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Safety evaluation of the food enzyme α-cyclodextrin glucanotransferase from *Escherichia coli* strain WCM105xpCM703

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Abstract

The food enzyme α-cyclodextrin glucanotransferase ((1→4)-α-D-glucan 4-α-D-[(1→4)-α-D-glucano]-transferase; EC 2.4.1.19) is produced with a genetically modified *Escherichia coli* strain WCM105xpCM703 by Wacker Chemie GmbH. The production strain harbours a self-replicating multicopy plasmid which contains genes conferring resistance to two highly important antimicrobials for human and veterinary medicine. The food enzyme is free from viable cells of the production organism, but not of its recombinant DNA. Therefore, the food enzyme poses a risk of promoting the spread of antimicrobial resistance genes. It is intended to be used in starch processing for the production of α-cyclodextrin. Residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of α-cyclodextrin; consequently, dietary exposure was not calculated. Genotoxicity tests, although not raising a safety concern, did not comply with the EFSA guideline. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The study was not supplied as a full report. The Panel identified the highest dose tested as the No Observed Adverse Effect Level, which according to the authors of the study corresponds to 260 mg TOS/kg body weight per day. In the absence of information about the sequence homology of this α-cyclodextrin glucanotransferase with known allergens, the Panel could not complete the assessment on the allergenicity of the food enzyme. The Panel concludes that the food enzyme α-cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM703 cannot be considered safe.

Keywords: food enzyme, α-cyclodextrin glucanotransferase, cyclomaltodextrin glucanotransferase, α-CGTase, EC 2.4.1.19, *Escherichia coli*, genetically modified microorganism

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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:

i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
ii) there is a reasonable technological need;
iii) 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 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 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.

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 subtilis* and by the companies "Meiji Seika Pharma Co., Ltd" for the authorisation of the food enzyme Polygalacturonase from *Talaromyces cellulolyticus*/*Talaromyces pinophilus*, "Yakult Pharmaceutical Industry Co., Ltd" for the authorisation of the food enzyme Beta-galactosidase from *Sporobolomyces singularis* (YIT 10047), and "Bioresco ltd." for the authorisation of the food enzymes Cyclomatodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM703) and Cyclomaltodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM6420).

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 five applications fall within the scope of the food enzyme Regulation and contain 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 assessments on the food enzymes Bacillolysin from *Bacillus subtilis*, Polygalacturonase from *Talaromyces cellulolyticus*/*Talaromyces pinophilus*, Beta-galactosidase from *Sporobolomyces singularis* (YIT 10047), and Cyclomatodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM703) and Cyclomaltodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM6420) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

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 food enzyme Cyclomatodextrin glucanotransferase from a genetically modified *E. coli* K12 strain WCM105xpCM703.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme α-cyclodextrin glucanotransferase from a genetically modified *E. coli* K12 strain WCM105xpCM703.

Additional information was requested from the applicant during the assessment process on 12 June 2019 and was consequently provided (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, 2009), as well as in the EFSA 'Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and following the relevant existing guidance's of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature: cyclomaltodextrin glucanotransferase
Systematic name: (1→4)-α-D-glucan 4-α-D-[(1→4)-α-D-glucano]-transferase (cyclising)
Synonyms: cyclodextrin glycosyltransferase, α-cyclodextrin glucanotransferase, α-CGTase
IUBMB No.: EC 2.4.1.19
CAS No.: 9030-09-5
EINECS No.: 618-522-8

The α-cyclodextrin glucanotransferase catalyses the transglycosylation of glucans by the formation of a (1→4)-α-D-glucosidic bond, resulting in the generation of α-, β- and γ-cyclodextrins and transglycosylated glucans. This α-cyclodextrin glucanotransferase is intended to be used in starch processing for the production of α-cyclodextrin, which consists of six glucosyl units.

3.1. Source of the food enzyme

The production strain is a genetically modified bacterium *Escherichia coli* strain WCM105xpCM703, which is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany) with deposit number DSM 15289.[4]

The production strain was taxonomically identified as *E. coli* by sequence alignment analysis of two house-keeping gene loci: the *gapA* gene (encoding glyceraldehyde-3-phosphate dehydrogenase) and

[4] Technical dossier/Annex 7.

the *tufB* gene (encoding protein chain elongation factor EF-Tu). The strain was shown to be a K-12 derivative by a K-12 specific polymerase chain reaction (PCR) using a method described in Kuhnert et al. (1995).[5]

3.1.1. Characteristics of the parental and recipient microorganisms

The recipient strain, *E. coli* WCM105, derives from the parental strain *E. coli* E610. Strain E610 (CGSC# 6669) is a derivative of *E. coli* K-12 with improved efficiency in protein secretion, and has been obtained by classical mutagenesis (Yem and Wu, 1978).[5] *E. coli* K-12 is well-characterised and its safety 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).

The recipient strain *E. coli* WCM105 was selected for resistance to low concentrations of the antibiotic cycloserine, leading to an increased protein secretion. The *traA* gene involved in conjugation was deleted by genetic modification. No details are provided on how this genetic modification was done, in particular on whether antimicrobial resistance marker genes were used. The deletion is reported to be confirmed by sequencing analysis, but no data were provided.[5]

3.1.2. Characteristics of introduced sequences

The sequence encoding the α-cyclodextrin glucanotransferase was derived from *Klebsiella oxytoca* strain M5a1 (Binder et al., 1986). It was placed under the control of [redacted] promoter and [redacted] terminator[6]

Plasmid pCM703 was derived from [redacted] as selectable markers.[7]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise the α-cyclodextrin glucanotransferase from *K. oxytoca*. For this purpose, plasmid pCM703 was introduced in the recipient strain *E. coli* WCM105 by transformation, resulting in the production strain WCM105xpCM703. Plasmid pCM703 remains in the cytoplasm of the production strain [redacted].

3.1.4. Safety aspects of the genetic modification

The production strain *E. coli* WCM105xpCM703 differs from the recipient strain WCM105 in its capacity to produce the α-cyclodextrin glucanotransferase from *K. oxytoca*. The production strain is resistant to [redacted], due to the presence of the respective resistance genes in the multicopy self-replicating plasmid pCM703. Uncertainty remains on the presence of other antimicrobial resistance genes possibly used in the genetic modification of the recipient strain. The presence of antimicrobial resistance genes in the production strain raises a safety concern. The presence of the [redacted] resistance gene in the food enzyme is further investigated in Section 3.3.4.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[5]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch fermentation system with conventional process controls in place. [redacted] is added to the culture medium to induce the expression of the α-cyclodextrin glucanotransferase gene.[9] After completion of the fermentation, the solid biomass is removed from the fermentation broth by centrifugation and filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated,

[5] Additional data April 2020.
[6] Technical dossier/Table 1.
[7] Technical dossier/Figure 1 and Annex 8.
[8] 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.
[9] Technical dossier/p. 21 & Additional data April 2020.

including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded.[10] 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.[11]

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

The α-cyclodextrin glucanotransferase consists of a single polypeptide chain of 655 amino acids. The molecular mass, derived from the amino acid sequence, is 69.0 kDa.[12] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. A consistent protein pattern was observed across all three batches. The gels showed a single dominant band migrating at the same position as the marker proteins of 72 kDa.[5] No relevant side activities have been reported.

The in-house determination of α-cyclodextrin glucanotransferase activity is based on the reaction with the substrate starch, forming cyclodextrin (reaction conditions: [redacted]). The enzymatic activity is determined by means of [redacted]. The α-cyclodextrin glucanotransferase activity is quantified relative to an internal enzyme standard and expressed in α-cyclodextrin glucanotransferase units (U)/g.[13]

The food enzyme has a temperature optimum around [redacted] and a pH optimum around pH [redacted].

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three production batches[14] and two batches produced for the toxicological tests[15] (Table 1). The average total organic solids (TOS) of the three food enzyme production batches was 1.2%. The average enzyme activity/TOS ratio of the three food enzyme production batches is 26.4 U/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches				
		1	2	3	4[(a)]	5[(b)]
α-cyclodextrin glucanotransferase activity	U/mL batch[(c)]	271	313	289	625	530
Protein	%	NA	NA	NA	0.50	0.36
Ash	%	0.31	0.30	0.40	0.55	0.60
Water	%	98.47	98.88	98.05	98.05	98.1
Total organic solids (TOS)[(d)]	%	1.22	0.82	1.55	1.40	1.30
Activity/mg TOS	U/mg TOS	22.2	38.2	18.7	44.6	40.8

NA: not analysed.
(a): Batch used for the bacterial reverse mutation test and *in vitro* mammalian chromosomal aberration test.
(b): Batch used for the repeated dose 90-day oral toxicity study in rodents.
(c): U: α-cyclodextrin glucanotransferase units (see Section 3.3.1).
(d): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

Despite being requested, no data on lead or microbiological parameters were available to establish the general compliance of the food enzyme with the FAO/WHO specification (FAO/WHO, 2006).

The concentration of [redacted] was quantified in three food enzyme batches in triplicate by liquid chromatography with tandem mass spectrometry, ranging [redacted].[5]

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

[10] Technical dossier/Section 3.2.2.1.
[11] Technical dossier/Annex 3 and 9; Additional data April 2020.
[12] Technical dossier/p. 8.
[13] Technical dossier/Annex 2.
[14] Technical dossier/p. 11.
[15] Technical dossier/p. 30.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent batches of the food enzyme. [redacted]. No colonies were found.[5]

The absence of recombinant DNA in the food enzyme was tested by PCR analysis of three batches, targeting a 500-bp fragment specific for the [redacted]resistance gene in the pCM703 plasmid. Recombinant DNA from the production strain was found in all three batches.[5]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats has been provided. The toxicological assays were performed with food enzyme batches 4 and 5 (Table 1). Both batches have higher enzymatic activity per mg TOS, when compared to the mean of the production batches. Therefore, the Panel considers that the toxicological tests provide limited evidence for the safety evaluation of this food enzyme.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a,b) and following Good Laboratory Practice (GLP) in four strains of Salmonella Typhimurium (TA1535, TA1537, TA98, TA100) and *Escherichia coli* WP2uvrA in the presence or absence of metabolic activation (S9-mix).[16] The plate incorporation method was applied and two separate experiments were carried out in triplicate using five different concentrations of the food enzyme (62, 185, 556, 1,667 and 5,000 μg/plate, corresponding to 0.9, 2.6, 7.8, 23.3 and 70 μg TOS/plate and 313, 625, 1,250, 2,500 and 5,000 μg/plate, corresponding to 4.4, 8.8, 18, 35 and 70 μg TOS/plate; Batch 4). No evidence of toxicity was observed under any of the conditions tested. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers. Although the Panel concluded that the food enzyme did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study, the highest concentration of TOS tested was 70 μg/plate and not the 5,000 μg/plate recommended in the guidance (EFSA, 2014).

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[17] The food enzyme was tested for its ability to induce chromosomal aberrations in human peripheral blood lymphocytes with and without metabolic activation (S9 mix) at concentrations up to 5,000 μg food enzyme/mL (corresponding to 70 μg TOS/mL) (Batch 4). Two separate experiments in duplicate cultures were performed. In the first experiment, the cultures were exposed to 1,250, 2,500 and 5,000 μg/mL (corresponding to 17.5, 35 and 70 μg TOS/mL) for 4 h followed by a 20 h recovery (short treatment) either in the presence and absence of the S9 mix and for 24 h continuous treatment in the absence of the S9 mix. In the second experiment, the cultures were exposed to 3,000, 4,000 and 5,000 μg/mL (corresponding to 42, 56 and 70 μg TOS/mL) for 24 h and 48 h continuous treatment in the absence of the S9 mix and 4 h followed by a 20 h or 44 h recovery in the presence of the S9 mix. No reduction of the mitotic indices was observed at any concentration tested. The frequency of structural and numerical (polyploidy) chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that the food enzyme did not induce structural and numerical chromosomal aberrations in cultured human blood lymphocytes, under the test conditions employed for this study, although the food enzyme, expressed as TOS, was not tested up to 10 mM or up to 55 ± 5% cytotoxicity, as recommended in OECD guideline 473.

[16] Technical dossier/Literature/Krul & van den Wijngaard, 2004; Bär et al., 2004.
[17] Technical dossier/Literature/de Vogel, 2004, Bär et al., 2004.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was provided only in the form of a publication (Bär et al., 2004) and not the full study report.

The repeated dose 90-day oral toxicity study was performed in accordance with requirements of FDA guidelines for testing of food additives and following GLP. Groups of 20 male and 20 female Wistar rats received by gavage 5, 10 or 20 mL food enzyme/kg body weight (bw) per day, corresponding to 65, 130 and 260 mg TOS/kg bw per day (Batch 5). Controls received the vehicle (tap water, 20 mL/kg bw per day).

There were two premature deaths: one control male died due to mis-dosing and one low-dose female died due to narcosis accident.

Clinical chemistry revealed some statistically significant differences to controls: increased plasma calcium concentration in low-dose females on day 30, lower albumin to globulin ratio in mid-dose males and lower plasma cholesterol concentration in high-dose males on day 60, and lower plasma concentration of triglycerides at termination in high-dose males. As these findings were transient or lacked a dose–response relationship or were limited to one sex, they were considered by the Panel as not of toxicological significance.

Histopatological examination revealed slight to moderate pulmonary changes such as alveolar haemorrhages with crystals (incidence statistically significantly increased in high-dose males), increased septal cellularity (incidence statistically significantly increased in low- and high-dose males) and accumulations of alveolar macrophages. The Panel considered these changes to be related to the gavage administration of test solutions (aspiration) and not of toxicological relevance.

No other statistically significant differences to controls were observed.

The Panel identified the highest dose tested as the no observed adverse effect level, which according to the study authors corresponds to 260 mg TOS/kg bw per day.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The comparison of the amino acid sequence with those of known allergens, despite being requested, has not been provided.[5] Consequently, the Panel cannot assess the potential allergenicity of the α-cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM703.

No information is available on oral and respiratory sensitisation or elicitation reactions of this α-cyclodextrin glucanotransferase.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[18]) are used as raw materials (soybean) in the growth medium fed to the production organism.[5] However, during the fermentation process, these products will be degraded and utilised by the bacteria for cell growth, cell maintenance and production of enzyme protein. In addition, the bacterial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

In the absence of information about the sequence homology of this α-cyclodextrin glucanotransferase against known allergens, as well as the lack of information about the general allergenicity of cyclomaltodextrin glucanotransferases, the Panel is not in the position to complete the assessment on the allergenicity of the α-cyclodextrin glucanotransferase expressed by the production strain *E. coli* WCM105xpCM703.

[18] 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.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in starch processing for the production of α-cyclodextrin at a recommended use level of up to 25 U/g starch, corresponding to 820 mg TOS/kg starch.[5]

A flowchart depicting the manufacturing process steps of cyclodextrin has been provided.[5] The food enzyme is added to the liquefied starch, where the cyclodextrin glucanotransferase catalyses a transglycosylation reaction to degrade the amylose in the starch to form a cyclodextrin mixture. Solvent is then added to recover the α-cyclodextrin/1-decanol complex.[19]

This complex is separated from the reaction mixture and purified in multiple steps, including dissolution in water and re-precipitation at 110–130°C, removal of 1-decanol in a stripping column at 100–130°C and crystallisation of α-cyclodextrin.

The Panel considered that the efficiency of the described purification steps in the production of α-cyclodextrin is essentially the same as those in the production of glucose syrups. Furthermore, the absence of protein in α-cyclodextrin was shown by polyacrylamide gel electrophoresis in three batches,[20] and by amino acid analysis after acid hydrolysis.[21]

3.5.2. Dietary exposure estimation

The technical information and experimental data provided on the removal of food enzyme TOS during starch processing for α-cyclodextrin production were considered by the Panel as sufficient to exclude this process from the exposure estimation (Annex B in EFSA CEF Panel, 2016). Consequently, a dietary exposure was not calculated.

4. Conclusions

The food enzyme contains DNA from the production strain, which harbours genes conferring resistance to two highly important antimicrobials for human and veterinary medicine in a self-replicating multicopy plasmid. Therefore, the enzyme α-cyclodextrin glucanotransferase poses a risk of promoting the spread of genes coding for antimicrobial resistance. The Panel concludes that the food enzyme α-cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM703 cannot be considered safe.

The food enzyme is free from viable cells of the production organism, but not from recombinant DNA.

5. Remarks

The food enzyme α-cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM703 is used solely for in-house production of the α-cyclodextrin. The assessment of the α-cyclodextrin is outside the remit of the CEP Panel. The applicant stated that this proprietary enzyme is not for sale to third parties.

Documentation provided to EFSA

1) Technical dossier 'Alpha-cyclodextrin glucanotransferase'. March 2015, submitted by Bioresco Ltd on behalf of Wacker Chemie GmbH.
2) Technical dossier. December 2017, amended by Bioresco Ltd.
3) Additional information. April 2020, submitted by Bioresco Ltd.

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[19] Technical dossier/pp. 13–16.
[20] Technical dossier/Annex 4, LoD = 5 mg protein/kg α-cyclodextrin.
[21] Technical dossier/Annex 3, LoD = 10 mg protein/kg α-cyclodextrin.

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Abbreviations

bw	body weight
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
DNA	Deoxyribonucleic acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GMbH
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	Genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology

kDa	kilo Dalton
LOD	Limit of Detection
OECD	Organisation for Economic Cooperation and Development
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization