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Safety evaluation of the food enzyme β -cyclodextrin glucanotransferase from *Escherichia coli* strain WCM105xpCM6420

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Jaime Aguilera, Natalia Kovalkovicova, Yi Liu, Joaquim Maia and Andrew Chesson

Abstract

The food enzyme β -cyclodextrin glucanotransferase ((1 \rightarrow 4)- α -D-glucan 4- α -D-[(1 \rightarrow 4)- α -D-glucano]-transferase; EC 2.4.1.19) is produced with a genetically modified *Escherichia coli* strain WCM105xpCM6420 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 γ -cyclodextrin. Residual amounts of total organic solids are removed by the purification steps applied during the production of γ -cyclodextrin; consequently, dietary exposure was not calculated. A bacterial reverse mutation test was not considered, because the representativeness of the test material could not be established. No other toxicological tests were provided. In the absence of information about the sequence homology of this β -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 β -cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM6420 cannot be considered safe.

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Correspondence: fip@efsa.europa.eu

Panel members: Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Coconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn and Andrew Chesson.

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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 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 1331/2008² 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:

- 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 234/2011³ 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 WCM105xpCM6420.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme β -cyclodextrin glucanotransferase from a genetically modified *E. coli* K12 strain WCM105xpCM6420.

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 with 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 \rightarrow 4)- α -D-glucan 4- α -D-[(1 \rightarrow 4)- α -D-glucano]-transferase (cyclising)
Synonyms:	cyclodextrin glycosyltransferase, β -cyclodextrin glucanotransferase, β -CGTase
IUBMB No.:	EC 2.4.1.19
CAS No.:	9030-09-5
EINECS No.:	618-522-8

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

3.1. Source of the food enzyme

The production strain is a genetically modified bacterium *Escherichia coli* strain WCM105xpCM6420, which is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany) with deposit number DSM 14053.⁴

⁴ Technical dossier/Annex 7.

The production strain was taxonomically identified as *E. coli* by sequence alignment analysis of two house-keeping genes: the *gapA* gene (encoding glyceraldehyde-3-phosphate dehydrogenase) and 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 *tra-A* 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 β -cyclodextrin glucanotransferase was derived from *Bacillus* sp. strain 1-1 (Schmid, 1989). It was placed under the control of [REDACTED] promoter and [REDACTED] terminator.⁶

Plasmid pCM6420 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 β -cyclodextrin glucanotransferase from *Bacillus* sp. strain 1-1. For this purpose, plasmid pCM6420 was introduced in the recipient strain by transformation, resulting in the production strain WCM105xpCM6420. Plasmid pCM6420 remains in the cytoplasm of the production strain [REDACTED].

3.1.4. Safety aspects of the genetic modification

The production strain *E. coli* WCM105xpCM6420 differs from the recipient strain WCM105 in its capacity to produce the β -cyclodextrin glucanotransferase from *Bacillus* sp. strain 1-1. The production strain is resistant to [REDACTED], due to their presence of the respective resistance genes in the multicopy self-replicating plasmid pCM6420. Uncertainty remains on the existence 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 potential 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 852/2004⁸, 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 β -

⁵ Additional data April 2020.

⁶ Technical dossier/Table 1.

⁷ Technical dossier, Figure 1 and Table 1.

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.

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, 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 β -cyclodextrin glucanotransferase consists of a single polypeptide chain of 655 amino acids. The molecular mass, derived from the nucleotide sequence, is 69.6 kDa.⁵ 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 protein of 72 kDa.⁵ No relevant side activities have been reported.

The in-house determination of β -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 β -cyclomaltodextrin glucanotransferase activity is quantified relative to an internal enzyme standard and expressed in β -cyclodextrin glucanotransferase units (U)/g.¹²

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

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three production batches¹³ and one batch produced for the toxicological tests¹⁴ (Table 1). The average total organic solids (TOS) of the three food enzyme production batches was 1.5%. The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 49.8 U/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches			
		1	2	3	4 ^(a)
β -cyclodextrin glucanotransferase activity	U/mL batch ^(b)	861	434	502	700
Protein	%	NA	NA	NA	NA
Ash	%	0.25	0.34	0.13	NA
Water	%	97.94	97.56	99.25	97.0
Total organic solids (TOS) ^(c)	%	1.81	2.10	0.62	NA
Activity/mg TOS	U/mg TOS	47.6	20.7	81.0	NA

NA: not analysed.

(a): Batch used for the bacterial reverse mutation test.

(b): U: cyclodextrin glucanotransferase units (see Section 3.3.1).

(c): TOS calculated as 100% – % water – % ash.

⁹ Technical dossier/p. 21 & Additional data April 2020.

¹⁰ Technical dossier/Section 3.2.2.1.

¹¹ Technical dossier/Annex 3 and 9 & Additional data April 2020.

¹² Technical dossier/Annex 2.

¹³ Technical dossier/p. 11.

¹⁴ Technical dossier/Literature/van Delft, 1997.

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.

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 pCM6420 plasmid. Recombinant DNA from the production strain was found in all three batches.⁵

3.4. Toxicological data

A bacterial gene mutation assay (Ames test) was made by using batch 4 (Table 1) as the test material.¹⁴ Despite more detailed analysis for the chemical composition of the batch 4 being requested, only enzymatic activity and water content were analysed in this batch.⁵ As insufficient data prevent the Panel from evaluating the suitability of this test material, the Ames test was not considered for this assessment.

3.4.1. 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 β -cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM6420.

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

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011¹⁵) 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 β -cyclodextrin glucanotransferase against known allergens expressed, 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 β -cyclodextrin glucanotransferase expressed by the production strain *E. coli* WCM105xpCM6420.

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 γ -cyclodextrin at a recommended use level of 15–20 U/g starch, corresponding to up to 403 mg TOS/kg starch.⁵

¹⁵ 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.

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 γ -cyclodextrin/8-cyclohexadecen-1-one complex.¹⁶

This complex is separated from the reaction mixture and purified in multiple steps, including dissolution in water and re-precipitation at 110°C, removal of 8-cyclohexadecen-1-one in a stripping column at 100–120°C and crystallisation of γ -cyclodextrin.

The Panel considered that the efficiency of the described purification steps in the production of γ -cyclodextrin is essentially the same as those in the production of glucose syrups. Furthermore, the absence of protein in γ -cyclodextrin was shown by polyacrylamide gel electrophoresis in three batches,¹⁷ and by amino acid analysis after acid-catalysed hydrolysis.¹⁸

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 β -cyclodextrin glucanotransferase poses a risk of promoting the spread of genes coding for antimicrobial resistance. The Panel concludes that the food enzyme β -cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM6420 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 β -cyclodextrin glucanotransferase produced with the genetically modified *E. coli* strain WCM105xpCM6420 is used solely for in-house production of the γ -cyclodextrin. The assessment of the γ -cyclodextrin is outside the remit of the CEF Panel. The applicant stated that this proprietary enzyme is not for sale to third parties.

Documentation provided to EFSA

- 1) Technical dossier 'Beta-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.

References

- EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 22 pp. <https://doi.org/10.2903/j.efsa.2009.1051>
- EFSA CEF Panel (EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the submission of a dossier on food enzymes. EFSA Journal 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Exposure assessment of food enzymes. EFSA Journal 2016;14(11):4581, 9 pp. <https://doi.org/10.2903/j.efsa.2016.4581> and Annex B – Process-specific technical data used in exposure assessment of food enzymes (Available online: https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4581&file=efs24581-sup-0001-Annex_B.pdf)

¹⁶ Technical dossier, 2nd submission/pp. 13–16.

¹⁷ Additional data April 2020, LoD = 10 ng/lane by silver staining.

¹⁸ Technical dossier/Annex 4, LoD = 5 mg protein/kg γ -cyclodextrin.

- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193, 54 pp. <https://doi.org/10.2903/j.efsa.2011.2193>
- FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs, 3, 63–67. Available online: <http://www.fao.org/3/a-a0675e.pdf>
- Gorbach SL, 1978. Risk assessment of recombinant DNA experimentation with *Escherichia coli* K12. The Journal of Infectious Diseases, 137, 613–714. <https://doi.org/10.1093/infdis/137.5.704>
- Hayashi K, Morooka N, Yamamoto Y, Fujita K, Isono K, Choi S, Ohtsubo E, Baba T, Wanner BL, Mori H and Horiuchi T, 2006. Highly accurate genome sequences of *Escherichia coli* K-12 strains MG1655 and W3110. Molecular Systems Biology, 2, 2006.0007. <https://doi.org/10.1038/msb4100049>
- Kuhnert P, Nicolet J and Frey J, 1995. Rapid and accurate identification of *Escherichia coli* K-12 strains. Applied Environmental Microbiology, 61, 4135–4139. <https://doi.org/10.1128/aem.61.11.4135-4139.1995>
- Schmid G, 1989. Cyclodextrin glycosyltransferase production: yield enhancement by overexpression of cloned genes. Trends in Biotechnology, 7, 244–248. [https://doi.org/10.1016/0167-7799\(89\)90015-2](https://doi.org/10.1016/0167-7799(89)90015-2)
- Van Delft JHM, 1997. Ames test with beta-CGTase Ec6420. Report No. V97.358. Revised final report of TNO Nutrition and Food Research Institute, Zeist, The Netherlands.
- Yem DW and Wu HC, 1978. Physiological characterization of an *Escherichia coli* mutant altered in the structure of murein lipoprotein. Journal of Bacteriology, 133, 1419–1426. Available online: <https://jb.asm.org/content/133/3/1419.long>

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
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
GMO	Genetically Modified Organism
IUBMB	International Union of Biochemistry and Molecular Biology
kDa	kilo Dalton
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