confidential Commercial Information**).

- Toxicological testing

To assess the safety of Dextransucrase, different endpoints of toxicity were investigated and are evaluated and assessed in this document:

- Ames test: no mutagenic activity under the given test conditions
- Chromosomal aberrations: no clastogenic activity under the given test conditions
- 90-day oral toxicity on rats: the NOAEL (no observed adverse effect level) is established at the highest dose tested, 1000 mg total organic solid (TOS)/kg bw/day in male and female rats.

The full study reports are provided as Annexes 11-13, and test summaries are provided in Annex 8.

In addition, safety was further assessed according to the decision tree in the Pariza-Johnson guidelines (2001) for assuring the safety of a new enzyme preparation. The Pariza-Johnson decision tree results are presented in Annex 10.

- Overall safety assessment

In the 90-day oral (gavage) study in rats, a NOAEL was established at 1000 mg TOS/kg bw/day for Dextransucrase produced with *B. subtilis*. The study was designed based on OECD guideline No. 408 and conducted in compliance with both the FDA Good Laboratory Practice Regulations and the OECD Good Laboratory Practice. Since human exposure to Dextransucrase is through oral ingestion, selection of this NOAEL is thus appropriate.

NOAEL = 1000 mg TOS/kg bw/day

Determination of margin of safety

It is prudent to ascertain that the most suitable available NOAEL is sufficiently high to accommodate the intended uses. The margin of safety was calculated by dividing the NOAEL obtained from the 90-day oral (gavage) study in rats by the human exposure (worst case scenario) assessed in Section F. As the margin of safety was determined to be greater than 100 (i.e.), it suggests that the available toxicology data support the proposed uses and application rates.

$$\text{Margin of safety} = \frac{\text{No observed adverse effect level}}{\text{Maximum daily exposure}}$$

$$\text{Margin of safety} = \frac{1000 \text{ mg TOS/kg bw/day}}{0.64 \text{ mg TOS/kg bw/day*}}$$

Margin of Safety = 1562.5

* Refer to Section F.2.1 Dietary exposure

C.3 Information on the potential allergenicity of the enzyme processing aid

The ingestion of food enzymes has been shown to be an unlikely concern regarding food allergy (Bindslev-Jensen et al., 2006). The most current allergenicity assessment guidelines developed by the Codex Commission (Codex, 2009) and Ladics et al. (2011); Ladics (2018) recommend the use of FASTA or BLASTP search for matches of 35% identity or more over 80 amino acids of a subject protein and a known allergen. Ladics et al. (2011); Ladics (2018) further discussed the use of the "E-score or E-value in BLAST algorithm that reflects the measure of relatedness among protein sequences and can help separate the potential random occurrence of aligned sequences from those alignments that may share structurally relevant similarities." High E-scores are indicative that any alignments do not represent biologically relevant similarity, whereas low E-scores ($<10^{-7}$) may suggest a biologically relevant similarity (i.e. in the context of allergy, potential cross reactivity). They suggest that the E-score may be used in addition to percent identity (such as > 35% over 80 amino acids) to improve the selection of biologically relevant matches. The past practice of conducting an analysis to identify short, six contiguous identical amino acid matches is associated with false positive results and is no longer considered a scientifically defensible practice.

The Codex Commission states:

"A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens."

The *S. salivarius* Dextransucrase amino acid sequence is given in Appendix D4 as "**Confidential Commercial Information**".

A full-length sequence alignment against known allergens in the Allergy Research and Resource Program (FARRP) AllergenOnline database, January 30, 2025 V23, containing 2334 peer reviewed allergen sequences listed in the database (using E-value <0.1) yielded no sequence matches (see Appendix B4, **Confidential Commercial Information**).

The FASTA full-length (conventional) sequence analysis was found to produce less false positive results when compared to the sliding 80-amino acid window analysis and was determined to be the more appropriate analysis when evaluating for potential allergenic cross-reactivity of proteins (Ladics *et al*., 2007; Cressman & Ladics, 2009). Nevertheless, a search for 80-amino acid stretches within the sequence matching to known allergens was performed using the same FARRP AllergenOnline database and yielded no sequence matches.

As cautioned in Codex (2009), researched by Herman *et al*. (2009) and further elaborated by Ladics *et al*. (2011); Ladics (2018) and on AllergenOnline.org, there is no evidence that a short identical contiguous amino acid match will identify a protein that is likely to be cross-reactive and could be missed by the conservative 80 amino acid match (>35%). The FARRP AllergenOnline database allows isolated identity matches of eight contiguous identical amino acids to satisfy demands by some regulatory authorities for this precautionary search. Performing the eight contiguous identical amino acid searches produced no matches (see Appendix B4, **Confidential Commercial Information**).

Additionally, microbial derived enzymes acting as environmental allergens have not been demonstrated to be active via the oral route. This concept was evaluated by Bindslev-Jensen *et al*. (2006) in which 19 microbial derived enzymes used in the food industry were tested for allergenicity in a double-blind placebo-controlled food challenge study in subjects with positive skin prick tests to inhalation allergens, food allergens, and bee and wasp allergens. None of the 19 microbial derived food enzymes were found to be food allergens and the authors concluded that ingestion of microbial derived food enzymes in general are not considered to be a concern regarding food allergy. Moreover, enzymes are processing aids and per definition, they are not present or active in the final food. Consequently, there is no exposure of the enzyme to the consumer.

In conclusion, bioinformatic analyses based on sequence homology determined that the expressed *Streptococcus salivarius* Dextransucrase variant is unlikely to pose a risk of food allergenicity.

As for all enzyme products, an MSDS for the Dextransucrase product would include a precautionary statement that inhalation of enzyme mist/dust may cause allergic respiratory reactions, including asthma, in susceptible individuals on repeated exposure.

An allergen statement for the enzyme preparation is given in Annex 6. Liquid glucose derived from wheat is used as a fermentation ingredient, however, according to Clause S9-3 to Standard 1.2.3, this substance would be exempt from mandatory allergen declaration.

C.4 Safety assessment reports prepared by international agencies or other national government agencies, if available

Documentation on the approval of Dextransucrase by other agencies or jurisdictions is provided in Section 3.1, Part J.2, and Appendix C (**Confidential Commercial Information).**

D. Additional information related to the safety of an enzyme processing aid derived from a microorganism

D.1 Information on the source microorganism

The source organism was obtained by genetic engineering of *Bacillus subtilis* strain a known safe host strain. The purpose of the modification is to produce the Dextransucrase enzyme of the donor strain *Streptococcus salivarius*.

D.1.1 The production organism

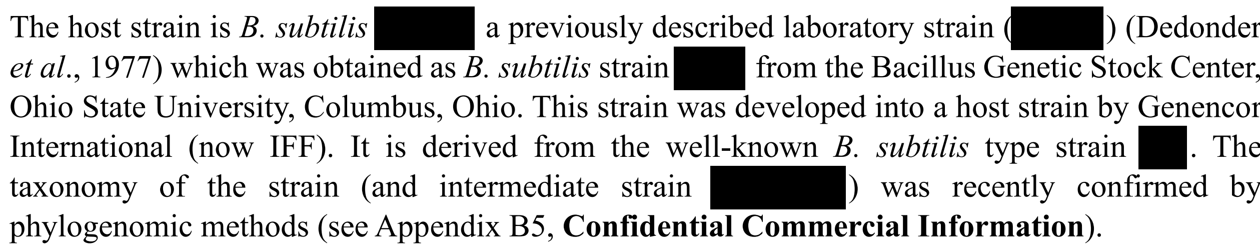
The production organism is a strain of *B. subtilis*, that has been genetically engineered by Danisco US Inc. to overexpress a protein engineered Dextransucrase gene from *Streptococcus salivarius*. The production organism is a strain of *B. subtilis* designated *B. subtilis* The history of the strain development, method of transformation, and the genetic tools used to transform the host strain are described in Appendix D (**Confidential Commercial Information**).

B. subtilis, including genetically engineered strains, is listed in Schedule 18 to Standard 1.3.3 of the FSANZ Food Standards Code as a permitted source for several enzymes, including α-Acetolactate decarboxylase, α-Amylase, β-Amylase, Asparaginase, Endo-1,4-beta-xylanase, β-Glucanase, Maltogenic α-amylase, Metalloproteinase, Pullulanase, Serine proteinase and Aqualysin 1. This species, and its genetically engineered strains are also listed in Table V of the Food Additive Table of Division 16 of the Canadian Food and Drug Regulations (http://www.hc-sc.gc.ca/fn-an/securit/addit/list/5-enzymes-eng.php) as approved sources for α-Amylase, Glucanase, Protease and Xylanase.

The species *Bacillus subtilis* is an accepted source of enzymes in the literature, and pathogenic strains are not described in the Bergey Manual (Bergey, 1986) or in the ATCC and other catalogues. The species *B. subtilis* does not appear on the Proposal for a Council Directive amending the "Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agent at work" and is also not present on the European Guideline 93/88/EEG. *B. subtilis* is accepted as a safe host for the construction of Risk Group I GMMs in several countries, like Germany, The Netherlands, and accepted as a host of certified host-vector systems under the NIH Guidelines in the USA.

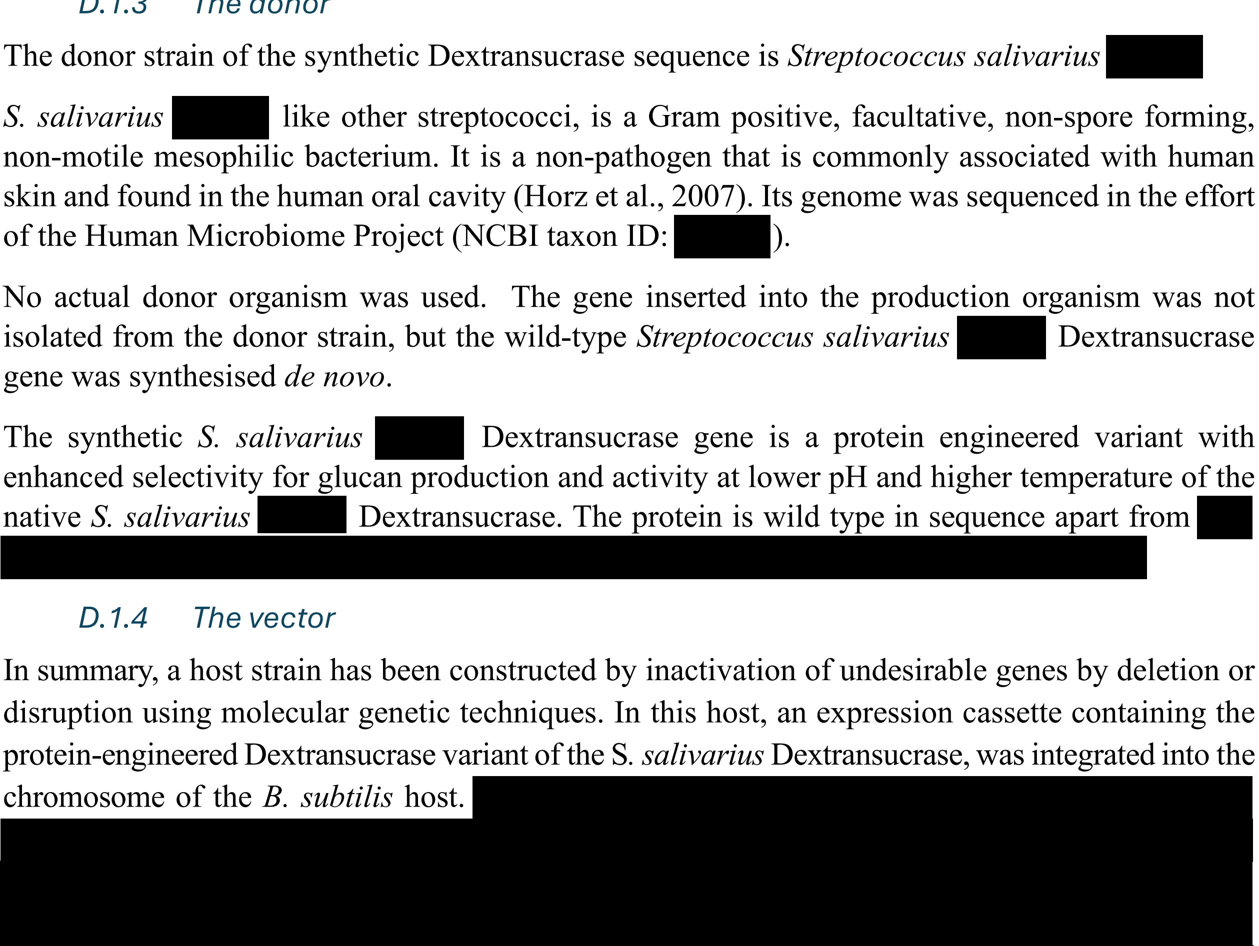
We have constructed a host strain by inactivation of undesirable genes by deletion or disruption using molecular genetic techniques. In this host, an expression cassette containing the protein-engineered Dextransucrase of the *S. salivarius* Dextransucrase, was integrated into the chromosome of the *B. subtilis* host. The cassette was inserted by homologous recombination. See Appendices D1-2 (**Confidential Commercial Information**) for details of the genetic modifications employed.

D.1.2 The host



The host strain is *B. subtilis* a previously described laboratory strain ( ) (Dedonder *et al.*, 1977) which was obtained as *B. subtilis* strain from the Bacillus Genetic Stock Center, Ohio State University, Columbus, Ohio. This strain was developed into a host strain by Genencor International (now IFF). It is derived from the well-known *B. subtilis* type strain . The taxonomy of the strain (and intermediate strain) was recently confirmed by phylogenomic methods (see Appendix B5, **Confidential Commercial Information**).

As discussed in Section 3.3.2, Part A, *B. subtilis* strain has been the host used for numerous enzymes assessed and approved by FSANZ, the relevant application details are provided in Appendix A2 (**Confidential Commercial Information**).



D.1.3 The donor

The donor strain of the synthetic Dextransucrase sequence is *Streptococcus salivarius*

S. salivarius like other streptococci, is a Gram positive, facultative, non-spore forming, non-motile mesophilic bacterium. It is a non-pathogen that is commonly associated with human skin and found in the human oral cavity (Horz et al., 2007). Its genome was sequenced in the effort of the Human Microbiome Project (NCBI taxon ID:).

No actual donor organism was used. The gene inserted into the production organism was not isolated from the donor strain, but the wild-type *Streptococcus salivarius* Dextransucrase gene was synthesised *de novo*.

The synthetic *S. salivarius* Dextransucrase gene is a protein engineered variant with enhanced selectivity for glucan production and activity at lower pH and higher temperature of the native *S. salivarius* Dextransucrase. The protein is wild type in sequence apart from

D.1.4 The vector

In summary, a host strain has been constructed by inactivation of undesirable genes by deletion or disruption using molecular genetic techniques. In this host, an expression cassette containing the protein-engineered Dextransucrase variant of the S. *salivarius* Dextransucrase, was integrated into the chromosome of the *B. subtilis* host.

The expression cassettes consist of:

	***Bacillus subtilis* ( ), *pksR* locus cassette**
Promoter	promoter (*B. subtilis*)
Dextransucrase AA mutations	(*S. salivarius* variant); chemically synthesised
Terminator	(*B. subtilis*)
Selective marker	(*B. subtilis*)
Other genes	none

No foreign bacterial vector DNA remained present in the final production strain. An intermediate strain in this construction, *B. subtilis* was recognised by the Dutch authorities as a Risk

Class 1 microorganism. All these modifications were performed in such a way that no bacterial vector DNA remained present in the strain. No antibiotic resistance markers were inserted into the new microorganism.

A detailed description is provided in Appendix D2 as **Confidential Commercial Information**.

D.2 Information on the pathogenicity and toxicity of the source organism

The safety of the production organism must be the prime consideration in assessing the safety of an enzyme preparation intended for use in food (Pariza and Foster, 1983). If the organism is nontoxigenic and non-pathogenic, then it is assumed that foods or food ingredients produced from the organism, using current Good Manufacturing Practices, are safe to consume. (IFBC, 1990). Pariza and Foster (1983) define a non-toxigenic organism as 'one which does not produce injurious substances at levels that are detectable or demonstrably harmful under ordinary conditions of use or exposure' and a non-pathogenic organism as 'one that is very unlikely to produce disease under ordinary circumstances. *Bacillus subtilis* strains used in enzyme manufacture meet these criteria for non-toxigenicity and non-pathogenicity. US FDA affirmed as GRAS the native subtilisin and neutral protease produced by *B. subtilis* (21 C.F.R. § 184.1150).

B. subtilis occurs ubiquitous in the environment (soil, water, plants and animals) and as a result can be also found in food (de Boer and Diderichsen, 1991). The bacterium has already been used for decades for the production of food enzymes with no known reports of adverse effects to human health or the environment (de Boer and Diderichsen, 1991). Alpha-amylase enzyme preparation from *B. subtilis* has been used commercially since 1929, when it was used in the manufacture of chocolate syrup to reduce its viscosity (Reed, 1966).

Recently the US Food and Drug Administration reviewed the safe use of food-processing enzymes from recombinant microorganisms, including *B. subtilis* (Olempska-Beer *et al*. 2006). An extensive risk assessment of *B. subtilis*, including its history of commercial use has been published by the US Environmental Protection Agency (US EPA, 1997). It was concluded that *B. subtilis* is not a human pathogen nor is it toxigenic.

B. subtilis is exempted as a host of certified host-vector systems under the NIH Guidelines in the USA since 1994 (NIH, 2016). The US EPA has added *B. subtilis* to the list of exempted organisms in 1997 (US EPA, 1997). *B. subtilis* is a low-risk-class microorganism, i.e., category 1 of the European Federation of Biotechnology (Frommer *et al*., 1989), and it can be used under the lowest containment level at large scale, GILSP, as defined by OECD (1992).

JECFA has evaluated food enzymes derived from *B. subtilis*, including some genetically engineered strains, and concluded that these food enzymes do not constitute a toxicological hazard (JECFA, 1972; JECFA, 1993; JECFA, 1999e; JECFA, 1999d; JECFA, 1999c; JECFA, 1999b; JECFA, 2004a; JECFA, 2004b; JECFA, 2009) as well as other substances like Riboflavin (vitamin B) (JECFA, 1999a). 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. The QPS list is reviewed and updated annually by the Panel on Biological Hazards (BIOHAZ). If a defined taxonomic unit does not raise safety concerns or if any possible concerns can be excluded, the QPS approach can be applied, and the taxonomic unit can be recommended to be included in the QPS list. The safety of *B. subtilis* as a production organism has been assessed by EFSA and was accorded QPS status in 2007 provided the qualification

requirements are met (EFSA, 2007). For *Bacillus* strains the specific requirement is absence of toxigenic activity.

Numerous oral toxicity, mutagenicity and carcinogenicity studies using enzyme products from *B. subtilis* 168-derived strains have been performed, and no evidence of a toxic or mutagenic effect has been observed.

Despite these facts *Bacillus* strains derived from the same parent were tested for pathogenicity and toxicity (see Appendices B2-3**, Confidential Commercial Information**). The conclusion of the research was that no toxic substances were produced by the strain, that it is not pathogenic, and not toxicogenic.

The validation of species classification and Whole Genome Sequencing analysis for production strain *B. subtilis* [redacted] is provided in Appendix B5 and 6, respectively as **Confidential Commercial Information**.

D.3 Information on the stability of the source organism

The parental strain of the production strain *B. subtilis* [redacted] and its derivatives have been used for industry scale enzyme manufacturing for decades by IFF and its parental companies and has demonstrated stable enzyme expression even at large scale fermentation. Please also refer to Appendix B3 for list of example enzyme preparations produced using *B. subtilis* [redacted] and its derivatives. Furthermore, the production strain has demonstrated to be 100% stable as confirmed by genome sequencing. A report on the stability of the inserted gene is provided in Appendix D3, (**Confidential Commercial Information**). Refer also section E.1.

E. Additional information related to the safety of an enzyme processing aid derived from a genetically modified organism

E.1 Information on the methods used in the genetic modification of the source organism.

The *B. subtilis* strain [redacted] was constructed for Dextransucrase production. The construction of the production strain consisted of:

- Expression cassette containing the Dextransucrase variant gene from *S. salivarius* under the control of the *B. subtilis* promoters described in Section D.1.4 above.
- Introduction of the expression cassette into the genome of *B. subtilis* [redacted] recipient strain.
- The gene donated to produce Dextransucrase from *B. subtilis* strain [redacted] is protein engineered.

The genetic stability of the inserted gene has been demonstrated by genome sequencing. Broth samples were taken prior and after prolonged fermentation mimicking commercial fermentation conditions. Samples were then used for genomic DNA extraction and next generation sequencing. The results demonstrate that the insertion cassette has been stably maintained during the fermentation process.

Full details of the genetic modifications and stability of the inserted genes are provided in Appendix D1-D3 as **Confidential Commercial Information**.

F. Information relating to the dietary exposure to the processing aid

F.1 A list of foods or food groups likely to contain the processing aid or its metabolites.

In the below applications Dextransucrase will be used as a processing aid where the enzyme is either not present in the final food, or present in insignificant quantities having no function or technical effect in the final food. The enzyme is intended for use in sucrose containing foods including, but not limited, to dairy products (excluding butter and fats), ice cream and edible ices, processed fruits and vegetables, confectionary, cereal products, breads and bakery products, sugars, honey and related products, and beverages.

F.2 The levels of residues of the processing aid or its metabolites for each food or food group.

F.2.1 *Level of use and residues in food*

Usage levels

The Dextransucrase enzyme preparation is used at the minimum level required to achieve the desired effect and in accordance with the principles of current Good Manufacturing Practice (GMP).

The intended use level in food ingredients and food products containing sucrose is 0.01-3 mg active enzyme protein/g sucrose.

Enzyme Residues in the Final Foods

The Dextransucrase enzyme will be deactivated or removed during the subsequent production and refining processes for all applications. In the rare case that inactive Dextransucrase enzyme is present in the processed food and is ingested, it will not be absorbed intact. Instead, the enzyme is broken down by the digestive system into small peptides and amino acids, with the latter being absorbed and metabolised, which poses no human health risk.

Dietary Exposure

Dextransucrase is used as processing aid in to convert sucrose in food ingredients and food products into polysaccharides, which provides sugar reduction and texture formation. While we expect the Dextransucrase to be not present in the final food or present as inactive residue in negligible amounts, the following conservative calculations assume that 100% of the enzyme remains in the processed food, as total organic solids (TOS).

The human exposure to the Dextransucrase can be calculated below.

On average, Australians consume 66.4 grams of sugar daily (Australian Bureau of Statistics 2023-24).

If assuming that 10% food producers would use the Dextransucrase to convert the sucrose (maximum projected business market share; Dextransucrase only works on sucrose) and that the daily sugar (66.4 g) consumed by Australians is all sucrose (worst-case as a portion of this would be assumed to be fructose, glucose, corn syrup, honey, etc.), it would result in a maximum exposure

of 200 mg enzyme/day. The dosage will be 0.01-3 mg active enzyme protein per g of sucrose.

Daily Sugar Consumption	66.4	g/day
Maximum Use Rate (AEP)	3	mg active enzyme protein/g sucrose
Maximum enzyme exposure (AEP)	200	mg active enzyme protein/day
Activity / Active Enzyme Protein ratio	5.5	GTFU/mg active protein
Maximum enzyme exposure (Activity)	1100	GTFU/day
TOS/Activity ratio	0.35	mg TOS/GTFU
Market share	10	%
Assumption on body weight	60	kg
Maximum enzyme exposure (TOS)	0.64	mg TOS/kg bw/day

F.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption.

Not applicable. Dextransucrase is not expected to be used in production of any foods or food groups that are currently not listed in NNSs. If such usage arises, an application would be made to inform FSANZ.

F.4 The percentage of the food group in which the processing aid is likely to be found or the percentage of the market likely to use the processing aid

The enzyme would be used as a processing aid in about 10% of the tonnage f sucrose containing food products sold in Australia and New Zealand.

F.5 Information relating to levels of residues in foods in other countries

Applications and levels of use of the Dextransucrase preparation in other countries is the same as presented in section F2.

F.6 For foods where consumption has changed in recent years, information on likely current consumption.

Not applicable. Consumption of foods produced with Dextransucrase is not expected to have a significant change.

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