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

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[43-18]

Supporting document 1

Risk assessment – Application A1146

A1146 Thermolysin (Protease) as a Processing Aid

Executive summary

Amano Enzyme Inc. (Amano) has submitted an Application seeking permission to use an enzyme, thermolysin, sourced from non-GM strain of *Anoxybacillus caldiproteolyticus,* as a processing aid in the manufacture of certain food products

Thermolysin converts proteins and peptides in various foods, resulting in the improvement of physiological properties (foaming ability, emulsifying ability, heat stability, viscosity) and organoleptic properties (taste and flavour).

Enzymes used in the production and manufacture of food are considered processing aids and are regulated by Schedule 18 of the *Australia New Zealand Food Standards Code* (the Code). Permitted enzymes with a specific technological purpose, such as thermolysin, are listed in Schedule 18—9(3) of the Code.

Thermolysin sourced from *A. caldiproteolyticus* was not genotoxic *in vitro* or *in vivo* and did not cause adverse effects in a subchronic toxicity study in rats. In the absence of any identifiable hazard an Acceptable Daily Intake 'not specified' is appropriate for the general population. A dietary exposure assessment is therefore not required.

Tuna protein from the culture medium may be present in thermolysin-treated foods at low levels. There is no established threshold for allergenic responses to fish proteins such that exposure to even low amounts of tuna protein in thermolysin-treated foods is potentially hazardous to individuals with an allergy to that food. Risk management measures are indicated for the protection of this sensitive subpopulation.

FSANZ is satisfied that that enzyme's use as a processing aid in the manner specified in the Application is technologically justified. FSANZ also concludes that, as the enzyme performs its technological purpose during processing and manufacture of food only, it is appropriately categorised as a processing aid rather than a food additive. The proposed maximum permitted level is GMP.

The enzyme also complies with the internationally accepted Joint Expert Committee on Food Additives (JECFA) specifications for chemical and microbiological purity.

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Thermolysin

1. Introduction and description

Thermolysin (EC 3.4.24.27) is an enzyme, which catalyses the hydrolysis of protein peptide bonds. Produced through fermentation, its microbial source is a non-GM strain of *Anoxybacillus caldiproteolyticus.* Thermolysin converts proteins and peptides in various foods, resulting in the improvement of physiological properties (foaming ability, emulsifying ability, heat stability, viscosity) and organoleptic properties (taste and flavour).

The enzyme is proposed to used by dairy, egg, meat and fish processors, yeast processors and flavouring manufacturers. The recommended use level is up to 0.24%, however food manufacturers typically fine-tune the use level based on Good Manufacturing Practice (GMP). Stable at 35-75°C and in a pH range of 6 to 10, thermolysin's peak activity occurs at 60-70°C and a pH of approximately 7-8.5. Inactivation occurs through thermal treatment or a change in pH. Once inactivated, thermolysin will not react with any protein present in the final food or non-target foods.

Thermolysin is produced under FSSC22000 quality control system, which is based on existing ISO Standards. It complies with international guidelines for the safe handling of microbial enzyme preparations published by the Association of Manufacturers of Fermentation Enzyme Products.

1.1 Identity

The production strain for thermolysin is a non-genetically modified strain of *A caldiproteolyticus* strain TP-7*,* which has a long history of safe use. Amano have produced this particular food enzyme since 1980.

The production strain is generated by several mutations from the original strain that was found in Japanese soil. N-methyl-N'-nitrosoguanidine (NTG) was used to obtain the current production strain.

The identity of the production strain has changed multiple times since 1989. The Applicant stated the strain used to produce thermolysin in 1989 was identified as *Bacillus thermoproteolyticus* Rokko and was equivalent to *Bacillus stearothermophilus*. In 1994, further biochemical testing and growth characteristics indicated the strain was *Bacillus stearothermophilus*. In 2001, *Geobacillus* was reported as a new genus, and *Bacillus stearothermophilus* was reclassified as *Geobacillus stearothermophilus* (Nazina et al 2001). The thermolysin production strain was regarded as *Geobacillus stearothermophilus* until 16s rDNA analysis performed in 2016 identified the production strain as *Anoxybacillus caldiproteolyticus.*

16s rDNA analysis confirms the production strain TP-7 is *A. caldoproteolyticus*, with the Applicant utilising a two-tiered cell banking system and other production mitigation measures in place to avoid genetic drift.

Thermolysin is produced via an initial cultivation step, with *A. caldiproteolyticus*, followed by several filtration and purification steps to yield a thermolysin concentrate. The concentrate is then blended with a sodium chloride carrier, ready for application directly to foods. There is currently no permission for enzymes derived from *A. caldiproteolyticus* in the Code.

Enzyme identity: Generic name: Thermolysin (Protease)
EC number: 3.4.24.27

CAS registry number: 9073-78-3
EINECS number: 232-973-4

Source microorganism identity:

Taxonomy:
Genus: Anoxybacillus
Species: caldiproteolyticus
Strain: TP-7
NCBI Taxonomy database ID: 247480
Synonyms: Geobacillus caldoproteolyticus

2 Food technology assessment

2.1 Technological purpose

Thermolysin, a metalloprotease[1], catalyses the hydrolysis of peptide bonds containing hydrophobic amino acids into amino acids and small peptides.

Some typical technological purposes and benefits of thermolysin are shown in Table 1.

Table 1: *Technological purposes of thermolysin*

Area of processing	Example foods	Resultant benefits of enzyme processing
Dairy	Cheese, butter, curds etc.	Wide variety of cheese and buttermilk flavours
Egg	Whole egg/white/yolk	Egg products with enhanced flavour and improved thermal tolerance, such as delayed denaturation
Meat & Fish	Meat/fish broth etc.	Meat/Fish extract with enhanced flavour, increased yield, improved nutritional content and superior functionality
Protein	Animal or vegetable sourced protein	Wide variety of flavour seasonings via hydrolysed animal or vegetable proteins
Yeast	Yeasts	Wide variety of nucleic acid, protein or trace element type yeast extracts for flavour enhancement
Flavourings	Enzymatic generation of food flavours	Wide variety of flavours possible

Thermolysin meets the definition of a processing aid as specified in Section 1.1.2—13 of the Code. If approved, the enzyme's full name, EC number and microbial origin will require listing in Schedule 18—9(3).

During the reaction between thermolysin and proteinaceous foods, low molecular weight peptides are generated and rarely, free amino acids, such as phenylalanine and isoleucine.Thermolysin however, has not been associated with the generation of free glutamate (MSG). FSANZ conclude that thermolysin does not increase the amount of MSG

[1] A metalloprotease, is any protease enzyme whose catalytic mechanism involves a metal.

upon protein digestion, when used as a processing aid.

2.2 Usage rates

Amano has provided suggested usage rates (Table 2) to guide food manufacturers, who will ultimately determine their own usage levels according to good manufacturing practice (GMP).

Table 2: *Suggested usage rates*

Application	Raw material (RM)		Recommended usage rates (mg TOS/kg RM)		Recommended usage rates (% w/w)	
			Min	Max	Min	Max
Dairy processing	Milk and milk derived proteinaceous ingredients		13	63	0.003	0.015
Egg processing	Eggs		13	101	0.003	0.024
Meat and fish processing	Meat and fish	Extract	13	63	0.003	0.015
		Softening	4	82	0.001	0.019
Protein processing	Proteins from various origin		13	63	0.003	0.015
Yeast processing	Yeast		10	1000	0.002	0.24
Flavouring production	Material of vegetable, animal or microbial origin		10	1000	0.002	0.24
TOS (%) =42.2%						

2.3 Technological justification

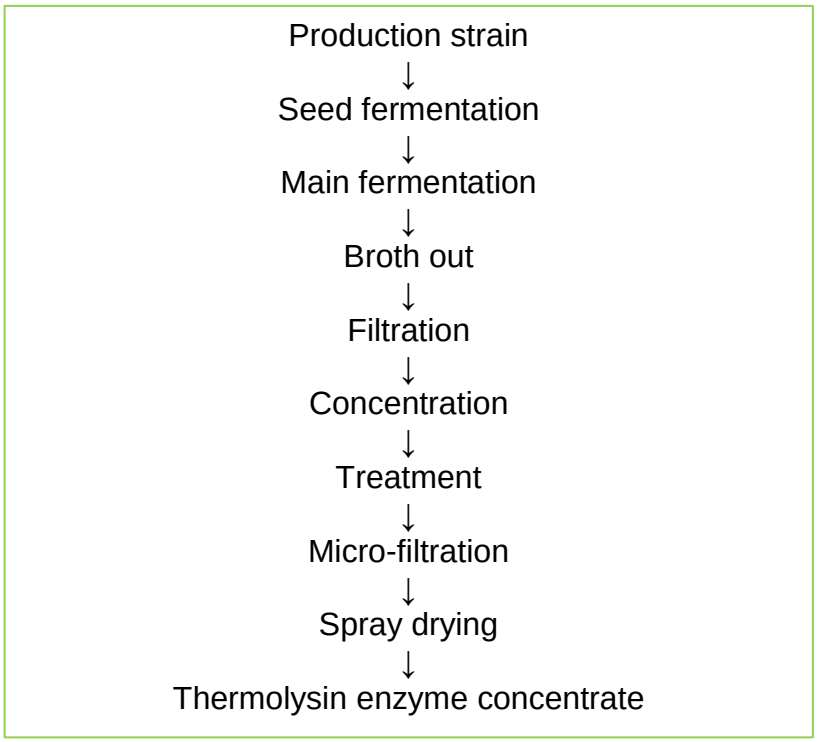
FSANZ considers the addition of thermolysin justified in improving the physiochemical and organoleptic properties of foods, when used at a level consistent with GMP.

2.4 Manufacturing process

Fermentation of *A. caldiproteolyticus* is conducted under standard culturing conditions using a carbohydrate rich fermentation media. Once the fermentation is complete, the broth is separated through a series of concentration steps, followed by a drying process. Once dry, the enzyme is blended with sodium chloride to produce the commercial preparation used by food manufacturers.

The fermentation process involves the use of some food additives, all of which are permitted in the Code. The enzyme preparation is produced according to the FSSC22000 quality control system and complies with international guidelines for the safe handling of microbial enzyme preparations published by the Association of Manufacturers of Fermentation Enzyme Products. GMP certification by the Japanese Food Additives Association for Amano's production plants and certificate of conformity to FSSC22000 are provided.

2.5 Manufacturing flow chart



2.6 Product specifications

There are international specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (JECFA 2016) and in the Food Chemicals Codex (Food Chemicals Codex 2014). These primary sources of specifications are listed in section S3—2 of the Code, for which enzyme preparations need to meet. Schedule 3—4 includes specifications for heavy metals if they are not included within specifications in sections S3—2 and S3—3. Table 3 shows the chemical and microbiological specifications for thermolysin, compared to three different production batches.

Table 3: *Product specifications for Thermolysin*

Chemical and Microbiological Specification		
Test subject	JECFA Specification	Mean of three batches of Amano Thermolysin enzyme powder
Lead	Not more than 5 mg/kg	0.024 mg/kg
Salmonella sp.	Absent in 25 g of sample	Absent in 25 g
Total coliforms	≤30 cfu/g	<10 cfu/g
Escherichia coli	Absent in 25 g of sample	Absent in 10 g
Antimicrobial activity	Not detected	Negative

2.7 Activity and stability of thermolysin

2.7.1 Activity 10 minute incubation (pH and thermal)

Peak activity of thermolysin occurs at a pH of approximately 7.7 – 8.0 and 60 – 70°C as shown in Figures 1 and 2, respectively.

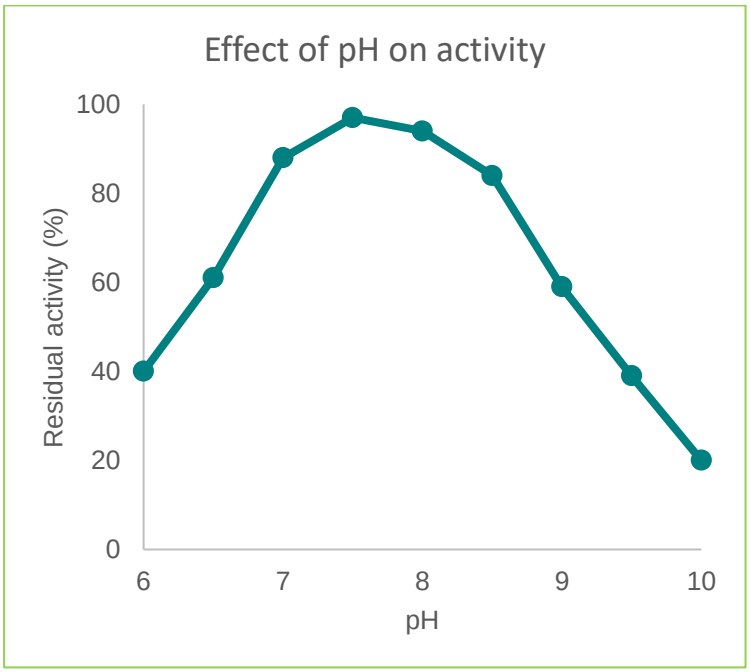


Figure 1. *Effect of pH on thermolysin activity (0.5% casein, 35°C, 10 min incubation)*

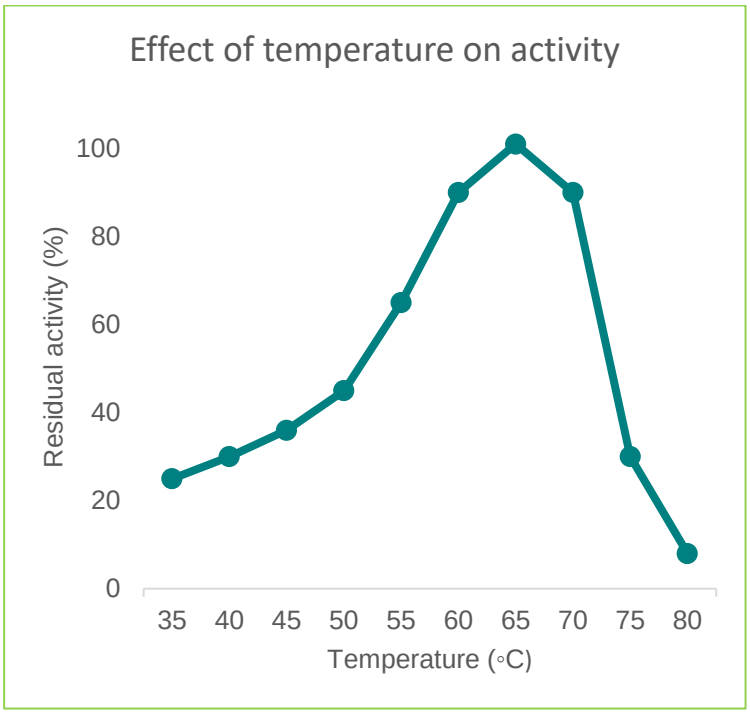


Figure 2. *Effect of temperature on thermolysin activity (0.5% casein, pH 7.2, 10 min incubation)*

2.7.2 Stability after 30 min extended incubation (pH and thermal)

Thermolysin is stable at a pH range of 6 to 10 and 35-75°C as shown in Figures 3 and 4.

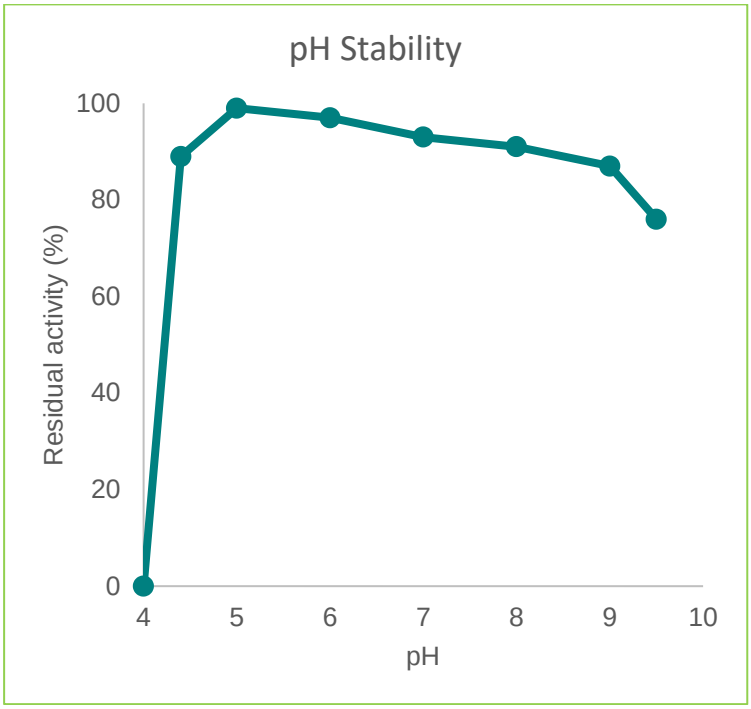


Figure 3. *pH stability of Thermolysin (60°C, 30 min incubation)*

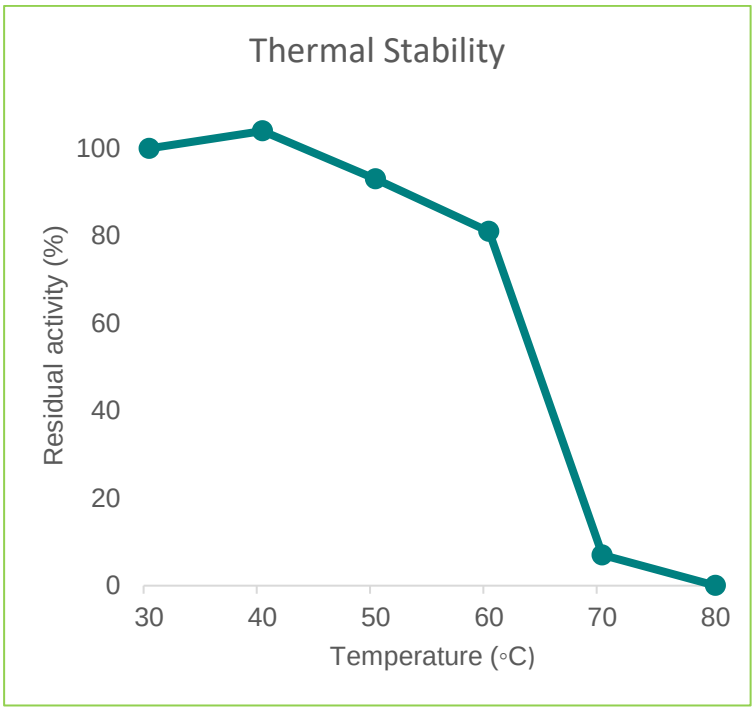


Figure 4. *Thermal stability of Thermolysin (30 min incubation)*

2.7.3 Long term stability

Storage trials conducted by Amano, at 15°C and 25°C, over 18 months, are shown in Figure 5. Thermolysin's activity remained at over 90% of the initial activity after 18 months at both temperatures.

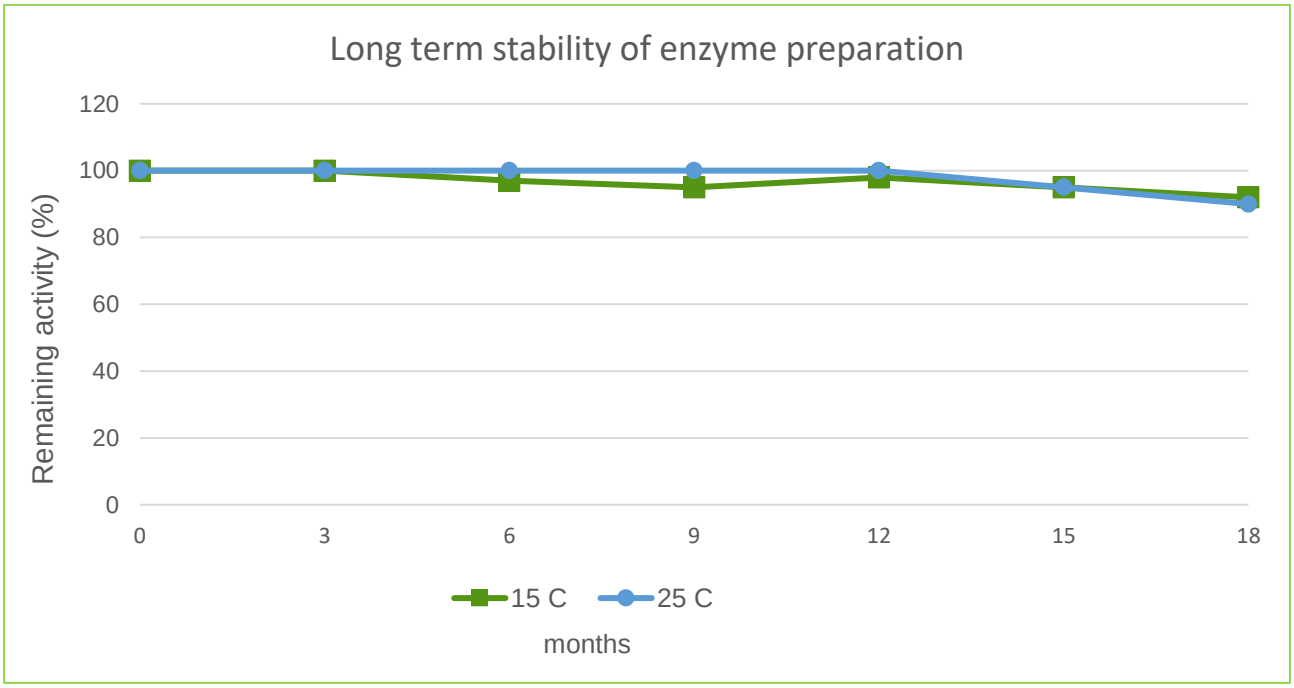


Figure 5. *Long term stability of Thermolysin*

2.8 Food technology conclusion

The stated purpose of this enzyme preparation, namely, for use as a processing aid in the processing and manufacture of dairy, egg, meat and fish products, yeast processing and flavouring production is clearly articulated in the Application. The evidence presented to support the proposed uses provides adequate assurance that the enzyme, in the form and prescribed amounts, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme performs its technological purpose during processing and manufacture of food after which it is inactivated so does not perform any technological function in the final food. It is therefore appropriately categorised as a processing aid and not a food additive. The enzyme preparation meets international purity specifications.

3 Hazard assessment

FSANZ has assessed the submitted evidence on the safety of thermolysin, including studies on genotoxicity, chronic toxicity in laboratory rats, and potential for allergenicity. The submitted data are considered adequate for hazard assessment for thermolysin.

3.1 Scope of the current hazard assessment

The Application is to seek an amendment to the Code for to permit thermolysin to be used as a processing aid in Australia and New Zealand.

FSANZ has not previously assessed the safety of thermolysin. Therefore, the aims of the current assessment were to:

- Review all of the available data on the toxicology of thermolysin to determine its safety as a processing aid, and
- If appropriate, establish a health-based guidance value for thermolysin

3.2 Hazard of the production organism

The applicant has provided information that the parent strain of the production organism that was isolated from Japanese soil is *Anoxybacillus caldiproteolyticus*. The production strain TP-7 is derived from the parent strain by seven rounds of chemical mutagenesis using N-methyl-N'-nitro-N-nitrosoguanidine. Strain TP-7 is not genetically modified.

The production strain TP-7 is not listed on the American type culture collection (ATCC) or Deutsche Sammlung von Mikroorganismen und Zelkulturen – DSMZ (German Collection of Micro-organisms and Cell Cultures). The applicant provided information to establish the identity of the production strain TP-7. Sequence analysis of the full 16S rDNA subunit identified strain TP-7 as *A. caldiproteolyticus* (99.7% homology to *A. caldiproteolyticus* R-35652).

Prior to a reclassification in 2012 (Coorevits et al 2012), *A. caldiproteolyticus* was previously known as *Geobacillus caldoproteolyticus*. Neither *A. caldiproteolyticus* nor *G. caldoproteolyticus* have previously been assessed by FSANZ.

The European Food Safety Authority (EFSA 2016) did not recommend *G. caldoproteolyticus* (i.e. *A. caldiproteolyticus*) for Qualified Presumption of Safety (QPS) status due to the lack of a sufficient body of knowledge on a safe history of use or presence in foods and feeds.

The Applicant supplied a toxicology study performed in 1989 using thermolysin produced by *Bacillus thermoproteolyticus*. In 2016, the microorganism used to produce thermolysin for this toxicology study was identified as *Anoxybacillus caldoproteolyticus* strain TP-7 based on analysis of the 16s rDNA subunit. The toxicology study showed that thermolysin produced by this production strain had no adverse effects. This implies the enzyme produced from the production organism did not contain toxin and the production strain TP-7 is non-toxigenic.

As stated in section 1.1 above, the Applicant provided information indicating the identity of the production strain has changed multiple times since 1989. The Applicant stated the strain used to produce thermolysin in 1989 was identified as *Bacillus thermoproteolyticus* Rokko and was equivalent to *Bacillus stearothermophilus*. In 1994, further biochemical testing and growth characteristics indicated the strain was *Bacillus stearothermophilus*. In 2001 *Geobacillus* was reported as a new genus, and *Bacillus stearothermophilus* was reclassified as *Geobacillus stearothermophilus* (Nazina et al 2001).The thermolysin production strain was regarded as *Geobacillus stearothermophilus*. Following 16s rDNA analysis in 2016 (as stated above), the production strain was identified as *Anoxybacillus caldiproteolyticus*.

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

3.3 Evaluation of Submitted Data

FSANZ has assessed the submitted evidence, including information on history of safe use of the source organism and the enzyme mixture, genotoxicity, subchronic toxicity in laboratory rats, and similarity to known proteins and allergens. The submitted data are considered suitable to assess the hazard of thermolysin.

3.4 History of human exposure and consumption

Thermolysin has been marketed for use in food since 1980. No adverse effects have been reported in workers exposed either the production strain of the microorganism, or to the enzyme preparation.

3.5 Genotoxicity studies

Genotoxicity studies include a mouse micronucleus test and a chromosomal aberration assay in Chinese hamster lung fibroblasts. An Ames test was not conducted for thermolysin, because it was anticipated that the presence of histidine in the enzyme might cause a false positive result.

Micronucleus Test in Mice. Unpublished report; Wada [Study Director] *1985*

This study was subject to Quality Assurance inspections and audits, although guideline(s) were not specified in the study report.

The test subjects were male ICR mice, 8 weeks of age and 28-36 g bodyweight at commencement of dosing. The test article was thermolysin powder from Daiwa Kasei KK. The protease level was not stated. However the applicant, Amano Enzyme, Inc., has certified that they acquired Daiwa Kasei KK in 2013 and the company now operates as the Shiga plant of Amano Enzyme, Inc. The thermolysin enzyme is the same that for which the application is made.

In a preliminary study, no increase in incidence of micronucleated polychromatic erythrocytes or polychromatic erythrocytes in bone marrow were found following single gavage doses of 1250, 2500 or 5000 mg/kg bw in 0.1% carboxymethylcellulose (CMC), or a repeat-dose regime of four doses of 1250 mg/kg bw over 24 hours. Sampling times were 24, 30 and 48 hours after the single doses, with two mice terminated at each timepoint. The repeat-dose mice were sampled 24 hours after the last dose. The control mice, which were gavaged with 0.1% CMC, were sampled at 24 hours. Dose volume was 10 mL/kg bw for all groups.

In the definitive study, 5 mice/group were administered 0, 1250, 2500 or 5000 mg/kg bw thermolysin in 0.1% CMC as a single dose by oral gavage, at a volume or 10 mL/kg bw, while 5 mice in a positive control group were treated with 2 mg/kg Mitomycin C by intraperitoneal injection. The femoral bone marrow samples were collected from negative control and treatment groups at 30 hours after dosing while those of the positive control group were collected 24 hours after injection.

The incidences of appearance of micronucleated polychromatic erythrocytes, micronucleated normochromatic erythrocytes and micronucleated erythrocytes were not different between negative control and thermolysin-dosed groups. Significant differences in incidences of micronucleated polychromatic erythrocytes and micronucleated erythrocytes in response to Mitomycin C treatment confirmed the sensitivity of the test system to a mutagen. Thus, it was concluded that thermolysin showed no evidence of mutagenic activity.

Chromosome Aberration test in Chinese hamster lung fibroblasts. Unpublished report; Sono [Study Director] *2010.*

The study was carried out in compliance with MHLW (Japan) and OECD standards of Good Laboratory Practice.

The test article for this study was Thermoase thermolysin powder from Daiwa Kasei KK, with a protease activity of 93%. As indicated for the mouse micronucleus study, this thermolysin is the same as that for which the application is made. On the basis of a preliminary cell growth inhibition test, concentrations used for the short term treatment and continuous treatment assays were 0.800, 0.677, 0.556, 0.463, 0.386 and 0.322 mg/mL. The vehicle, and negative control article, was sterile water for injection.

For the short-term treatment, each group comprised four plates of cells and a negative control group (0.5 mL water), the six treatment groups (0.5 mL test solution) and a positive control group were assayed. Assays were conducted with or without addition of S9 fraction (0.833 mL) for the purpose of metabolic activation. The positive control articles were cyclophosphamide (0.1 mL of 0.7 mg/mL solution) when S9 fraction was present and Mitomycin C (0.150 mL of 0.0025 mg/mL) in the absence of S9 fraction. All plates were examined and rinsed with isotonic sodium chloride after 6 hours of incubation, fresh culture medium added and incubation continued for a further 12 hours. Two plates per group were treated with addition of colcemid approximately 2 hours before the end of incubation. At the end of incubation, cells from these plates were harvested, processed and stained with 2% Giemsa for examination of chromosomes, while cells were examined microscopically and cell density measured in the other two plates.

Continuous treatments were conducted for 24 and for 48 hours, and, like the short term assay, were performed in quadruplicate. Groups included negative controls to which 0.5 mL of sterile water was added, the treatment groups to which 0.5 mL of the test solutions were added, and a positive control group treated with 0.1 mL of Mitomycin C (0.0025 mg/mL). As for the short-term assay, two plates/group were used for examination of chromosomes and two were used for measurement of cell density.

No evidence of chromosomal aberrations attributable to thermolysin was found. There was some evidence of cell growth inhibition and cytotoxicity, attributed to the protease activity of the test article.

3.6 Studies in Experimental Animals

91-day oral gavage study in rats. Unpublished report; Tyerman [Study Director] *1989*

Regulatory compliance of this study was with OECD, US EPA and US FDA GLP standards.

This study was conducted using Sprague-Dawley rats, 20/sex/group. Rats were gavaged once daily with 0, 40, 200 or 1000 mg/kg bw thermolysin powder for 91 days. The Thermoase thermolysin powder was technical grade and was estimated to have >85% activity by weight. As indicated for the genotoxicity studies, this thermolysin is the same as that for which the application is made. The balance of a batch of thermolysin powder from Daiwa Kasei KK, now the Shiga plant of Amano Enzyme, Inc., that is not enzyme is principally sodium chloride. Thermolysin powder was administered to the rats at a volume of 10 mL/kg bodyweight in 1% methylcellulose as the vehicle. The rats were approximately 6 weeks old at the start of the treatment phase. Endpoints included mortality, clinical observations, bodyweight changes, food consumption, ophthalmic findings, haematology, clinical biochemistry, gross necropsy findings, selected organ weights (adrenal glands, kidneys, liver and testes) and histopathology of a comprehensive tissue list.

All rats survived to scheduled termination. There were no effects on clinical findings, bodyweight changes, food consumption, ophthalmology, clinical biochemistry, gross necropsy findings or histopathology.

Males in the 1000 mg/kg/day group had slight but statistically significant decreases in erythrocyte numbers, haematocrit, platelet count and numbers of large unstained cells. Males in the 200 mg/kg/day group also had a statistically significant decrease in platelet count. The individual and group mean values for these parameters remained within the normal historical range for the laboratory, there were no corresponding changes in the haematology of the female rats, and no supporting changes in gross necropsy or histopathological findings. The findings were therefore considered to be unrelated to treatment.

Weights of adrenal glands, relative to bodyweight, showed a statistically significant increase in treated rats when compared to controls, but the difference was slight and adrenal weights remained within the normal range for rats of the age and strain. The statistical difference was therefore considered to be spurious.

The highest dose used on the study, 1000 mg/kg bw/day, was therefore identified as the No Observed Adverse Effect Level (NOAEL). Assuming a TOS concentration of 42.2% (from Table 2) in thermolysin powder, this is equivalent to 422 mg TOS/kg bw/day.

3.7 Potential for Allergenicity

The use of *A. caldiproteolyticus* for processing aid production has been approved in France. There are no allergenicity warnings associated with the use of this organism for this purpose.

The amino acid sequence of Thermolysin has been determined. Two searches of the Allergen Database for Food Safety (http://allergen.nihs.go.jp/ADFS/) were conducted; a search for matches to 8 consecutive amino acid sequences, and an 80 amino acid sliding window search. There were no matches found with the 80 amino acid sliding window search.

One match for 8 consecutive amino acids was found. The match was with ‘Sola t 2’ (aspartic protease inhibitor 11; Cathepsin D inhibitor PDI) which is derived from potato. However, there is no overall homology between thermolysin and this allergen.

Potential allergens in the culture medium include soybean flour, milk casein, and fish, specifically tuna. Results of analysis of thermolysin powder for residual soy and milk protein found “not more than the detection limit (1µg/g)”. An analytical test on thermolysin powder indicated that the level of cod parvalbumin protein was less than 1.4 mg/kg. However it is unclear how this result relates to residual tuna protein levels in the thermolysin powder

The Applicant has estimated that assuming that all fermentation medium material remains in the thermolysin protein preparation, the powder could contain up to 0.3% fish extract. The maximum use level of thermolysin is 1,000 mg total organic solids (TOS)/kg food ingredient. Thermolysin powder contains 42.2% TOS, and therefore the maximum amount of powder used would be 2,369 mg/kg, or 0.24%. The use of thermolysin powder at 0.24% w/w would lead to a maximum of 7.2 mg fish extract/kg food ingredient. This level would be lower in the final food.

3.8 Discussion

The submitted data are considered adequate to define the hazard of thermolysin.

Thermolysin has been marketed for use in food since 1980. There have been no reported adverse effects of thermolysin in food industry workers or in consumers. Genetic stability of the enzyme is ensured by production under manufacturing processes which are in compliance with AMFEP guidelines for the safe handling of microbial enzyme preparations.

The test article used in the genotoxicity studies and the subchronic rat study is the same enzyme as that for which the application is made. Batches of thermolysin differ in enzyme activity, but the balance of any batch that is not enzyme, is principally sodium chloride.

There was no evidence of mutagenic activity of thermolysin in a mouse micronucleus assay, and no evidence of chromosomal aberrations in Chinese hamster lung fibroblasts exposed to thermolysin *in vitro*. Thermolysin is not a suitable candidate for a bacterial reverse mutation assay (Ames test) because it contains histidine, which could give rise to false positive results.

Daily gavage of rats with a thermolysin preparation with high enzyme activity for 91 days identified a NOAEL of 1000 mg/kg bodyweight/day, the highest dose level tested. Assuming a TOS concentration of 42.2% (from Table 2) in thermolysin powder, this is equivalent to 422 mg TOS/kg bw/day. The applicant has estimated Theoretical Maximum Daily Intakes (TMDIs) for thermolysin of 1.03 mg TOS/kg bw/day for food, and 0.5 mg TOS/kg bw/day in beverage, for an overall TMDI of 1.53 mg TOS/kg bw/day. These TMDIs are based on very conservative assumptions, including that all producers of foods in which thermolysin may be used, use thermolysin; that all producers use the maximum level of thermolysin; that the foods and beverages consumed are those containing the highest levels of thermolysin; and that thermolysin is not denatured or destroyed during the production process. Even with these conservative assumptions, there is a 276-fold safety margin between the TMDI and the NOAEL.

No matches with known allergens were found in an 80 amino acid sliding window search of the Allergen Database for Food Safety. One match of 8 consecutive amino acids was found with Sola t 2, a protease inhibitor of potato, but there was no overall homology. Sola t 2 is one of five potato proteins identified by Seppälä *et al*. (2001) that may bind with IgE from sera of atopic infants and children with suspected food allergy. Potato is not one of the major food allergens, and reported clinical allergic responses in sensitive individuals are not life-threatening, but generally restricted to local reactions of mucosa and skin.

The Applicant has estimated that the use of thermolysin powder at 0.24% w/w would lead to a maximum of 7.2 mg fish extract/kg food ingredient. FSANZ notes that there is no established threshold for allergenic responses to fish proteins (EFSA et al 2014, Taylor et al 2014) such that exposure to even low amounts of tuna protein in thermolysin-treated foods is potentially hazardous to individuals with an allergy to that food.

3.9 Hazard assessment Conclusion

Based on the reviewed toxicological data, it is concluded that in the absence of any identifiable hazard to health and safety of the general population, an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate. A dietary exposure assessment is therefore not required.

Fish (Tuna) products are used in the fermentation media. There is no established threshold for allergenic responses to fish proteins, such that exposure to even low amounts of fish protein in thermolysin-treated foods is potentially hazardous to individuals with an allergy to

that food. Risk management measures are indicated for the protection of this sensitive subpopulation.

References

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