A Subtilisin Enzyme
from a recombinant strain of *Bacillus subtilis*

PROCESSING AID APPLICATION

Food Standards Australia New Zealand

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

26th July 2023

CONTENTS

1. General information

1.1 Applicant details

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

(b) Company:
[redacted]

(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 affliate of Genencor International Ltd, the manufacturer of the prodcut and another subsidiary of International Flavors and Fragrances Inc (IFF). Entity Relationship letter, Section 1.2.

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

1.2 Entity relationship statement

September 11, 2022

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Re: Statement Regarding Subsidiary Entities

To Whom It May Concern,

We are pleased to inform you that on February 1, 2021, International Flavors & Fragrances Inc. ("IFF") completed its previously announced combination of IFF and the Nutrition & Biosciences business (the "N&B Business") of DuPont de Nemours, Inc. ("DuPont") (the "Merger").

As part of the Merger, various legal entities associated with the N&B business, were transferred by DuPont to IFF. The N&B legal entities listed on the following website are among the legal entities associated with the N&B business that were transferred by DuPont to IFF.

https://www.iff.com/where-we-operate/subsidiaries

For your purposes, please consider the following N&B legal entities as operating under IFF ownership as IFF Health & Biosciences business.

[redacted]

[redacted]

Thank you for your attention.

Sincerely,

[redacted]

1.3 Purpose of the application

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a new Processing Aid, subject of this application. The intended use of the processing aid is for protein processing.
This application is made solely on behalf of IFF (trading as Danisco Australia Pty Ltd), 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.
Subtilisin, subject of this application, is intended for use in protein processing in a wide variety of foods to facilitate protein hydrolysis.
Currently no Subtilisin from *B.clausii* expressed in *B. subtilis* is permitted as a Processing Aid, however Subtilisin from B. licheniformis, and other enzymes including α-Acetolactate decarboxylase, α-Amylase, β-Amylase, Asparaginase, Endo-1,4-beta-xylanase, β-Glucanase, Hemicellulase multicomponent enzyme, Maltogenic α-amylase, Metalloproteinase, Pullunase, and Serine protease 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 2.3 and Appendix A.

1.4 Justification for the Application

1.4.1 Regulatory Impact Information

A. Costs and Benefits of the application

Subtilisin is an enzyme produced by submerged fermentation of *B. subtilis* carrying the gene encoding the Subtilisin gene from *B.clausii.* The enzyme is characterised as an Subtilisin (EC 3.4.21.62). A collection of information detailed in Section 3 supports the safety of the production organism and the enzyme for use in the applications outlined in Section 4.

The enzyme is intended to be used for protein hydrolysis in the processing of various protein containing foods.

More information on the benefit of this enzyme can be found in Section 2.2 and Appendix A.

Enzyme preparations are widely used as processing aids in the manufacture of food products. Currently no Subtilisin from *B. clausii* 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.

B. Impact on international trade

The inclusion of Subtilisin from *B. clausii* expressed in *B. subtilis* in the Australia New Zealand Food Standards Code as a processing aid may promote international trade on products produced with this enzyme product and reduce technical barriers to trade.

1.5. Support for the application

No marketing or promotional activities have been undertaken for Subtilisin derived from *B. subtilis* containing the gene for Subtilisin from *B. clausii* in the Australia/New Zealand market. Hence at this stage, no requests from food manufacturers are provided in support of this application. However, the need and justification for use of the processing aid are discussed in Section 1.3, and it is anticipated that support from the food processing industry will be

submitted during the period for public comment on the application Draft Regulatory Measure/Assessment Report.

1.6. 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 350 hours) to be the appropriate procedure for assessment of the application.

1.7. Confidential Commercial Information (CCI)

Certain (identified) technical and manufacturing information included in Appendices B1, B3, -B6, Appendices E1-E5 and 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.

1.8. 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).

1.9. International and other National Standard

Refer to Appendix D for further details

1.9.1. Codex Standards

Subtilisin from *B. clausii* produced by *B. subtilis* has not been reviewed by JECFA; there is no specific Codex Standard relevant to this application.

1.9.2. International legislation

Subtilisin derived from *B. subtilis* containing the gene for Subtilisin from *B. clausii* has been determined to be Generally Recognized as Safe (GRAS) in the United States as a food processing aid for protein processing by a panel of scientific experts in the USA.

1.10. Statutory declaration

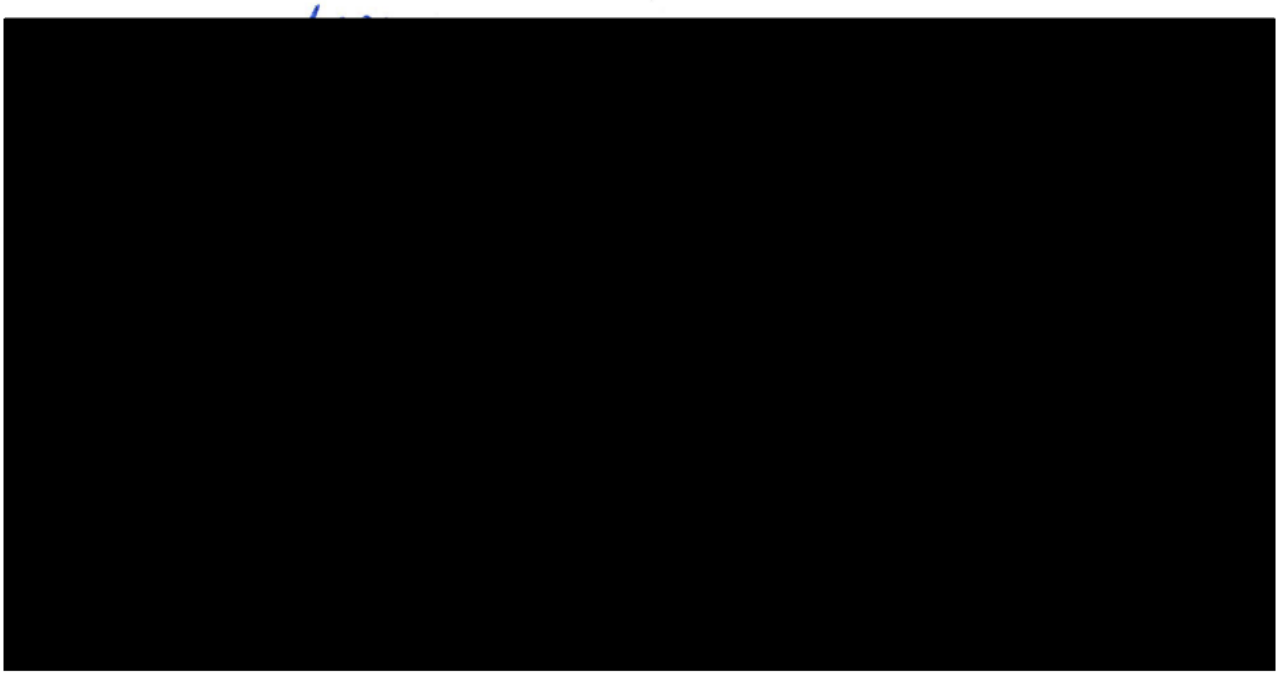
I, Caroline Elizabeth Gray,

of 12B Elstree Avenue, Glen Innes, Auckland 1072, New Zealand, Regulatory Affairs Manager/Director

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.



1.11. Checklist

	Mandatory Requirements	Check	Page Number	Remarks
General requirements for applications	A. Form of the application	✓	N.A.	
	Table of contents	✓	1	
	Executive summary	✓	N/A	Supplied separately
	B. Applicant details	✓	2	Section 1.1
	C. Purpose of application	✓	4	Section 1.3
	D. Justification for the application	✓	4	Section 1.4
	D.1 Regulatory impact information	✓	4	Section 1.4.1
	D.1.1 Costs and benefits of the application	✓	4	Section 1.4.1
	D.1.2 Impact on international trade	✓	4	Section 1.4.1
	E Information to support the application	✓	4	Section 1.5
	E.1 Data requirements	✓	N.A.	
	F. Assessment procedure	✓	5	Section 1.6
	G. Confidential commercial information (CCI)	✓	5	Section 1.7
	H. Other confidential information	✓	5	
	I. Exclusive capturable commercial benefit (ECCB)	✓	5	Section 1.8
	J. International and other national standards	✓	5	Section 1.9
	J.1 International Standards	✓	5	Section 1.9.1
	J.2 Other national standards or regulations	✓	5	Section 1.9.2
	K. Statutory declaration	✓	6	Section 1.10
	L. Checklist	✓	7	Section 1.11
3.3.2. Processing aids	A. Technical information on the processing aid	✓	9	Section 2
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	A.2 Information on the identity of the processing aid	✓	9	Section 2.2
	A.3 Information on the chemical and physical properties of the processing aid	✓	9	Section 2.3
	A.4 Manufacturing process	✓	10	Section 2.4
	A.5 Specification for identity and purity	✓	10	Section 2.5
	A.6 Analytical method for detection	×		Not applicable for enzymes used as processing aids
	C. Information related to the safety of an enzyme processing aid	✓	11	Section 3
	C.1 General information on the use of the enzyme as a food processing aid in other countries	✓	11	Section 3.1
	C.2 Information on the potential toxicity of the enzyme processing aid	✓	12	Section 3.2
	C.3 Information on the potential allergenicity of the enzyme processing aid	✓	12	Section 3.3

	C.4 Safety assessment reports prepared by international agencies or other national government agencies, if available	✓	12	Section 3.4
	D. Additional information related to the safety of an enzyme processing aid derived from a microorganism			Section 3
	D.1 Information on the source microorganism	✓	13	Section 3.5
	D.2 Information on the pathogenicity and toxicity of the source microorganism	✓	13	Section 3.6
	D.3 Information on the genetic stability of the source organism	✓	13	Section 3.7
	E. Additional information related to the safety of an enzyme processing aid derived from a genetically-modified microorganism			Section 3
	E.1 Information on the methods used in the genetic modification of the source organism	✓	14	Section 3.8
	F Information related to the dietary exposure to the processing aid		15	Section 4
	F.1. A list of foods or food groups likely to contain the processing aid or its metabolites	✓	15	Section 4.1
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	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	✓	15	Section 4.3
	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	✓	16	Section 4.4
	F.5 Information relating to the levels of residues in foods in other countries	✓	16	Section 4.5
	F.6 For foods where consumption has changed in recent years, information on likely current food consumption	✓	16	Section 4.6

2. Technical information

Please refer to Appendix A for further details

2.1. Type of processing aid

The Subtilisin enzyme is an enzyme produced by submerged fermentation of *B. subtilis*, carrying the subtilisin gene from *B. clausii*. This Processing Aid falls into the category "Enzymes of microbial origin" from the Food Standard Code section 1.3.3-6 Enzymes.

2.2. Identity

2.2.1 Chemical/Common Name:

The systematic name of the principle enzyme activity is Subtilisin. Other names used are alcalase, bacillopeptidase, alkaline proteinase, protease, thermoase, and subtilopeptidase.

- EC number: 3.4.21.62
- CAS number: 9014-01-1

Biological source: The Subtilisin enzyme is an enzyme produced by submerged fermentation of *B. subtilis*, carrying the subtilisin gene from *B. clausii*.

2.2.2 Marketing Name of the Processing Aid:

The marketing name of this enzyme preparation will depend on the application. An example marketing name of Subtilisin is FoodPro® PXT.

2.2.3 Molecular and Structural Formula:

Subtilisin is a protein. The amino acid sequence is known. Please refer to Appendix E.

2.3. Chemical and physical properties

The function of Subtilisin is to hydrolyse proteins with broad specificity for peptide bonds and a preference for a large uncharged residue in P, and the hydrolysis of peptide amides with the release of protein fragments of various lengths, peptides, and free amino acids.

When added to foods for the purpose of protein hydrolysis Subtilisin will degrade the component proteins of the food into peptides resulting in protein hydrolysates with improved and desirable properties. The benefit of this can be better production efficiencies, improved yields, increased throughput, and more efficient raw material usage, and generally a more desirable product.

Substrate specificity:
The function of Subtilisin is to catalyse the hydrolysis of proteins with broad specificity for peptide bonds and a preference for a large uncharged residue in P, and the hydrolysis of peptide amides with the release of protein fragments of various lengths, peptides, and free amino acids.

Activity:
The activity of the Subtilisin is defined in units/g. The assay is based on the ability of a protease to cleave p-nitroanilide from a synthetic peptide, succinyl-ala-ala-ala-p-nitroanilide (suc-AAApNA), resulting in an increase in absorbance at 405 nm. This increase in absorbance is related directly to enzyme activity via use of an enzyme standard.

The activity of the food enzyme Subtilisin from *Bacillus subtilis* was measured under various pH and temperature conditions. It was concluded that the food enzyme Subtilisin from a genetically modified strain of *Bacillus subtilis* exhibits activity from pH 6 till pH 11, and from 20ºC till 80˚C.

The optimum pH range lies between pH 8-9, whereas the optimum temperature range lies between 45 and 60˚C. No enzyme activity is left at temperatures above 80˚C after 4 minutes. Please refer to Appendix EA for further information.

Interaction of the enzyme with different foods:
The Subtilisin enzyme preparation will be used as a processing aid where the enzyme is not present or active in the final food or present in negligible amounts with no technical function in the final food.

Nutritional implication:
Subtilisin is a protein and any residual amounts remaining in food consumed would accordingly have the same nutritional value. However, the use levels of Subtilisin are very low, and as with other enzymes that are currently approved and used as Processing Aids, use of this preparation would not have any nutritional significance.

2.4. Manufacturing process

The enzyme is produced by a submerged fermentation process using appropriate substrate and nutrients. When fermentation is complete, the biomass is removed by centrifugation/filtration. The remaining fermentation broth containing the enzyme is filtered and concentrated. The concentrated enzyme solution is then standardised and stabilised with diluents. Finally, a polish filtration is applied.

Full details on the raw materials used for the production are provided in Appendix E. Note that this information is proprietary and "**Confidential Commercial Information**" status is requested.

The production of Subtilisin 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.

2.5. Specification for identity and purity

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

Metals:	
Lead	less than 5 mg/kg
Microbiological:	
Total viable count	less than 10,000 CFU/g
Total coliforms	less than 30 CFU/g
E. coli	absent in 25g
Salmonella	absent in 25g
Antibiotic activity	Negative by test
Production strain	Negative by test
Physical properties:	
Appearance	Off white powder

Standard for identity:

Subtilisin meets the specifications laid down by the Joint FAO/WHO Expert Committee on Food Additives and the Food Chemicals Codex.

3. Safety

Refer to Appendix B for further details

3.1. 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 1 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 1. Variation of enzymes in nature.

The expressed mature enzyme amino acid sequence of subtilisin shows a clear conserved peptidase_S8_S53 superfamily sequence domain, which includes endopeptidase and exopeptidase activities.

Subtilisin enzyme, the subject of this dossier, is one of the permitted processing aids on Schedule 18 of the ANZ Food Standards Code, i.e. from *Bacillus licheniformis*. In our case the enzyme protein is expressed from *Bacillus subtilis*.

The subtilisin enzyme derived from *Bacillus subtilis* carrying the subtilisin gene from *Bacillus clausii* (formerly taxonomically classified as *Bacillus lentus*) has been determined to be GRAS in the United States and is has been approved by several countries such as JECFA, Brazil, Canada, China, France, Denmark, Mexico. There have not been any adverse events reported since this protease has been in commercial use in these countries.

Please refer to section 1.8 and Appendix D for details on the different approval procedures in the countries listed above.

3.2. Toxicity of the enzyme

Toxin homology study
A BLAST search for homology of the Subtilisin sequence against the complete Uniprot database (http://www.uniprot.org/), was performed, with a threshold E-value of 0.1. In addition, a specific BLAST search for homology of the mature Subtilisin sequence was performed against the Uniprot animal toxin database. This yielded no matches. Therefore, the Subtilisin 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.

Although *B. subtilis* is scientifically determined by IFF as a Safe Strain Lineage, the food enzyme object of the current dossier is also supported by a 90-day oral toxicity study on the specific food enzyme object of this dossier. This toxicological test on *B. subtilis* CF520B are thus one of the pillars supporting the IFF *B. subtilis* Safe Strain Lineage. The position of the food enzyme in the IFF *B. subtilis* Safe Strain Lineage is presented in Appendix B3.

Toxicological testing
To assess the safety of Subtilisin a 90-day oral toxicity study was carried out on rats . This study established the NOAEL (no observed adverse effect level) at the highest dose tested, 420 mg total protein/kg bw/day, equivalent to 480.6 mg total organic solid (TOS)/kg bw/day in male and female rats. A summary of the results of this study can be found in Appendix B.

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.

3.3. Allegenicity of the enzyme

Bioinformatic analyses based on sequence homology determined that the *B. clausii* Subtilisin is unlikely to pose a risk of food allergenicity. Refer to Appendix B for additional information on the safety of the enzyme as to its allergenicity potential.

An allergen statement is given in Appendix A9.

3.4. Safety Assessment reports prepared by international agencies or other national government agencies, if avaiable

As discussed in section 1.8, Subtilisin from *B. clausii* expressed in *B. Subtilis* has not been reviewed by JECFA; there is no specific Codex Standard relevant to this application. It has, however, been determined to be GRAS by expert opinion in the United States. Refer Appendix D for safety reports/approval letters.

3.5. Information on the source microorganism

The production organism strain CF520B is a strain of *B. subtilis* which has been genetically modified by IFF to express a subtilisin gene from *B. clausii*.

B. subtilis has a long history of safe use in industrial-scale enzyme production. The long industrial use and wide distribution of *B. subtilis* in nature has never led to any pathogenic symptoms. Moreover, no case demonstrating invasive properties of the species has been found in the literature. The donor strain used as a source for the Subtilisin gene is *Bacillus clausii* also known as ATCC21536, and formerly taxonomically classified as *B. lentus*, Nielsen *et al*. (1995). The donor strain, *Bacillus clausii* ATCC21536, was only used as the donor of the subtilisin gene, which obtained from the American Type Culture Collection.

Full details of the gene and recombinant microorganism are provided in Appendix E. Note that this information is proprietary and "Confidential Commercial Information" status is requested.

3.6. Pathogenicity and toxcitiy of the source microorgansim

B. subtilis occurs ubiquitously in the environment (soil, water, plants and animals) and as a result can be also found in food. 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). For example, alpha-amylase enzyme preparations from *B. Subtilis* have been used commercially since 1929, when they were used in the manufacture of chocolate syrup to reduce its viscosity.

Recently, scientists with the US Food and Drug Administration (FDA) 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 (1997). It was concluded that *B. subtilis* strains used for enzyme manufacture are neither pathogenic nor toxigenic to humans. It is, however, prudent to ascertain the safety of the production strain as certain foodborne illness related strains may produce surfactin, a membrane spanning lipopeptide and amylolysin, a heat-stable toxin regarded to be a virulence factor (Apetroaie-Constantin *et al*., 2009).

Further details are discussed in Appendix B.

3.7. Genetic stability of the source microorganism

The parental strain of the production strain *B. subtilis* BG125 and its derivatives have been used for industry scale enzyme manufacturing for decades by IFF 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 BG125 and its derivatives. Furthermore, the production strain has demonstrated to be 100% stable as confirmed by genome sequencing. Refer also section 3.6.

3.8. Method used in the genetic modification of the source microorganism

The production organism of the Subtilisin preparation, the subject of this submission, is *B. subtilis* strain CF520B.

In summary, the genetic modification of the *B. subtilis* host involved recombinant DNA techniques to introduce multiple copies of the gene, encoding the *B. clausii* alkaline protease, into the chromosome of the *B. subtilis* host.

The modification employed a method by which only an expression cassette, consisting of the gene and the chloramphenicol resistance marker gene from plasmid pC194 (originally isolated from *S. aureus* but widely recognised to be naturally present in Bacillus), is introduced into the host genome, at the site of the endogenous alkaline protease aprE gene, without any vector sequences remaining in the final production strain.

Full details of the genetic modifications and stability of the inserted genes are provided in Appendix E1-E3. Note that this information is proprietary and "**Confidential Commercial Information**" status is requested.

4. Dietary exposure

Refer to Appendix C for further details

4.1. List of food or food groups likely to contain the enzyme or its metabolites

Subtilisin is intended for use in any foods which naturally contain protein. Typical processes which typically may employ Subtilisin for protein hydrolysis include:

- Baking
- Dairy processing
- Egg processing
- Meat and fish processing
- Protein processing
- Yeast processing

4.2. Levels of residues in food

The proposed application rate of Subtilisin in its intended application is listed below.

	Maximum use rate (kg Product /MT substance)	Resulting Exposure (mg TOS/kg RM)
Protein Processing	10	569

IFF expects the Subtilisin to be inactivated or removed during the subsequent production and refining processes for all applications.

In a variety of foods, Subtilisin performs a technological function to hydrolyse proteins into peptides. The Subtilisin is expected to be deactivated or not present in the final food.

The most appropriate way to estimate the human consumption in the case of food enzymes is using the Budget Method (Hansen, 1966; Douglass *et al.*, 1997). This method enables one to calculate a Theoretical Maximum Daily Intake (TMDI) based on conservative assumptions regarding physiological requirements for energy from food and the energy density of food rather than on food consumption survey data. The Budget Method was originally developed for determining food additive use limits and is known to result in conservative estimations of the daily intake.

Based on the raw materials used in the various food processes, the recommended use levels of the enzyme Subtilisin, for the calculation of the TMDI, the maximum use levels are chosen. The TMDI is calculated on basis of the maximal values found in food and beverages multiplied by the average consumption of food and beverages per kg body weight/day. Consequently, the TMDI will be: 2.73 mg TOS/kg body weight/day. The NOAEL has been determined for Subtilisin to be at 480.6 mg TOS/kg bw/day). Based on a worst-case scenario of daily food consumption, the NOAEL would offer a 175-fold margin of safety. It should be stressed that this Total TMDI is based on conservative assumptions and represents a highly exaggerated value. Please refer to Appendix C for details.

4.3. Likely level of consumption of foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs)

Not applicable. Subtilisin 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.

4.4. 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

There is no information on the expected use of this enzyme preparation in Australia/New Zealand or imported product currently sold in Australia/New Zealand. Zealand.

4.5. Levels of residues in food in other countries

Applications and levels of use of the Subtilisin preparation in other countries is the same as presented in section 4.2.

4.6. Likely current food consumption for foods where consumption has changed in recent years

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

5. References

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