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SCIENTIFIC OPINION

Safety evaluation of the food enzyme sucrose phosphorylase from the genetically modified *Escherichia coli* strain LE1B109-pPB129

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Abstract

The food enzyme sucrose phosphorylase (sucrose: phosphate α-D-glucosyltransferase; EC 2.4.1.7) is produced with the genetically modified *Escherichia coli* strain LE1B109-pPB129 by c-LEcta GmbH. The genetic modifications do not give rise to safety concerns. The food enzyme was free from viable cells of the production organism. It is intended to be used in combination with a cellobiose phosphorylase in the production of the specialty carbohydrate cellobiose. Since residual amounts of food enzyme–total organic solids are removed by the downstream purification steps, the Panel considered that toxicological studies other than assessment of allergenicity were unnecessary and a dietary exposure was not estimated. 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 upon 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

E. coli, EC 2.4.1.7, EFSA-Q-2021-00291, food enzyme, genetically modified microorganism, sucrose glucosyltransferase, sucrose phosphorylase, sucrose: phosphate α-D-glucosyltransferase

CONTENTS

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 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 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.

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 Union 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 "c-LEcta GmbH" for the authorisation of the food enzyme Sucrose phosphorylase from a genetically modified strain of *E. coli* K12 (strain LE1B109-pPB129).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] 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.

1.1.2 | Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Sucrose phosphorylase from a genetically modified strain of *E. coli* K12 (strain LE1B109_pPB129) in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and flavourings.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme sucrose phosphorylase from the genetically modified *Escherichia coli* strain LE1B109_pPB129.

[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.

Additional information was requested from the applicant during the assessment process on 21 June 2022 and received on 23 January 2023 (see 'Documentation provided to EFSA').

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, 2009a) 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, 2009b) 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. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	Sucrose phosphorylase
Systematic name	Sucrose:phosphate α-D-glucosyltransferase
Synonyms	Sucrose glucosyltransferase; Disaccharide glucosyltransferase
IUBMB no	2.4.1.7
CAS no	9074-06-0
EINECS no	851-947-9

Abbreviations: CAS, Chemical Abstracts Service; EINECS, European Inventory of Existing Commercial Chemical Substances; IUBMB, International Union of Biochemistry and Molecular Biology.

Sucrose phosphorylases catalyse the phosphorolysis of sucrose, producing α-D-glucose-1-phosphate and fructose. The enzyme under application is intended to be used in combination with a cellobiose phosphorylase in the production of the specialty carbohydrate cellobiose.

3.1 | Source of the food enzyme

The sucrose phosphorylase is produced with the genetically modified bacterium *Escherichia coli* strain LE1B109_pPB129, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany), with the deposit number [redacted].[4]

The production strain was identified as *E. coli* using [redacted].[5] No acquired antimicrobial resistance genes or virulence factors of concern were identified in its genome.

3.1.1 | Characteristics of the parental and recipient microorganism

The parental microorganism is [redacted], a strain that has been well characterised and whose safety (non-pathogenicity) has been documented (Gorbach, 1978). *E. coli* K-12 was shown to be ineffective in colonising the human gut and its genome has been fully sequenced (Hayashi et al., 2006). [redacted].[6]

[4]Technical dossier/Confidential Dossier/Annex V_b.

[5]Technical dossier/Confidential Dossier/Annex V_c.

[6]Technical dossier/Confidential Dossier/Annex V_c.

3.1.2 | Characteristics of introduced sequences

The sequence encoding the sucrose phosphorylase [redacted].[7]

3.1.3 | Description of the genetic modification

The purpose of the genetic modification was to enable the production strain to synthesise the sucrose phosphorylase [redacted].[8]

3.1.4 | Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *E. coli* LE1B109_pPB129 differs from the recipient strain in its capacity to produce the sucrose phosphorylase [redacted].

No issues of concern arising from the genetic modification were identified by the Panel.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[10]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. [redacted] is added to the fermentation medium to induce the production of the sucrose phosphorylase. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration and/or centrifugation and the cells are mechanically lysed to release the intracellular enzyme. The lysate containing the enzyme is treated with [redacted] and further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[11] 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.[12]

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

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The sucrose phosphorylase is a single polypeptide chain of [redacted] amino acids.[13] The molecular mass of the mature protein, calculated from the amino acid sequence, is 56.1 kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A consistent protein pattern was observed across all batches. The gels showed the target protein migrating between the marker proteins of 45 and 66 kDa in all batches, consistent with the expected mass of the enzyme.[15] No other enzymatic activities were reported.[16]

[7]Technical dossier/Confidential Dossier/Annex V_c.
[8]Technical dossier/Confidential Dossier/Annex V_c.
[9]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.
[10]Technical Dossier/Risk assessment document/p. 20 and additional information January 2023/Reply to question 3.
[11]Technical Dossier/Risk assessment document/p. 22–30 and Annex VI.
[12]Technical Dossier/Annex VII.
[13]Technical Dossier/Risk assessment document/p. 11 and Annex I.
[14]Technical Dossier/Risk assessment document/p. 11.
[15]Technical Dossier/Risk assessment document/p. 11,13.
[16]Technical Dossier/Risk assessment document/p. 17.

The in-house determination of sucrose phosphorylase activity is based on the hydrolysis of sucrose (reaction conditions: pH 7.0, 30°C, 5 min) by measuring the release of *α*-D-glucose-1-phosphate by means of a coupled reaction that forms NADPH, which is then detected spectrophotometrically at 340 nm. The enzyme activity is expressed in U/mL. One Unit (U) corresponds to the formation of 1 µmol glucose-1-phosphate per minute under the conditions of the assay.[17]

The food enzyme has a temperature optimum around 49°C (pH 7.0) and a pH optimum around pH 7.0 (30 C).[18] Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 7.0). The sucrose phosphorylase activity decreased sharply above 65°C, showing no residual activity after pre-incubation at 70°C.[19]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three batches used for commercialisation (Table 1).[20] The mean total organic solids (TOS) was 8.7% and the mean enzyme activity/TOS ratio was 35.3 U/mg TOS.

TABLE 1 Composition of the food enzyme preparation.

Parameters	Unit	Batches		
		1	2	3
Sucrose phosphorylase activity	U/mL batch[a]	1607	3791	3718
Protein	%	6.2	9.0	7.3
Ash	%	0.8	0.9	0.8
Water	%	43.1	41.4	37.0
Excipient (glycerol)	%	50.0	50.0	50.0
Total organic solids (TOS)[b]	%	6.1	7.7	12.2
Activity/TOS ratio	U/mg TOS	26.3	49.2	30.5

[a]U: Unit (see Section 3.3.1).

[b]TOS calculated as 100% – % water – % ash % excipient.

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg,[21] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, cadmium, mercury and arsenic were below the limit of detection (LoD) of the employed methods.[22,23]

The food enzyme preparation 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).[24] No antimicrobial activity was detected in any of the tested batches.[25]

[redacted] was used as an inducer in the fermentation process. Its concentration in three batches of the food enzyme was below the LoD of the analytical method. The Panel noted that this method had a LoD of [redacted].[26] Considering the absence of food enzyme–TOS in the final food (Section 3.5.1), any residual [redacted] would also have been removed during the downstream purification steps.

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

3.3.4 | Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in quintuplicate. One millilitre of product was incubated in non-selective enrichment medium [redacted]. Subcultures were then plated onto selective agar and incubated at [redacted]. No colonies were produced.[27] A positive control was included.

[17]Technical Dossier/Annex II a.

[18]Technical Dossier/Risk assessment document/p. 16–17.

[19]Technical Dossier/Risk assessment document/p. 16–17.

[20]Technical Dossier/Risk assessment document/p. 12 and Annexes III a-c.

[21]Technical Dossier/Annexes III a-c.

[22]LoDs: Pb = 0.02 mg/kg; As = 0.05 mg/kg; Cd = 0.01 mg/kg; Hg = 0.01 mg/kg.

[23]Technical Dossier/Annexes III a-c.

[24]Technical Dossier/Annexes III a-c.

[25]Technical Dossier/Annexes III a-c.

[26]Technical dossier/Additional data January 2023/Answer to question 1 and Annex II Section 1.

[27]Technical dossier/Additional data January 2023/Answer to question 4 and Annex II Section 4.

The absence of recombinant DNA in the food enzyme was analysed by polymerase chain reaction analysis of two batches of the food enzyme and two batches of the food enzyme after formulation with 50% glycerol, in triplicate.[28] No DNA was detected with primers that would have amplified a [redacted] in the production strain. However, the LoD of 10 ng spiked DNA/g food enzyme was determined in one sample only, for which information regarding the content of glycerol was not provided.[29] Consequently, the Panel was unable to conclude on the absence of recombinant DNA in the food enzyme.

3.4 | Toxicological data

In the course of the food manufacturing process, the food enzyme is removed by the purification steps applied (see Section 3.5). Consequently, in the absence of exposure, the Panel considered that no toxicological studies other than assessment of allergenicity were needed for the assessment of this food enzyme.

3.4.1 | Allergenicity

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

The potential allergenicity of the sucrose phosphorylase produced with the *E. coli* strain LE1B109_pPB129 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]

No information was available on oral and respiratory sensitisation or elicitation reactions of this sucrose phosphorylase.[31]

Some phosphorylases have shown food allergenic potential (Rangkakulnuwat et al., 2023). However, a literature search performed by the applicant did not identify any publication on allergic reactions to sucrose phosphorylase.

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

The food enzyme is intended to be used in the production of a specialty carbohydrate cellobiose at a recommended use level of 24,500 Unit/kg sucrose, corresponding to 931.56 mg TOS/kg sucrose.[32]

In the manufacturing of cellobiose, the food enzyme is used in combination with a cellobiose phosphorylase and is added to sucrose, glucose and phosphate. The successive action of sucrose phosphorylase and cellobiose phosphorylase converts sucrose and glucose into cellobiose. The reaction product is then passed through a membrane ([redacted]) to retain the enzyme in the reactor. The cellobiose-containing permeate is subject to electrodialysis to remove charged constituents. The cellobiose is then obtained by crystallisation.[33]

To establish the extent of the food enzyme–TOS removal, the applicant measured the amount of proteins present in the cellobiose product by SDS-PAGE analysis with silver staining, showing a removal of > 99%.[34] These data were considered by the Panel as sufficient to confirm the absence of TOS in the final product.

3.5.2 | Dietary exposure estimation

The Panel accepted the evidence provided as sufficient to conclude that the residual amounts of food enzyme–TOS in the cellobiose final products is negligible. Consequently, a dietary exposure was not calculated.

[28]Technical dossier/Additional data January 2023/Answer to question 5 and Annex II Section 5.

[29]Technical dossier/Additional data January 2023/Answer to question 5 and Annex II Section 5.

[30]Technical Dossier/Risk assessment document/p. 35–36/Annex IX.

[31]Technical Dossier/Additional information January 2023/Answer to question 6.

[32]Technical dossier/Additional information January 2023/Answer to question 7.

[33]Technical dossier/Annex VIII.

[34]Technical dossier/Additional information January 2023/Answer to question 8 and Annex II Section 8.

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 and on the removal of TOS during the intended food manufacturing process, the Panel concluded that the food enzyme sucrose phosphorylase produced with the genetically modified *Escherichia coli* strain LE1B109_pPB129 does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism but could not establish the absence of recombinant DNA.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Sucrose phosphorylase from the GM *E. coli* strain LE1B109_pPB129. Submitted by c-LEcta GmbH. The dossier was updated on 28 May 2021.

Additional information. January 2023. Submitted by c-LEcta GmbH.

ABBREVIATIONS

CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
IPTG	isopropyl *β*-D-1-thiogalactopyranoside
IUBMB	International Union of Biochemistry and Molecular Biology
LoD	limit of detection
kDa	kiloDalton
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

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

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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.

[†]Deceased.

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