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SCIENTIFIC OPINION

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Safety evaluation of the food enzyme bacillolysin from the non-genetically modified *Bacillus amyloliquefaciens* strain DP-Cyb74

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Abstract

The food enzyme bacillolysin (EC 3.4.24.28) is produced with the non-genetically modified *Bacillus amyloliquefaciens* strain DP-Cyb74 by Genencor International B.V. The production strain met all requirements for the qualified presumption of safety (QPS) approach to safety assessment. The food enzyme is intended to be used in six food manufacturing processes. Dietary exposure to the food enzyme total organic solids (TOS) was estimated to be up to 1.536 mg TOS/kg body weight per day in European populations. As the production strain qualifies for the QPS approach and no issue of concern arose from the production process of the food enzyme, the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

KEYWORDS

bacillolysin, *Bacillus amyloliquefaciens*, EC 3.4.24.28, food enzyme, non-genetically modified microorganism

CONTENTS

Abstract 1
1. Introduction 1
1.1. Background and Terms of Reference as provided by the requestor 3
1.1.1. Background as provided by the European Commission 3
1.1.2. Terms of Reference 3
1.2. Interpretation of the Terms of Reference 4
2. Data and methodologies 4
2.1. Data 4
2.2. Methodologies 4
3. Assessment 4
3.1. Source of the food enzyme 5
3.2. Production of the food enzyme 5
3.3. Characteristics of the food enzyme 5
3.3.1. Properties of the food enzyme 5
3.3.2. Chemical parameters 6
3.3.3. Purity 6
3.4. Toxicological data 6
3.4.1. Allergenicity 6
3.5. Dietary exposure 7
3.5.1. Intended use of the food enzyme 7
3.5.2. Dietary exposure estimation 8
3.5.3. Uncertainty analysis 8
3.6. Margin of exposure 8
4. Conclusions 9
5. Documentation as provided to EFSA 9
Abbreviations 9
Conflict of interest 9
Requestor 9
Question number 9
Copyright for non-EFSA Content 9
Panel members 10
Note 10
References 10
Appendix A 11
Appendix B 12

1 | INTRODUCTION

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008[1] on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the EU market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) lays down the administrative, technical and toxicological data required.

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008[1] on food enzymes.

Five applications have been introduced by "the Association of Manufacturers and Formulators of Enzyme Products (AMFEP)" for the authorisation of the food enzyme Bacillolysin from *Bacillus amyloliquefaciens*, and the companies "Danisco US Inc." for the authorisation of food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), Beta-galactosidase from a genetically modified strain of *Bacillus subtilis* (DP-Ezg29) and Endo-1,4-beta-xylanase from a genetically modified strain *Bacillus subtilis* (DP-Ezd31), and "Intertek Scientific & Regulatory Consultancy" for the authorization of the food enzyme Beta-fructofuranosidase from *Aspergillus fijiensis* (strain ATCC®20611™).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008,[2] the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb44), Bacillolysin from *Bacillus amyloliquefaciens*, Beta-galactosidase from a genetically modified strain of *Bacillus subtilis* (DP-Ezg29), Endo-1,4-beta-xylanase from a genetically modified strain *Bacillus subtilis* (DP-Ezd31) and Beta-fructofuranosidase from *Aspergillus fijiensis* (strain ATCC®20611™) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

[1]Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3]Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.03.2011, pp. 15–24.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme bacillolysin from the non-genetically modified *Bacillus amyloliquefaciens* strain DP-Cyb74, submitted by Genencor International B.V.

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2015-00837. During a meeting between EFSA, the European Commission and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP),[5] it was agreed that the joint dossier will be split into six individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00837. This data package, identified as EFSA-Q-2022-00527, concerns the food enzyme bacillolysin that is produced with the *Bacillus amyloliquefaciens* strain DP-Cyb74 and submitted by Genencor International B.V.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme bacillolysin from a non-genetically modified *Bacillus amyloliquefaciens* (strain DP-Cyb74). The data package was submitted on 22 August 2022.

Additional information was requested from the applicant during the assessment process on 28 June 2023 and received on 28 August 2023 (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 28 June 2023, the applicant requested a clarification teleconference on 25 August 2023, after which the applicant provided additional data on 28 August 2023.

2.2 | Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	Bacillolysin
Systematic name	–
Synonyms	*Bacillus* metalloendopeptidase; *Bacillus subtilis* neutral proteinase
IUBMB No	EC 3.4.24.28
CAS No	9080-56-2
EINECS No	232-991-2

Bacillolysins catalyse the hydrolysis of the peptide bonds of proteins with broad specificity, releasing peptides and amino acids. The enzyme under assessment is intended to be used in six food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): (1) processing of cereals and other grains for the production of brewed products; processing of dairy products for the production of (2) fermented dairy products and (3) modified milk proteins; (4) processing of meat and fish products for the production of protein hydrolysates and processing of plant- and fungal-derived products for the production of (5) protein hydrolysates and 6) plant-based analogues of milk and milk products.[6]

[4]Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes Text with EEA relevance. OJ L 168, 28.6.2012, p. 21–23.

[5]The full detail is available at the https://www.efsa.europa.eu/en/events/event/ad-hoc-meeting-industry-association-amfep-joint-dossiers-food-enzymes.

[6]Technical dossier/Additional information, 28 August 2023/Annex 1.

3.1 | Source of the food enzyme

The bacillolysin is produced with the non-genetically modified bacterium *Bacillus amyloliquefaciens* strain DP-Cyb74,[7] which is deposited [redacted] with the deposit number [redacted].[8] The production strain was identified as *B. amyloliquefaciens* [redacted].[9]

The species *B. amyloliquefaciens* is included in the list of organisms for which the qualified presumption of safety (QPS) approach may be applied, provided that the absence of acquired antimicrobial resistance (AMR) genes and toxigenic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2020).

The production strain was shown not to be cytotoxic [redacted].[10] [redacted]. No genes of concern were identified.[11]

Therefore, the production strain was considered to meet the requirements for the QPS approach to safety assessment.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[12] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[13]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[14] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[15]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme[16]

The enzyme is a single polypeptide chain of [redacted] amino acids.[17] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa. The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[18] A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about [redacted] kDa,[19] consistent with the expected mass of the enzyme. No other enzymatic activities were reported.[20]

The in-house determination of bacillolysin activity[21] is based on the hydrolysis of azocasein (reaction conditions: pH 7.5, 30°C, 5 min). The release of low molecular mass dye-tagged peptides not precipitated by trichloroacetic acid is measured spectrophotometrically at 420 nm. The enzyme activity is quantified relative to an internal enzyme standard and expressed in AZO (azocasein) neutral protease units/g.[22]

[7] Synonyms: GICC03129, NP104-12.
[8] Technical dossier/Annex N.
[9] Technical dossier/Annex Q.
[10] Technical dossier/Annex O.
[11] Technical dossier/Annex R.
[12] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[13] Technical dossier/p. 8–9, 15, 44–45, 50–51; Technical dossier/Annex E.
[14] Technical dossier/p. 8, 15, 45–52; Technical dossier/Annex F.
[15] Technical dossier/p. 45–48; Technical dossier/Annex G.
[16] Technical dossier/Annex K; Annex J; Technical dossier/Additional information, 28 August 2023/Annex 1.
[17] Technical dossier/p. 34; Technical dossier/Annex H; Annex K.
[18] Technical dossier/p. 32; Technical dossier/Annex J.
[19] Technical dossier/p. 32; Technical dossier/Annex J.
[20] Technical dossier/p. 7, 34–35.
[21] Technical dossier/Annex B.
[22] Technical dossier/Annex B.

The food enzyme has a temperature optimum around 60°C (pH 7.5) and a pH optimum around pH 6.5 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at temperatures between 25 and 85°C (pH 6.5). No enzyme activity was detected after pre-incubation at above 65°C for 30 min.[23]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[24] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 17.8% and the mean enzyme activity/TOS ratio was 28.1 AZO/mg TOS.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches		
		1	2	3
Bacillolysin activity	AZO/g[a]	5966	5916	3297
Protein	%	13.9	12.0	8.4
Ash	%	0.3	0.9	0.8
Water	%	78.5	80.3	85.9
Total organic solids (TOS)[b]	%	21.2	18.8	13.3
Activity/TOS ratio	AZO/mg TOS	28.1	31.5	24.8

[a]AZO: Azocasein unit (see Section 3.3.1).
[b]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content in all batches was below 0.05 mg/kg,[25,26] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[27] No antimicrobial activity was detected in any of the tested batches.[28]

The Panel considered that the information provided on the purity of the food enzyme was sufficient.

3.4 | Toxicological data[29]

As the production strain qualifies for the QPS approach of safety assessment and no issue of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary (EFSA CEP Panel, 2021).

3.4.1 | Allergenicity

The allergenicity assessment considered only the food enzyme and not carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the food enzyme bacillolysin produced with the non-genetically modified *B. amyloliquefaciens* strain DP-Cyb74 was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[30]

[23]Technical dossier/Annex B; Technical dossier/Additional information, 28 August 2023/Annex 1.
[24]Technical dossier/p. 30–31; Technical dossier/Annex A; Annex B; Annex C.
[25]Technical dossier/p. 7, 33–34, 73; Technical dossier/Annex C; Annex D.
[26]Technical dossier/Annex C: LoD: Pb = 0.05 mg/kg.
[27]Technical dossier/p. 7, 33–34, 73; Technical dossier/Annex C; Annex D.
[28]Technical dossier/p. 7, 33–34, 73; Technical dossier/Annex C; Annex D.
[29]Technical dossier/p. 11, 16, 63.
[30]Technical dossier/p. 11, 64–65; Technical dossier/Annex H; Annex I; Annex K.

No information was available on oral and respiratory sensitisation or elicitation reactions of this bacillolysin. In addition, no allergic reactions upon dietary exposure to any bacillolysin have been reported in the literature.[31]

[redacted] (listed in the Regulation (EU) No 1169/2011[32,33]), as well as [redacted], known sources of allergens, are present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from these sources are present in the food enzyme.

The Panel considered that a risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme[34]

The food enzyme is intended to be used in six food manufacturing processes at the recommended use levels summarised in Table 2.

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[c]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of cereals and other grains		
• Production of brewed products	Cereals	20.41–**61.23**
Processing of dairy products		
• Production of fermented dairy products	Milk	0.01–**0.13**
• Production of modified milk proteins	Milk proteins	133.62–**534.47**
Processing of meat and fish products		
• Production of protein hydrolysates from meat and fish proteins	Meat and fish proteins	133.62–**534.47**
Processing of plant- and fungal-derived products		
• Production of protein hydrolysates from plants and fungi	Plant and fungi	133.62–**400.85**
• Production of plant-based analogues of milk and milk products	Plant-based raw materials	133.62–**534.47**

[a]The name has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]The numbers in bold represent the maximum recommended use levels which were used for calculation.

[c]Technical dossier/p. 84; Technical dossier/Additional information, 28 August 2023/Annex 1/Answer 2.

In brewing, the food enzyme is added to cereals during the mashing step.[35] The bacillolysin hydrolyses proteins in the cereals to release free amino nitrogen for the optimal growth of the brewer's yeast during fermentation. In addition, the partial degradation of proteins improves the clarity of beer. The food enzyme-TOS remains in the beer.

To produce fermented dairy products, the food enzyme is added to milk during inoculation to facilitate protein hydrolysis.[36] The food enzyme-TOS remains in the final products.

To manufacture protein hydrolysates, the food enzyme is added to the proteins isolated from the following sources: milk (e.g. whey proteins, caseins), plant (e.g. soy, wheat, maize), fungal or animal sources (e.g. meat, fish, collagen, gelatine).[37] Bacillolysin is used to hydrolyse and enhance the flavour of the resulting protein hydrolysates,[38] which are subsequently used as ingredients in a variety of foods, including infant formula, follow-on formula and foods for special medical purposes. The food enzyme-TOS remains in these protein hydrolysates.

[31]Technical dossier/p. 11, 64–65; Technical dossier/Annex I.

[32]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[33]Technical dossier/Annex G.

[34]Technical dossier/Additional information, 28 August 2023/Annex 1.

[35]Technical dossier/p. 55.

[36]Technical dossier/p. 56; Technical dossier/Additional information, 28 August 2023/Annex 1/Answer 2.1.

[37]Technical dossier/p. 57; Technical dossier/Additional information, 28 August 2023/Annex 1/Answer 2.2.

[38]Technical dossier/p. 79.

In the production of plant-based analogues of milk and milk products, the food enzyme is added to the slurry of plant materials (e.g. oat flour) during the saccharification step.[39] The food enzyme can also be added to plant-based beverages prior to fermentation to produce fermented plant-based milk analogues.[40] The hydrolysis by bacillolysin is used to increase the yield and to enhance flavours. The food enzyme-TOS remains in these plant-based analogues.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in food processes, it is expected that the enzyme is inactivated in most of the food manufacturing processes listed in Table 2, but may remain active in fermented dairy products.

3.5.2 | Dietary exposure estimation

Chronic exposure to the food enzyme-TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme-TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 1.536 mg TOS/kg body weight per day in infants at the 95th percentile.

TABLE 3 Summary of the estimated dietary exposure to food enzyme–TOS in six population groups.

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**Infants**	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.063–0.670 (12)	0.072–0.447 (15)	0.048–0.125 (19)	0.009–0.071 (21)	0.015–0.090 (22)	0.006–0.065 (23)
Min–max 95th percentile (number of surveys)	0.154–1.536 (11)	0.240–1.185 (14)	0.133–0.439 (19)	0.032–0.271 (20)	0.061–0.360 (22)	0.024–0.206 (22)

3.5.3 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

TABLE 4 Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate.

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme-TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

Abbreviations: +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

[39]Technical dossier/p. 58; Technical dossier/Additional information, 28 August 2023/Annex 1/Answer 2.3.

[40]Technical dossier/Additional information, 28 August 2023/Annex 1/Answer 2.3.

The conservative approach applied to the exposure estimate to food enzyme-TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

3.6 | Margin of exposure

Since no toxicological assessment was considered necessary by the Panel, a margin of exposure was not calculated.

4 | CONCLUSIONS

Based on the data provided, the QPS status of the production strain and the absence of issues of concern arising from the production process, the Panel concluded that the food enzyme bacillolysin produced with the non-genetically modified *Bacillus amyloliquefaciens* strain DP-Cyb74 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier "Application for authorisation of bacillolysin from *Bacillus amyloliquefaciens* (DP-Cyb74). 7 November 2014 (joint dossier). Individual data package was submitted on 22 August 2022 by Genencor International B.V.

Additional information. 28 August 2023. Submitted by Genencor International B.V.

ABBREVIATIONS

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
AMR	antimicrobial resistance gene
[redacted]	[redacted]
AZO	azocasein
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EFSA BIOHAZ Panel	EFSA Panel on Biological Hazards
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation of the United Nations
FoodEx	standardised food classification and description system
GM	genetically modified
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
kDa	kiloDalton
LoD	limit of detection
non-GM	non-genetically modified
QPS	qualified presumption of safety
RM	raw material
TOS	total organic solids
[redacted]	[redacted]
WHO	World Health Organisation

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2022-00527

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NOTE

The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

REFERENCES

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting information' section). The file contains two sheets, corresponding to two tables.

TABLE 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

TABLE 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[a], Serbia[a], Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[a], Serbia[a], Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
The elderly[b]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden

[a]Consumption data from these pre-accession countries are included for testing purpose.

[b]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).