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Updated safety evaluation of the food enzyme isoamylase from the *Dyella* sp. strain MU 1174

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Abstract

The food enzyme isoamylase (glycogen α -1,6-glucanohydrolase, EC 3.2.1.68) is produced with *Dyella* sp. strain MU 1174 by Hayashibara Co. Ltd. In a previous opinion, the Panel on Food Contact Materials, Enzymes and Processing Aids of EFSA could not conclude on the safety of this food enzyme due to uncertainties about the presence of a gene conferring resistance to antimicrobials in the genome of the production strain and its potential transfer to the food enzyme. New whole genome sequence data provided by the applicant showed that the production strain *Dyella* sp. MU 1174 does not contain antimicrobial resistance genes of concern. Based on the new data provided and the evaluation of the data previously submitted, the Panel concludes that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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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.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (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 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 1331/2008² 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 European Union 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, 2009a) 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 on food enzymes.

An application has been introduced by the applicant "Hayashibara Co., Ltd." for the authorisation of the food enzyme isoamylase from a non-genetically modified strain of *Pseudomonas amyloclavata* (current name: *Dyella* sp.) strain MU 1174.

The application relates to the food enzyme for which, according to the scientific opinion of 9 September 2020, EFSA cannot conclude on the safety. The new application contains supplementary information to address the data gaps identified in that scientific opinion (EFSA-Q-2016-00524).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011³ implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

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, 11.3.2011, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: isoamylase from a non-genetically modified strain of *Pseudomonas amyloclavata* (*Dyella* sp.) strain MU 1174 in accordance with Article 29 of Regulation (EC) No 178/2002, and Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

In October 2020, EFSA could not conclude on the safety evaluation of the food enzyme isoamylase produced from a non-genetically modified *Dyella* sp. strain (EFSA CEP Panel, 2020). The present opinion is a follow-up of the previous assessment and assesses only supplementary information provided in the new dossier. Other aspects of the safety of the food enzyme were addressed in the previous Scientific Opinion.

2. Data and methodologies

2.1. Data

The applicant has submitted a revised dossier in support of the application for authorisation of the isoamylase preparation from *Dyella* sp. strain MU 1174. New information not present in the original application was underlined in the technical dossier and four new Annexes were included.

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, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidances of EFSA Scientific Committees.

3. Assessment

IUBMB nomenclature	Isoamylase
Systematic name	Glycogen α -1,6-glucanohydrolase
Synonyms	Debranching enzyme
IUBMB No	EC 3.2.1.68
CAS No	9067-73-6

The enzyme catalyses the hydrolysis of 1,6- α -D-glucosidic branch linkages in α -glucans, such as glycogen and amylopectin, releasing amylose. It is intended to be used in starch processing for the production of various starch hydrolysates.

3.1. Source of the food enzyme

Previous data based on whole genome sequence (WGS) analysis indicated the presence in the genome of the production strain *Dyella* sp. MU 1174 of a sequence with homology to a gene encoding [REDACTED] resistance. Resistance to [REDACTED] may show cross-resistance to [REDACTED], which is a critically important antimicrobial according to the World Health Organization (WHO, 2016). The Panel noted that the WGS obtained was incomplete and the database used for the search no longer maintained. However, as DNA from the production strain was found in the food enzyme, the Panel concluded that the possible presence of at least one antimicrobial resistance gene in the food enzyme could not be excluded and could present a safety concern (EFSA CEP Panel, 2020).

The applicant has now provided a second and complete WGS analysis of the [REDACTED] chromosome, obtained using a [REDACTED], in which the determined sequence is significantly longer (ca. [REDACTED]) than the one obtained in the first experiment.⁴ A search of this new WGS was made against a maintained database (CARD).⁵ A 81% identity was found to a gene referring resistance to elfamycin,

⁴ Technical dossier/Annex 34.a.

⁵ <https://card.mcmaster.ca/home>

an antimicrobial not classified as highly or critically important for human or animal medicine (WHO, 2016).⁶ Therefore, the new data indicate that the production strain *Dyella* sp. MU 1174 does not contain antimicrobial resistance genes of concern.

3.2. Toxicology

Toxicological studies other than allergenicity are not required in the absence of exposure when the food enzyme is used in starch processing for the production of various starch hydrolysates.

3.2.1. Allergenicity

The new WGS data revealed that the amino acid sequence of the isoamylase encoded by the production strain *Dyella* sp. MU 1174 showed some minor differences with respect to the one provided in the initial application. The differences did not alter the total number of amino acids or the calculated molecular weight. The applicant repeated the comparison of the amino acid sequence using the updated data 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.⁷ Therefore, the conclusions of the Panel on the potential allergenicity of the isoamylase produced with *Dyella* sp. MU 1174 previously published (EFSA CEP Panel, 2020) remain the same.

4. Conclusions

Based on the new data showing the absence of antimicrobial resistance genes of concern from the production strain, and the evaluation of the data previously submitted, the Panel concludes that the food enzyme isoamylase produced with *Dyella* sp. MU 1174 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Application for authorisation of Isoamylase preparation from *Pseudomonas amyloclavata* (current name: *Dyella* sp.) strain MU 1174 in accordance with Regulation (EC) No 1331/2008. May 2021. Submitted by Hayashibara Co. Ltd.

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⁶ Technical dossier/Annex 33.

⁷ Technical dossier/Annex 24.

[REDACTED]

WHO (World Health Organization), 2016. Critically important antimicrobials for human medicine– 4th rev. WHO Press. ISBN 978-92-4-151146-9.

Abbreviations

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
EINECS	European Inventory of Existing Commercial Chemical Substances
IUBMB	International Union of Biochemistry and Molecular Biology
WGS	whole genome sequence
WHO	World Health Organization