



**Petition to Amend Schedule 18 of the Australia New Zealand Food
Standards Code to Include
 β -Galactosidase from *Papiliotrema terrestris* as a Processing Aid**

- Executive Summary -

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Petition to Amend Schedule 18 of the Australia New Zealand Food Standards Code to Include β -Galactosidase from *Papiliotrema terrestris* as a Processing Aid

Executive Summary

Amano Enzyme Inc. (referred to Amano Enzyme hereafter) is proposing to amend Schedule 18 of the Australia New Zealand Food Standards Code to include β -Galactosidase derived from *Papiliotrema terrestris* as an enzyme of microbial origin. β -Galactosidase (EC 3.2.1.23, CAS number 9031-11-2) is an enzyme catalyzing the hydrolysis of β -Galactosides.

It is used as an enzyme for producing galactooligosaccharide (GOS) from lactose. β -Galactosidase is proposed for use as a processing aid in food productions at levels up to 0.03%. GOS belongs to the group of prebiotics and can be utilized to various foods. Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of beneficial bacteria in the colon.

The β -Galactosidase is an enzyme derived from non-genetically modified strain of *Papiliotrema terrestris*. The production strain is obtained by several mutations from the original strain that was found Japanese soil. N-methyl-N'-nitrosoguanidine (NTG) was used to obtain the current production strain. The production process of the β -Galactosidase enzyme comprises a cultivation step with *Papiliotrema terrestris*, followed by several filtration and purification steps to result in β -Galactosidase concentrate.

All of the raw materials used in the manufacture of the β -Galactosidase are safe and suitable for use. The enzyme is produced according to the FSSC22000 quality control system. Production controls are in place to monitor the strain during the fermentation and ensure the avoidance of genetic drift. Furthermore, the product specifications along with extensive batch analysis of β -Galactosidase demonstrate the purity of the enzyme preparation, including the absence of microbiological and heavy metal contaminants, as well as the lack of antibiotic activity.

β -Galactosidase is stable for at least 6 months at under 25°C from the manufacturing date under the sealed condition. The enzyme exhibits activity from pH 3.0 till pH 8.0, and from 40°C till 75°C. The optimum pH is pH 5.0 and the optimum temperature is 70°C. Also, as far as Amano Enzyme is aware, Amano Enzyme's β -Galactosidase described in this dossier does not have any enzymatic side activities which might cause adverse effect.

β -Galactosidase is listed in IPA Database by CCFA (Codex Committee on Food Additives). *Cryptococcus laurentii* is known as a closely related species with *Papiliotrema terrestris*. It is reported that β -Galactosidase from *Cryptococcus laurentii* has been used for manufacturing of GOS for over 25 years in Japan. Since the enzyme, β -Galactosidase in this submission is Amano's new-developed product, there is no approved status in other countries.

The safety of β -Galactosidase derived from *Papiliotrema terrestris* can be supported by toxicity studies. The food enzyme has been subjected to a standard package of toxicological tests, with the



following results:

- Bacterial reverse mutation: No mutagenic activity under the given test conditions
- Chromosomal aberrations: No clastogenic activity under the given test conditions
- Systemic toxicity: The No Observed Adverse Effect Level (NOAEL) is greater than 2,000 mg/kg-bw/day (1,800mg TOS/kg-bw/day), which is the high dose in the study.

β -Galactosidase derived from *Papiliotrema terrestris* also does not pose any allergenicity concerns, given the long history of use of the enzyme. Additionally, the amino acid sequence of β -Galactosidase does not indicate that β -Galactosidase has a potency of any allergenicity concerns.

Theoretical Maximum Daily Intake was calculated using the Budget Method and Food Consumption Database. Based on this method, the Total TMDI of β -Galactosidase was calculated as 4.75 mg TOS/kg-bw/day for adult and 2.1 mg TOS/kg-bw/day for infant and baby. As described above, NOAEL of the enzyme is 1,800 mgTOS/kg/day. Consequently, the safety margin of β -Galactosidase is 379 for adult (1,800/4.75) and 857 for infant and baby (1,800/2.1), respectively.

As such, no safety concerns are anticipated with the proposed use of β -Galactosidase as a processing aid in Australia/New Zealand.



References

IPA Database by CCFA. Available at: http://ipa.ccfa.cc/substance?task=detail&substance_id=589

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