An Aminopeptidase Enzyme Preparation

Produced With

Trichoderma reesei

Expressing the Aminopeptidase Gene

From

Aspergillus clavatus

Is Generally Recognized As Safe

For Use in Food Processing

Where science
& creativity meet

Notification Submitted by Danisco US Inc.
(a Wholly Owned-Subsidiary of
International Flavors & Fragrances)

December 30, 2021

TABLE OF CONTENTS

1. GENERAL INTRODUCTION, STATEMENT AND CERTIFICATION

In accordance with 21 C.F.R. §170. 225, Danisco US Inc. submits this GRAS Notice for an aminopeptidase produced by submerged fermentation of *Trichoderma reesei* carrying the gene encoding the aminopeptidase enzyme from *Aspergillus clavatus*.

Aminopeptidase catalyzes the cleavage of the N-terminal peptide bond in proteins and peptides, and releases of an N-terminal residue (amino acid, Xaa+Yaa-). The enzyme will be used in protein processing, yeast processing, and flavoring production. In these applications, the aminopeptidase will be used as a processing aid and will either not be present or will be present in insignificant quantities as inactive residue, having no function or technical effect in the final food.

The accepted name of this enzyme is aminopeptidase.

The systematic name of the principle enzyme activity is also aminopeptidase.

The IUB nomenclature is aminopeptidase.

Synonoms of aminopeptidase include: aminopolypeptidase, and peptidase, amino-.

The EC number of the enzyme is 3.4.11.15, and the CAS number is 114796-97-3.

Our safety evaluation is consistent with the recent publication by the Enzyme Technical Association (Sewalt *et. al*., 2016),[1] which includes an evaluation of the production strain, the enzyme, and the manufacturing process, as well as a determination of dietary exposure. This generally recognized methodology, based on the decision tree by Pariza and Johnson (2001) and inclusive of published safety information, provides the common knowledge element of the GRAS status to the FDA (Sewalt *et al.,* 2017).[2]

The safety of the production organism is the prime consideration in assessing the safety of an enzyme preparation intended for food uses (Pariza & Johnson, 2001; Pariza & Foster, 1983). An essential aspect of the safety evaluation of enzymes produced with genetically engineered microorganisms is the identification and characterization of the inserted genetic material (Pariza & Johnson, 2001; Pariza & Foster, 1983; IFBC, 1990; SCF, 1991; OECD, 1993; Berkowitz & Maryanski, 1989). The safety evaluation shows no evidence to indicate that any of the cloned DNA sequences and incorporated DNA code for or express a harmful toxic substance.

[1] https://doi.org/10.1089/ind.2016.0011
[2] https://www.sciencedirect.com/science/article/abs/pii/S0278691517303605?via%3Dihub

1.1 § 170.225 (c)(2) Name and Address of Notifier

Danisco US Inc.
(A Wholly Owned-Subsidiary of International Flavors and Fragrances)
925 Page Mill Road
Palo Alto, CA 94304
United States

1.2 § 170.225 (c)(3) Common or Usual Name of Substance

The aminopeptidase enzyme preparation is produced with a *Trichoderma reesei* strain expressing the gene encoding the aminopeptidase from *Aspergillus clavatus.*

1.3 § 170.225 (c)(4) Applicable Conditions of Use

The aminopeptidase is intended to be used as a processing aid in protein processing at 140-2,125 mg TOS/kg protein, in yeast processing at 723-4,340 mg TOS/kg yeast, and flavoring production at 140-7,024 mg TOS/kg protein.

1.4 §170.225 (c)(5) Basis for GRAS Determination

This GRAS Notice is based upon scientific procedures in accordance with 21 C.F.R. §170.30 (a) and (b).

1.5 §170.225 (c)(6) Exemption from Pre-Market Approval

Pursuant to the regulatory and scientific procedures established in 21 C.F.R. §170.325, Danisco US Inc. has determined that its aminopeptidase enzyme preparation produced with a genetically engineered strain of *T. reesei* expressing the aminopeptidase enzyme from *A. clavatus* is a Generally Recognized As Safe ("GRAS") substance for the intended applications (protein processing, yeast processing, and flavoring production) and is, therefore, exempt from the requirement for pre-market approval.

1.6 §170.225 (c)(7) Availability of Information for FDA Review

A notification package providing a summary of the information that supports this GRAS determination is enclosed with this notice. The package includes a safety evaluation of the production strain, the enzyme, and the manufacturing process, as well as an evaluation of dietary

exposure. The complete data and information that are the basis for this GRAS determination are available for review and copying at 925 Page Mill Road, Palo Alto, CA 94304 during normal business hours or can be sent to the Food and Drug Administration upon request.

1.7 §170.225 (c)(8) and (c)(9) Disclosure and Certification

This GRAS notice does not contain any data and/or information that is exempt from disclosure under the Freedom of Information Act (FOIA; 5 U.S.C §552).

We confirm that the data and information in this GRAS notice satisfactorily addresses Part 2-7 of a GRAS notice per 21 C.F.R. §170.230 to 170.255 as copied below.

§170.230	Part 2 of a GRAS Notice:	Identity, method of manufacture, specifications, and physical or technical effects
§170.235	Part 3 of a GRAS Notice:	Dietary exposure
§170.240	Part 4 of a GRAS Notice:	Self-limiting levels of use
§170.245	Part 5 of a GRAS Notice:	Experience based on common use in food before 1958
§170.250	Part 6 of a GRAS Notice:	Narrative
§170.255	Part 7 of a GRAS Notice	List of supporting data and information in your GRAS notice

Danisco US Inc. certifies that to the best of our knowledge this GRAS notice is a complete, representative, and balanced submission that includes unfavorable and favorable information known to us as well as relevant to the evaluation of the safety and GRAS status of the use of the notified substance.

December 30, 2021

Annie Han — Date
Global Regulatory Affairs
Danisco US Inc. (A Wholly Owned-Subsidiary of International Flavors & Fragrances)
925 Page Mill Road
Palo Alto, CA 94304
Work: 650-846-4040
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2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATION AND PHYSICAL OR TECHNICAL EFFECT

2.1 PRODUCTION ORGANISM

2.1.1 Production Strain

The production organism is a strain of *Trichoderma reesei*, that has been genetically engineered by Danisco US Inc. to overexpress an aminopeptidase gene from *Aspergillus clavatus.*

T. reesei has a long history of safe use in industrial scale enzyme production. The safety of this species as an industrial enzyme producer has been reviewed by Nevalainen *et al.* (1994), Blumenthal (2004), Olemska-Beer *et al.* (2006), and Frisvad *et al.* (2018). The organism is considered non-pathogenic for humans and does not produce fungal toxins or antibiotics under conditions used for enzyme production. It is generally recognized as a safe production organism and is the source organism of a range of enzyme products that are used as processing aids in the international food and feed industries. *T. reesei* is classified as a Biosafety Level 1 (BSL1) microorganism by the American Type Culture Collection (ATCC) based on assessment of the potential risk using U. S. Department of Public Health guidelines with assistance provided by ATCC scientific advisory committees, and also considered as suitable for Good Industrial Large Scale Practice (GILSP) worldwide and meets the criteria for a safe production microorganism as described by Pariza and Johnson (2001).

The production strain contains copies of a synthetic codon optimized gene encoding an aminopeptidase placed under the control of the highly efficient promoter and terminator sobtained from endogenous *T. reesei* CBHI encoding gene. Copies of the expression cassette were integrated into the recipient chromosome by using the native *Trichoderma reesei pyr2* gene and the *Aspergillus nidulans amdS* gene as selectable markers.

2.1.2 Recipient Organism

The host organism *T. reesei* strain RL-P37 was obtained from Dr. Montenecourt. The derivation and characterization of strain RL-P37 has been published (Sheir-Neiss and Montenecourt, 1984). Strain RL-P37 is a cellulase over-producing strain that was obtained through several classical mutagenesis steps from the wild-type *T. reesei* strain (QM6a). Strain QM6a is present in several public culture collections, *e.g.* in the American Type Culture Collection as ATCC 13631. *T. reesei* has more recently been identified as a clonal derivative or anamorph of *Hypocrea jecorina* (Khuls *et al*., 1996; Dugan, 1998).

2.1.3 Aminopeptidase Expression Plasmid

The genetic modification of the *T. reesei* host involved recombinant DNA techniques to introduce multiple copies of the gene encoding the *A. clavatus* aminopeptidase gene into the *T. reesei* host.

The expression cassettes consisted:

- native *Trichoderma reesei cbh1* (cellobiohydrolase) promoter
- the codon optimized *A. clavatus* aminopeptidase encoding gene
- native *Trichoderma reesei cbh1* terminator
- endogenous *Trichoderma reesei pyr2* or exogenous *Aspergillus nidulans amdS* gene used as a selectable marker.

All these modifications were performed in such a way that no bacterial vector DNA remains present in the strain. No antibiotic resistance markers were inserted into the new microorganism. The genetic construction was evaluated at every step to assess the incorporation of the desired functional genetic information and the final construct was verified by Southern blot analysis and PCR analyses to confirm that only the intended genetic modifications to the *T. reesei* strain had been made.

2.1.4 Stability of the Introduced Genetic Sequences

The production strain is expected to be stable after industrial scale fermentation as chromosomal integration of the aminopeptidase gene is generally recognized as a stable transformation method compared to expression systems such as plasmids.

2.1.5 Absence of Production Microorganism in Product

Absence of the production organism in the product is a standard product specification, which is confirmed with an analytical method that has a detection limit of <1 CFU/g of product.

2.2 ENZYME IDENTITY AND SUBSTANTIAL EQUIVALENCE

2.2.1 Enzyme Identity

Classification	Aminopeptidase
IUB Nomenclature	Aminopeptidases
IUB Number	3.4.11.15

CAS Number	114796-97-3
Reaction catalyzed	Catalyze the cleavage of the N-terminal peptide bond in proteins and peptides and release of an N-terminal residue (amino acid, Xaa Yaa-)
Molecular weight	50 kDa

2.2.2 Amino Acid Sequence

The published amino acid sequence of aminopeptidase from *Aspergillus clavatus* NRRL.1 is known and included in Appendix 1, which is 100% identical to aminopeptidase Y from *Aspergillus clavatus* NRRL.1 (XP 001273779.1).

2.3 MANUFACTURING PROCESS

This section describes the manufacturing process for this aminopeptidase enzyme which follows standard industry practice (Kroschwitz, 1994; Aunstrup *et al*., 1979; Aunstrup, 1979). For a diagram of the manufacturing process, see Appendix 2. The quality management system used in the manufacturing process complies with the requirements of ISO 9001. The enzyme preparation is also manufactured in accordance with FDA's current Good Manufacturing Practices ("cGMP") as set forth in 21 C.F.R. §110.

2.3.1 Raw Materials

The raw materials used in the fermentation and recovery process for this aminopeptidase concentrate are standard ingredients used in the enzyme industry (Kroschwits, 1994; Aunstrup *et al.,* 1979; Aunstrup, 1979). All the raw materials conform to the specifications of the Food Chemicals Codex (FCC), 12th edition (US Pharmacopeia, 2020), except for those raw materials that do not appear in the FCC. For those not appearing in the FCC, internal requirements have been set in line with FCC and acceptability of use for food enzyme production. Danisco US Inc. uses a supplier quality program to qualify and approve suppliers. Raw materials are purchased only from approved suppliers and are verified upon receipt.

The antifoam (also known as defoamers) and flocculant used in the fermentation and recovery is used in accordance with cGMP per the Food and Drug Administration (FDA) correspondence to Enzyme Technical Association (ETA) acknowledging the listed antifoam dated September 11, 2003.

Regarding potential major food allergens, glucose (which may be derived from wheat) will be used in the fermentation process and is consumed by the microorganism as a nutrient. No other major allergen substances will be used in the fermentation, recovery process, and the formulation of the product.

2.3.2 Fermentation Process

The aminopeptidase enzyme is manufactured by submerged fermentation of a pure culture of the genetically engineered strain of *T. reesei* described in Part 2. All equipment is carefully designed, constructed, operated, cleaned, and maintained to prevent contamination by foreign microorganisms. During all steps of fermentation, physical and chemical control measures are taken, and microbiological analyses are conducted periodically to ensure absence of foreign microorganisms and confirm production strain identity.

2.3.3 Recovery Process

The recovery process is a multi-step operation, which starts immediately after the fermentation process.

The enzyme is recovered from the culture broth by the following series of operations:

1. Primary separation –centrifugation or filtration;
2. Concentration – ultrafiltration;
3. Addition of stabilizers/preservatives; and
4. Polish filtration.

2.3.4 Formulation and Standardization Process

The ultra-filtered concentrate is stabilized by final formulation to contain 40% glycerol, 5% sodium chloride, 0.2% sodium benzoate, 0.2% potassium sorbate, and 0.1% calcium chloride. The remaining portion of the formulation is water.

The final aminopeptidase liquid concentrate is analyzed in accordance with the general specifications for enzyme preparations used in food processing as established by the Joint FAO/WHO Expert Committee on Food Additives ("JECFA") in 2006 and FCC, 12th edition (USP, 2020). These specifications are set forth in Section 2.4.

2.4 COMPOSITION AND SPECIFICATIONS

2.4.1 Quantitative Composition

The liquid concentrate is stabilized with formulation ingredients listed below and tested to demonstrate that it meets the specification. Various commercial formulations exist with a range of enzyme activities. The following is a representative composition for the commercialized product:

Glycerol	30 - 40% w/w
Aminopeptidase	13 - 15% w/w
Potassium sorbate	0.15 - 0.25% w/w
Sodium benzoate	0.15 - 0.25% w/w
Calcium chloride	0.08 - 0.12% w/w

The remainder is water.

The preparation includes TOS (total organic solids resulting from fermentation), which is approximately 26.7% of the liquid concentrate.

2.4.2 Specifications

As mentioned, aminopeptidase preparation meets the purity specifications for enzyme preparations set forth in FCC, 12th edition (USP, 2020). In addition, it also conforms to the General Specifications for Enzyme Preparations Used in Food Processing as proposed by JECFA (2006).

The results of analytical testing of the 3 lots of product is given in Appendix 3 verifying that it meets USP (2020) and JECFA (2006) specifications for enzyme preparations.

2.5 APPLICATION

2.5.1 Mode of Action

As noted above, the function of aminopeptidase is to catalyze the cleavage of the N-terminal peptide bond in proteins and peptides.

2.5.2 Use Levels

The aminopeptidase is intended to be used as a processing aid in protein processing at 140-2,125 mg TOS/kg protein, in yeast processing at 723-4,340 mg TOS/kg yeast, and flavoring production at 140-7,024 mg TOS/kg protein.

Protein processing:

Aminopeptidase can be used in protein processing for production of protein hydrolysates which can be further processed to provide liquid or powdered protein hydrolysates to be used in a wide range of food products. Aminopeptidase can be added to the protein hydrolysis step. The proposed application rate of aminopeptidase in protein processing is 140-2,125 mg TOS/kg protein.

Yeast processing:

Aminopeptidase can be used in the production of processed yeast products such as but not limited to, yeast autolysates, yeast extracts, and yeast cell walls. Processed yeast products are predominantly used as savory ingredients in a variety of food products, including bouillon cubes or powder, sauces, gravy, ready to consume meals, and processed cheese. Aminopeptidase can be added in the autolysis step. The proposed application rate of aminopeptidase in yeast processing is 723-4,340 mg TOS/kg yeast.

Flavoring production:

Aminopeptidase can be used in the production of flavoring substances. These substances can be used as ingredients in a wide variety of final foods and drinks (*e.g.*, bouillon cubes or powder, sauces, gravy, ready to consume meals, and processed cheese). The proposed application rate of aminopeptidase in flavorings production is 140-7,024 mg TOS/kg protein.

2.5.3 Enzyme Residues in the Final Applications

Aminopeptidase will be deactivated or removed during the subsequent production and refining processes for all applications. In the rare case that inactive aminopeptidase enzyme is present in the processed food and is ingested, it will not be absorbed intact. Instead, the enzyme is expected to be broken down by the digestive system into small peptides and amino acids, with the latter being absorbed and metabolized, which is not expected to pose any human health risk.

3. DIETARY EXPOSURE

Aminopeptidase will be used as a processing aid in protein processing, yeast processing, and flavoring production. The table below shows the range of recommended use levels for each application where the food enzyme may be used:

Application	Raw material (RM)	Recommended use levels (mg TOS/kg RM)	Maximal recommended use levels (mg TOS/kg RM)
Protein processing	Proteins	140-2,125	2,125
Yeast processing	Yeast culture or yeast extract, cell walls and autolysed yeast	723-4,340	4,340
Flavorings production	Material of vegetable, animal or microbial origin	140-7,024	7,024

While we expect the aminopeptidase to be absent 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 exposure to aminopeptidase via protein processing, yeast processing, and flavoring production is outlined below via the Budget Method (Hansen, 1966; Douglass *et al.*, 1997). This method has been used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001). The method enables 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. The Budget Method is based on the following assumed consumption of targeted important foodstuffs and beverages (for less important foodstuffs, *e.g.,* snacks, lower consumption levels are assumed). The assumption is for Processed food (50% of total solid food) and for soft drinks (25% of total beverages).

Average consumption over the course of a lifetime/kg body weight/day	Total solid food (kg)	Total non-milk beverages (l)	Processed food (50% of total solid food) (kg)	Soft drinks (25% of total beverages) (l)
	0.025	0.1	**0.0125**	**0.025**

The recommended use level of the aminopeptidase is given, based on the raw materials used in the food process. The calculation considers how much solid or liquid food is obtained per kg raw material, and it is assumed that all the TOS will end up in the final product. Therefore, the concentration of TOS from aminopeptidase in the applications can be calculated/summarized as in the table below:

Application		Raw material (RM)	Maximal use level (mg TOS/kg RM)	Example Final food (FF)	Ratio RM/FF	Maximal level in FF (mg TOS/kg food)
Solid food	Protein processing	Proteins	2,125	Protein hydrolysates used in *e.g.* soups, bouillons, dressings.	0.085	180.6
	Yeast processing	Yeast culture, yeast extract, cell walls, autolysed yeast	4,340	Savoury snacks, ready meals, noodles, processed cheese, soups and bouillons etc.	0.02	86.8
	Flavorings production	Material of vegetable, animal or microbial origin	7,024	Savoury snacks, ready meals, noodles, processed cheese, soups and bouillons etc.	0.01	70.24

HUMAN EXPOSURE ASSESSMENT

In this assessment, the Budget method is used. This method was previously used by JECFA (FAO/WHO, 2001) and contains the following assumptions:

1) Level of consumption of foods and beverages:

For solid foods, the daily intake is set at 25 g/kg bw based on a maximum lifetime energy intake of 50 Kcal/kg bw/day. For non-milk beverages, a daily consumption of 100 ml/kg bw is used corresponding to 6 liters per day for a 60-kg adult.

2) Concentration of enzymes in foods and beverages:

The concentration of enzyme in foods and beverages is the maximum application rate.

3) Proportion of foods and beverages that contain the enzymes:

a) A default of 50% of all solid foods is used to represent processed foods (*i.e.,* 12.5 g/kg bw/day).

b) A default of 25% is used to represent non-milk beverages that may contain the enzyme (*i.e.,* 25 ml/kg bw/day).

4) Estimation of the theoretical maximum daily intake (TMDI)

To represent a worst-case scenario, TMDI for solid foods will be combined with the TMDI for beverages in the risk assessment. The TDMI from protein processing represents the worse-case scenario and will be used in the Human Intake Risk Assessment.

TMDI in food (mg TOS/kg body weight/day)	**TMDI in beverage (mg TOS/kg body weight/day)**	**Total TMDI (mg TOS/kg body weight/day)**
180.6x0.0125=2.26	NA	**2.26**

4. SELF-LIMITING LEVELS OF USE

As the enzyme will be used as processing aid in the food manufacturing process, there is no notable oral intake for humans. Therefore, self-limiting levels of use are not applicable.

In addition, as a processing aid the use levels are limited for economical reasons. The customers are unlikely to use more enzyme than is needed to achieve the technical effects in order to minimize production costs.

5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Information regarding this enzyme's common use in food before 1958 is not provided as the statutory conclusion of our GRAS status, which is based on scientific procedures rather than common use before 1958.

6. SAFETY EVALUATION

6.1 SAFETY OF THE PRODUCTION STRAIN

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 non-toxigenic 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." *T. reesei* strains used in enzyme manufacture meet these criteria for non-toxigenicity and non-pathogenicity.

6.1.1 Safety of the host

T. reesei was first isolated from nature in 1944. The original isolate, QM6a, and its subsequent derivatives have been the subject of intense research due to their usefulness in the production of cellulases. In the 1980s, it was suggested by Bissett (1984) that *T. reesei* be placed into synonymy with *Trichoderma longibrachiatum*. Later however, evidence emerged indicating that the two species are not identical (Meyer *et al.,* 1992; Dugan, 1998). The proposal by Khuls *et al.* (1996) that *T. reesei* was a clonal derivative of *Hypocrea jecorina* is being generally accepted in the scientific community, and the US National Center for Biotechnology Information (NCBI) refers to *T. reesei* as the anamorph of *H. jecorina*. Therefore, the names *T. reesei* and *H. jecorina* are in

use in the scientific literature to refer to essentially the same microorganism species (Samuels *et al*, 2012). Unfortunately, the name *T. longibrachiatum* is also still used in various regulations (including 21 C.F.R. §184.1250) and various enzyme positive lists around the globe and continued use of this name as a synonym for *T. reesei* has begun to result in questions from regulators as *T. longibrachiatum* is increasingly associated with infection of immune-compromised individuals. The U.S. EPA's risk assessment on *T. reesei* (Federal Register / Vol. 77, No. 172 / September 5, 2012 / pages 54499-54411) stresses that it is not the species associated with infection of immune-compromised individuals, but rather this is *T. longibrachiatum*, hence the continued use on various national and international regulatory positive lists of *T. longibrachiatum* rather than *T. reesei* as an approved / acceptable enzyme production host needs to be revisited.

A review of the literature search on the organism (1972-2021) uncovered no reports that implicate *T. reesei* in any way with a disease situation, intoxication, or allergenicity among healthy adult human and animals. The species is not present on the list of pathogens used by the EU (Council Directive 90/679/EEC, as amended) and major culture collections worldwide. It is classified as Biosafety Level 1 (BSL1) microorganism by the American Type Culture Collection (ATCC) based on assessment of the potential risk using U.S. Department of Public Health guidelines with assistance provided by ATCC scientific advisory committees. BSL1 microorganisms are not known to cause diseases in healthy adult humans.

Brückner and Graf (1983) reported the isolation from *T. reesei* strain QM9414 a peptaibol compound (*e.g.,* paracelsin) that exhibited antibiotic activity. Their work was confirmed by another group that found evidence of peptaibol production in two other *T. reesei* strains (Solfrizzo *et al*., 1994). However, peptaibols' antibiotic activity is clinically and commercially irrelevant and the growth conditions under which the compounds were produced are very different from those in standard enzyme manufacturing. Frisvad *et al.* (2018) reviewed the secondary metabolite potential of the major fungal species including *T. reesei* and concluded that *Trichoderma reesei* cannot produce any recognized mycotoxins and is one of the most important production organisms for safe enzyme production in the industry.The US EPA published a risk assessment (EPA, 2012) to support tiered exemption status for *T. reesei* QM6a and its derivatives (including QM9414), in which the Agency acknowledged that under normal submerged fermentation conditions paracelsin is not produced. Strain QM9414 and its derivatives have been safe producers of commercial cellulase enzyme preparations for food applications. The enzyme manufacturers still confirm the industrial enzyme preparations do not to have antibiotic activity per the specifications recommended by the JECFA (2006).

Trichoderma reesei has a long history of safe use in industrial scale enzyme production. The safety of this species as an industrial enzyme producer has been reviewed by Nevalainen *et al*. (1994), Blumenthal (2004), Olemska-Beer *et al.* (2006), and Frisvad *et al.* (2018). The organism is

considered non-pathogenic for humans and does not produce fungal toxins or antibiotics under conditions used for enzyme production. It is generally considered a safe production organism and is the source organism of a range of enzyme products that are used as processing aids in the international food and feed industries. It is listed as a safe production organism for cellulases by Pariza and Johnson (2001) and Olempska-Beer *et al.* (2006). Various strains have been reviewed in approval dossiers for commercial enzyme products internationally, for example, in Canada (Food and Drugs Act Division 16, Table V, List of Permitted Food Enzymes), the United States (21 C.F.R. §184.1250), Mexico, Brazil, France, Denmark, Australia/New Zealand, China, and Japan. To date, at least 23 enzymes produced in *T. reesei* have been notified to FDA/CFSAN as GRAS for their intended uses and received a "no questions" letter,[1] of which eight were for enzymes produced by members of Danisco's *T. reesei* Safe Strain Lineage.

The production organism of the aminopeptidase enzyme preparation, the subject of this submission, is *T. reesei* strain [redacted] which was produced from strain *T. reesei* RL-P37 using recombinant DNA methods. The purpose of this genetic modification is to express the aminopeptidase from *A. clavatus* in *T. reesei* RL-P37, a commercial production strain produced from several classical mutagenesis steps from the well-known wild-type strain QM6a. Virtually all *T. reesei* strains used all over the world for industrial cellulase production today are derived from QM6a. Danisco US, Inc. has used strain RL-P37 to produce cellulases for over fifteen years and has developed many production strains from it using recombinant DNA techniques. The strain has been determined to be non-pathogenic and non-toxigenic through an acute intraperitoneal study in rats. All the food/feed grade products produced by this lineage were determined to be safe for their intended uses and are the subject of numerous GRAS determinations. Eight GRAS Notices were filed for the products from this strain lineage, in which FDA issued "no questions" letters (see GRN 230, GRN 315, GRN 333, GRN 372, GRN 567, GRN 703, GRN 727, GRN 808, and GRN964).[2]

From the information reviewed, it is concluded that the organism *T. reesei* strain provides no specific risks to human health and is safe to use as the production organism of the aminopeptidase. The strain is therefore non-pathogenic and non-toxigenic.

[1] http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=basic&search=reesei
[2] https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices

6.1.2 Safety of the Donor Source

The source for the aminopeptidase is *Aspergillus clavatus*. However, no actual strain was used as donor. The published amino acid sequence of aminopeptidase from *Aspergillus clavatus* NRRL 1 was used to synthetisize a gene ([redacted]) codon optimized for expression in *Trichoderma reesei*.

The species *Aspergillus clavatus* is a deuteromycetes with a full taxonomic lineage as:

> *Cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; Eurotiomycetes, Eurotiomycetidae, Eurotiales, Aspergillaceae, Aspergillus, Aspergillus clavatus*

The species *A. clavatus* is found in soils and animal manure (Al-Doory Y and Domson JF, 1984). *Aspergillus clavatus* NRRL 1 is Biosafety Level of 1 in the ATCC database.[1]

A literature search was conducted on October 05, 2021 in PubMED using the searching term "*Aspergillus clavatus*" and "food safety OR toxin OR toxicology OR pathogen" on PubMED resulting in 70 records, which were reviewed and several of which were used in a brief literature review below.

Aspergillus clavatus is a fungus of the genus *Aspergillus*. Black-spored Aspergillus section Nigri species has been identified for production of the mycotoxins ochratoxin A (OTA) and fumonisin B2 (FB2) which are toxic for human and animals. Ochratoxins and fumonisins are a small group of chemically related toxic fungal metabolites (mycotoxins).

The pathogenicity of *A. clavatus* is not relevant as only one published amino acid sequence of aminopeptidase from *Aspergillus clavatus* NRRL 1 was used to synthetisize a gene codon optimized for expression in *Trichoderma reesei*. The full genome sequence of this strain is available at the U.S. Department of Energy (DOE) Joint Genome Institute (JGI).[2]

Aspergillus nidulans acetamidase (*amdS*) gene was used as a selectable marker, to enable growth on acetamide medium. Only the *amdS* gene in isolated form was used. The gene was first described by Hynes *et al*. (1983). The strain was not described further than "a strain of genotype biA1" but it is certainly a derivative of the original *Aspergillus nidulans* isolate (Glasgow wild-type) deposited as strain A4 at the Fungal Genetics Stock Center, Kansas City, USA. Meanwhile, the description of the gene in GenBank (Accession number M16371) mentions the Glasgow wild-type

[1] https://genomes.atcc.org/genomes/5c93dfc481894c67
[2] https://mycocosm.jgi.doe.gov/Aspcl1/Aspcl1_home.html

Aspergillus nidulans strain as the source. Sequencing and PCR experiments verified that the gene Danisco US Inc. used is the same as published by Corrick *et al*. (1987).

6.2 SAFETY OF THE MANUFACTURING PROCESS

The manufacturing process to produce aminopeptidase will be conducted in a manner similar to other food and feed enzyme production processes. It consists of a pure-culture fermentation process, cell separation, concentration, and formulation, resulting in a liquid aminopeptidase enzyme preparation. The process is conducted in accordance with food good manufacturing practice (GMP) as set forth in 21 C.F.R. Part 110. The resultant product meets the purity specifications for enzyme preparations of the Food Chemicals Codex, 12th Edition (US Pharmacopeia, 2020) and the general specifications for enzyme preparations used in food processing proposed by FAO/WHO (JECFA, 2006).

Regarding potential major food allergens, glucose (which may be derived from wheat) will be used in the fermentation process and is consumed by the microorganism as a nutrient. No other major allergen substances will be used in the fermentation, recovery processes, or formulation of this product.

6.3 SAFETY OF AMINOPEPTIDASE

The aminopeptidase enzyme, the subject of this GRAS Notice is produced with a non-pathogenic, non-toxigenic strain of *Trichoderma reesei* (formerly *Trichoderma longibrachiatum*), which has been genetically engineered by Danisco US Inc. to overexpress the aminopeptidase gene from *Aspergillus clavatus*. Aminopeptidases derived from other microorganisms are approved in France, Australia, Canada, China, Brazil and Mexico. Additionally, FDA issued "No Questions" letter for several peptidases (IUBMB 3.4.x.x) (GRN 345, GRN 817, and GRN 832).

6.3.1 Allergenicity

According to Pariza and Foster (Pariza and Foster 1983), there have been no confirmed reports of allergies in consumers caused by enzymes used in food processing.

In 1998 the Association of Manufacturers of Fermentation Enzyme Products (AMFEP, 1998) Working Group on Consumer Allergy Risk from Enzyme Residues in Food reported on an in-depth analysis of the allergenicity of enzyme products. They concluded that there are no scientific indications that small amounts of enzymes in bread and other foods can sensitize or induce allergy reactions in consumers, and that the enzyme residues in bread and other foods do not represent any unacceptable risk to consumers. Further, in an investigation of possible oral allergenicity of 19

commercial enzymes used in the food industry, there were no findings of clinical relevance even in individuals with inhalation allergies to the same enzymes, and the authors concluded "that ingestion of food enzymes in general is not considered to be a concern with regard to food allergy" (Bindslev-Jensen *et al*., 2006).

Despite this lack of general concern, the potential that aminopeptidase could be a food allergen was assessed by comparing the amino acid sequence with sequences of known allergens in a public database, which is described in more detail below. To conduct the bioinformatic analysis of aminopeptidase, three FASTA searches were performed: 1) a full-length amino acid sequence search and 2) a sliding 80-amino acid window search, and 3) an 8-amino acid search. Based on the sequence homology alone, it was concluded that the aminopeptidase is unlikely to pose a risk of food allergenicity.

The most current allergenicity assessment guidelines developed by the Codex Commission (2009) and Ladics *et al.* (2011) 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) 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 to eight, 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 aminopeptidase seuquence is give in Appendix 1. A full length amino acid sequence search with greater than 35% identity and an E-value of < 0.1 to known allergens using the Food Allergy Research and Resource Program (FARRP) on the AllergenOnline database[1]February 14, 2021 V20 which contains 2233 peer-reviewed allergen sequences[2] confirmed no hits.

[1] http://www.allergenonline.org/index.shtml
[2] http://www.allergenonline.org/databasebrowse.shtml

There was also no match to allergens by identity across 80 amino acids exceeding 35%. FASTA alignment of the above sequence with known allergens also using the AllergenOnline database[1] revealed no match (using E-value <0.1 as the cut-off) to sequences in the database using the full sequence search capabilities.

Although cautioned in Codex Commission (2009), researched by Herman *et al*. (2009) and further elaborated by Ladics *et al.* (2011) and on AllergenOnline.org that there is no evidence that a short contiguous amino acid match will identify a protein that is likely to be cross-reactive and that could be missed by the conservative 80 amino acid match (35%), this database does allow for isolated identity matches of 8 contiguous amino acids to satisfy demands by some regulatory authorities for this precautionary search. Performing the 8 contiguous amino acids search produced no sequence matches with known allergens.

Microbial enzymes acting environmental allergens have yet to be conclusively demonstrated to be active via the oral route. This concept was evaluated extensively in a recently published study (Bindslev-Jensen *et al.*, 2006) that failed to indicate positive reactions to 19 orally challenged commercial enzymes in a double-blind placebo controlled food challenge study with subjects with positive skin prick tests for the same allergens. The authors concluded that positive skin prick test results are of no clinical relevance to food allergenicity, and that ingestion of food enzymes in general is not a food allergy concern.

In conclusion, based on the sequence homology alone, the *A. clavatus* aminopeptidase produced with *T. reesei* production strain is unlikely to pose a risk of food allergenicity.

6.3.2 Safety of Use

As noted in the Safety section 6.1, *T. reesei*, and enzyme preparations derived there from, including acid fungal protease, alpha-amylase, alpha-glucosidase, beta-glucanase, cellulase, chymosin, glucoamylase, lipase, phytase, transglucosidase, trehalase, and xylanase enzyme preparations, are well recognized by qualified experts as being safe. Published literature, government laws and regulations, reviews by expert panels such as JECFA, as well as Danisco US Inc.'s own unpublished safety studies, support a conclusion of safety.

[1] http://www.allergenonline.org/index.shtml

T. reesei is widely used by enzyme manufacturers around the world for the production of enzyme preparations for use in human food, animal feed, and numerous industrial enzyme applications. It is a known safe host for enzyme production.

In addition to the allergenicity assessment described above, the safety of amonopeptidase and production organism has also been established using the Pariza and Johnson (2001) decision tree:

1. Is the production strain[1] genetically modified?[2,3] Yes. *Go to 2.*

2. Is the production strain modified using rDNA techniques? Yes. *Go to 3a.*

3a. Does the expressed enzyme product which is encoded by the introduced DNA[4,5] have a history of safe use in food?[6] Yes. Aminopeptidases derived from other microorganisms are approved throughout the world, most notably *Lactococcus lactic*, *Aspergillus oryzae*, and *Aspergillus niger*. Aminopeptidases have been used in protein processing, brewing and cheese production. In addition, the enzyme will be inactivated in the food manufacture process. The safety of the enzyme in the intended uses was supported by 1) a thorough investigation of the aminopeptidase sequence, identifying no potential risk for food allergenicity, 2) the production strain pertains to *T. reesei* safe strain lineage. Go to 3c.

3c. Is the test article free of transferable antibiotic resistance gene DNA?[7] Yes. No antibiotic resistance genes were used in the construction. *Go to 3e.*

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[1] Production strain refers to the microbial strain that will be used in enzyme manufacture. It is assumed that the production strain is nonpathogenic, nontoxigenic, and thoroughly characterized; steps 6–11 are intended to ensure this

[2] The term "genetically modified" refers to any modification of the strain's DNA, including the use of traditional methods (e.g., UV or chemically-induced mutagenesis) or rDNA technologies.

[3] If the answer to this or any other question in the decision tree is unknown, or not determined, the answer is then considered to be NO.

[4] Introduced DNA refers to all DNA sequences introduced into the production organism, including vector and other sequences incorporated during genetic construction, DNA encoding any antibiotic resistance gene, and DNA encoding the desired enzyme product. The vector and other sequences may include selectable marker genes other than antibiotic resistance, noncoding regulatory sequences for the controlled expression of the desired enzyme product, restriction enzyme sites and/or linker sequences, intermediate host sequences, and sequences required for vector maintenance, integration, replication, and/or manipulation. These sequences may be derived wholly from naturally occurring organisms or incorporate specific nucleotide changes introduced by *in vitro* techniques, or they may be entirely synthetic.

[5] If the genetic modification served only to delete host DNA, and if no heterologous DNA remains within the organism, then proceed to step 5.

[6] Engineered enzymes are considered *not* to have a history of safe use in food, unless they are derived from a safe lineage of previously tested engineered enzymes expressed in the same host using the same modification system.

[7] Antibiotic resistance genes are commonly used in the genetic construction of enzyme production strains to identify, select, and stabilize cells carrying introduced DNA. Principles for the safe use of antibiotic resistance genes in the

3e. **Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products?** Yes, inserted DNA is well characterized and free of unsafe attributes. *Go to 4.*

4. Is the introduced DNA randomly integrated into the chromosome? Yes. *Go to 5.*

5. Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects which may result in the synthesis of toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed? Yes. The inserted DNA is well characterized. The production strain does not produce toxic metabolites of concern as confirmed by T-2 toxin analysis. *Go to 6.*

6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?[1] Yes. The *T. reesei* production strain pertains to the *T. reesei* safe strain lineage (Appendix 4). *T. reesei* safety as a production host and methods of modification are well documented and their safety has been confirmed through toxicology testing (Appendix 5).

Conclusion: Article is accepted.

6.3.3 Safety Studies

Aspergillus clavatus aminopeptidase is an enzyme preparation produced with *T. reesei* that can be used as a processing aid in protein processing, yeast processing, and flavoring processing.

Danisco US Inc. has determined by scientific procedures that this production organism *T. reesei* pertains to a safe strain lineage (Appendix 4). A review of all toxicology studies conducted with enzyme preparations produced by different strains of Danisco US Inc.'s *T. reesei* (Appendix 5) indicates that, regardless of the production organism strain, all enzyme preparations were found to have the following conclusions:

manufacture of food and feed products have been developed (IFBC, 1990; "FDA Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants

[1] In determining safe strain lineage one should consider the host organism, all of the introduced DNA, and the methods used to genetically modify the host (see text). In some instances the procedures described by Pariza and Foster (1983) and IFBC (1990) may be considered comparable to this evaluation procedure in establishing a safe strain lineage

1) Negative as a dermal irritant;
2) Negative as an ocular irritant;
3) Negative as a mutagen, clastogen, and aneugen in genotoxicity studies; and
4) Not observed to adversely affect any specific target organs.

Therefore, due to the consistency of the findings supporting the safety of enzyme preparations derived from different *T. reesei* strains, it is reasonable to expect that most enzyme preparation produced from *T. reesei* strains would have a similar toxicological profile (Appendix 5).

In addition to the decision tree analysis and the availability of multiple toxicology studies for the safe strain lineage, different endpoints of toxicity of this aminopeptidase were investigated as part of our safety program to satisfy international and external requirements globally. This battery of tests included:

A. 90-Day Repeated Dose Toxicity Study Administered by Oral Gavage in Sprague-Dawley Rats
B. *In Vitro* Mammalian Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL)
C. Bacterial Reverse Mutation Assay

The results are evaluated, interpreted, and assessed in this document. The test material, Ultra-Filtered Concentrate (UFC), used in all toxicology investigations has the following characteristic,

Lot No.:	4903281829
Physical:	Fermentation liquid, filtered
Enzyme:	Aminopeptidase
CAS No:	114796-97-3
Enzyme activity:	6321 KAPU/g
pH:	4.67
Specific gravity:	1.089 g/mL
Total protein (TP):	267.92 mg/g
TOS:	27.84%

A. Repeated Dose 90-day Oral toxicity in rats (2019)

The objective of this study was to investigate the potential toxicity of the aminopeptidase to induce systemic toxicity after repeated daily oral administration to Charles River CD rats of both sexes for 90 continuous days. Three groups of young adult male and female rats (10/sex/group) were dosed by oral gavage with Phytase B, diluted in deionized water, at doses of 250, 500, or 1000 mg

total organic solids (TOS)/kg body weight/day. The control group was dosed with deionized water. This study was conducted in accordance with OECD guideline No. 408 (June 2018).

No test article-related effects were reported among clinical observations, ophthalmic observations, body weight measurements, food consumption or food efficiency values, functional observation battery tests, locomotor activity evaluations, hematology, coagulation, clinical chemistry, hormone analysis, urinalysis parameters, or organ weight, macroscopic or microscopic pathology findings. Under the conditions of this study, the no-observed-adverse-effect-level (NOAEL) was established at the high dose of 1000 mg TOS/kg bw/day.

B. In vitro Mammalian Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL) (2019)

The purpose of this study was to evaluate the potential of aminopeptidase and/or its metabolites to induce structural chromosomal aberrations in Human Peripheral Blood Lymphocytes (HPBL) in the presence and absence of an exogenous metabolic activation system. A preliminary toxicity test was performed to establish the dose range for testing in the cytogenetic test. This assay was conducted in accordance with OECD guideline No. 473 (July 2016). In the preliminary toxicity assay, the highest concentration tested was the OECD recommended limit dose of 5000 µg/ml. The cells were exposed to 9 concentrations of the test substance ranging from 0.5 to 5000 µg/ml, as well as to a vehicle control. Test substance precipitation was not observed and substantial toxicity (i.e., ≥ 50% mitotic reduction in relation to the vehicle control) was not observed at any concentration in any test condition. The concentrations chosen for the assay ranged from 625 to 5000 µg/ml for all test conditions. Neither test substance precipitation nor substantial toxicity was observed at any concentration in any test condition. Under the conditions of the assay described in this test, aminopeptidase was concluded to be negative for the induction of structural and numerical chromosome aberrations in both the non-activated and S9-activated test systems. Aminopeptidase was negative in the In Vitro Mammalian Chromosome Aberration Assay in HPBL.

C. Bacterial reverse mutation assay (2019)

The test article, aminopeptidase was tested in the Bacterial Reverse Mutation Assay using Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 and Escherichia coli tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. This assay was conducted in accordance with OECD guideline No. 471 (1997). In the toxicity-mutation test, the maximum dose evaluated was 5000 µg/plate for the tester strains. All tester strains and test conditions were evaluated at 8 dose levels along with the negative (vehicle) and positive controls. The dose levels used in the test were 1.50, 5.00, 15.0, 50.0, 150, 500, 1500 and 5000 µg per plate

for all tester strains. No positive mutagenic responses were observed at any dose level or with any tester strain in either the absence or presence of S9 metabolic activation. No appreciable toxicity was observed at any dose level with any tester strain in either the absence or presence of S9. Based on the toxicity-mutation test, the maximum dose evaluated in the mutagenicity test was 5000 µg/plate for each of the tester strains in the absence and presence of S9 metabolic activation. All tester strains and test conditions were evaluated at 6 dose levels along with the negative (vehicle) and positive controls. The dose levels used were 15.0, 50.0, 150, 500, 1500 and 5000 µg/plate for all tester strains. No positive mutagenic responses, appreciable toxicity, or precipitation was observed at any dose level or with any tester strain in either the absence or presence of S9 metabolic activation. All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that, under the conditions of this study, aminopeptidase did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9. Therefore, the test article was concluded to be negative in this assay.

6.4 OVERALL SAFETY ASSESSMENT

6.4.1 Identification of the NOAEL

In the 90-day oral (gavage) study in rats for the aminopeptidase, a NOAEL was established at 1000 TOS kg bw/day. 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 *Aspergillus clavatus* aminopeptidase produced with *T. reesei* production strain is through oral ingestion, selection of this NOAEL is thus appropriate.

NOAEL: 1000 mg TOS/kg bw/day

6.4.2 Conclusion

Determination of the margin of safety

The margin of safety is calculated by dividing the NOAEL obtained from the 13-weeks oral (gavage) study in rats by the human exposure (worst case scenario). If the margin of safety is greater than 100, it suggests that the available toxicology data support the proposed uses and application rates.

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Aminopeptidase enzyme preparation produced with Trichoderma reesei expressing aminopeptidase gene from Aspergillus clavatus

2. Submission Format: *(Check appropriate box(es))*

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8) and 170.250(d) and (e))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

The enzyme is aminopeptidase (IUBMB 3.4.11.15) which catalyzes the cleavage of the N-terminal peptide bond in proteins and peptides, and releases of an N-terminal residue (amino acid, Xaa+Yaa-). The enzyme is intended to be used as processing aid in protein processing at 140-2125 mg TOS/kg protein, yeast processing at 723-4340 mg TOS/kg yeast, and flavoring production at 140-7024 mg TOS/kg protein.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Danisco US Inc.
(name of notifier)

has concluded that the intended use*(s)* of Aminopeptidase enzyme preparation produced with Trichoderma reesei expressing aminopep
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Danisco US Inc. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

925 Page Mill Road, Palo Alto, CA 94304, USA
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Annie Han Digitally signed by Annie Han Date: 2021.12.30 11:18:01 -08'00'	Annie Han, Senior Specialist, Global Regulatory Affairs	12/30/2021