STATEMENT



Taxonomic identity of the Bacillus licheniformis strains used to produce food enzymes evaluated in published EFSA opinions

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Abstract

Bacillus paralicheniformis, a species known to produce the antimicrobial bacitracin, could be misidentified as Bacillus licheniformis, depending on the identification method used. For this reason, the European Commission requested EFSA to review the taxonomic identification of formerly assessed *B. licheniformis* production strains. Following this request, EFSA retrieved the raw data from 27 technical dossiers submitted and found that the taxonomic identification was established by 16S rRNA gene analyses for 15 strains and by whole genome sequence analysis for 12 strains. As a conclusion, only these 12 strains could be unambiguously identified as B. licheniformis.

KEYWORDS

B. licheniformis, B. paralicheniformis, taxonomy

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CONTENTS

Ab	stract.		1				
1.	Intro	Introduction					
	1.1.	Background and Terms of Reference as provided by the requestor	3				
		.1.1. Background as provided by the European Commission					
		.1.2. Terms of Reference	4				
2.	Data	nd methodologies	4				
	2.1.	Data					
	2.2.	Nethodologies					
3.							
4.		isions					
5.		nentation as provided to EFSA					
Ab		ons					
Со	nflict	interest	7				
		umber					
		for non-EFSA content					
		bers					
		mer					

1 | INTRODUCTION

The species *Bacillus licheniformis* is a Gram-positive, rod-shaped bacterium that belongs to the *Bacillus* genus, commonly found in soils.

B. paralicheniformis, classified in 2015 as a new species, is closely related to *B. licheniformis* within the *Bacillus* genus. Among the few differences between the two species, it is notable the capacity of *B. paralicheniformis* to produce bacitracin, fengycin and a lantipeptide (Dunlap et al., 2015). Bacitracin is considered a medical important antimicrobial and it has been previously shown to induce cross-resistance to colistin (Xu et al., 2018), an important antimicrobial (as defined by the 'WHO List of Medically Important Antimicrobials, 2024').¹

Strains of both species may qualify for the qualified presumption of safety (QPS) approach, provided that certain criteria (the qualifications) are met. The QPS is a scientific approach developed by EFSA to simplify the safety assessment framework of a microorganism for its use in the food chain (EFSA, 2007; EFSA BIOHAZ Panel, 2022). The QPS list was published at the species level since 2008 and updated regularly thereafter (EFSA BIOHAZ Panel, 2023). Following an unambiguous taxonomic identification of the strain, the QPS qualifications are applied at the strain level. For *Bacillus* species, these include the absence of acquired antimicrobial resistance (AMR) genes to clinically relevant antimicrobials and the absence of toxigenic activity. Due to the ability of some strains of *B. paralicheniformis* to produce bacitracin, which presence in food products is a hazard, an additional qualification applies for strains of this species which is the inability to synthesise bacitracin.

The most recent guidance on the risk assessment of food enzymes (EFSA CEP Panel, 2021c) specifies that taxonomical identification for bacteria should be exclusively conducted through a computational approach using whole genome sequence (WGS) data. Before this guidance, although EFSA encouraged WGS-based approaches (EFSA CEP Panel, 2019), identification through the comparison of sequences classically used for taxonomic identification (such as the 16S rRNA gene) or other characteristic genes (e.g. housekeeping genes) were considered acceptable. Consequently, scientific opinions published before the implementation of the EFSA's scientific guidance of 2021 may contain a less robust taxonomical identification, potentially misidentifying strains of *Bacillus paralicheniformis* as *Bacillus licheniformis*.

Several studies indicate that taxonomic identification through phylogenetic analysis based solely on the 16S rRNA gene sequence may be insufficient to distinguish between different species within the *Bacillus* genus (Burgess et al., 2010; Branquinho et al., 2014; Olajide et al., 2021). Conversely, taxonomic identifications based on WGS analysis are capable of unequivocally identifying *Bacillus* species and discriminating between *B. licheniformis* and *B. paralicheniformis*.

Recognising the importance of unequivocal taxonomic identification of *B. paralicheniformis* from *B. licheniformis*, in December 2023, the European Commission requested EFSA to re-examine all the published scientific opinions on food enzymes in which *B. licheniformis* was reported as the production strain. In this statement, EFSA re-examined the data provided in the technical dossiers supporting the publication of the EFSA's opinions to document whether the taxonomic identifications were based on WGS.

1.1 Background and Terms of Reference as provided by the requestor

1.1.1 Background as provided by the European Commission

On 8 March and 5 July 2023, EFSA adopted two opinions on the safety evaluation of the food enzyme subtilisin from the non-genetically modified strains of *Bacillus paralicheniformis* LMG S-30155² and DP-Dzx96.³

The opinions state that the presence of bacitracin, a medical important antimicrobial, in the food enzyme represents a risk for the development of resistance in bacteria. The food enzyme cannot therefore be considered safe.

In light of the adopted opinions and in accordance with article 19(4) of Regulation (EC) No 178/2002, the Commission sent a letter asking the applicant to take the necessary follow-up actions and notably to withdraw the food enzyme from the market.

EFSA further clarified that *Bacillus paralicheniformis* is the only species of the *Bacillus subtilis* group that can produce bacitracin.

Whole genome sequence (WGS) analysis is required for the identification and characterisation of bacterial strains intended for use as food enzyme production strains by the 'Scientific Guidance for the submission of dossiers on Food Enzymes' published in 2021. Due to the possibility to re-identify a *Bacillus licheniformis* strain as a *Bacillus paralicheniformis* based on the internationally accepted classification, EFSA systematically checks the data and methodology used in the initial submission and whenever needed, requests the applicant to redo the species identification by a WGS analysis.

Only food enzymes produced with a production strain unambiguously identified as *Bacillus paralicheniformis* by WGS analysis are subject to further scrutiny regarding the presence of bacitracin.

¹WHO List of Medically Important Antimicrobials, 2024, https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf?sfvrsn=3320dd3d_2.

²https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.7910.

³https://www.efsa.europa.eu/en/efsajournal/pub/8155.

⁴https://doi.org/10.2903/j.efsa.2021.6851.

Between 2017 and December 2023, several opinions were adopted for food enzymes produced by *Bacillus licheniformis* strains. As WGS data were not always available in the applications submitted before the date of entry into force of the guidance, the Commission considers that there is a need for checking the data and methodology used for the identification of the production strain for those already adopted opinions of food enzymes from *Bacillus licheniformis* and to confirm their identity.

The new rules on transparency introduced by Regulation (EU) 2019/1381 of the European Parliament and of the Council on the transparency and sustainability of the EU risk assessment in the food chain shall not apply to those applications, as they were submitted before Regulation (EU) 2019/1381 entered into application.

1.1.2 | Terms of Reference

In accordance with Article 29 of Regulation (EC) No 178/2002, the Commission requests the assistance of the European Food Safety Authority in the re-assessment of adopted opinions for food enzymes from *Bacillus licheniformis* to confirm the taxonomic identity of the production strains in accordance with the 'Scientific Guidance on Food Enzymes' published in 2021. In that assessment, the Authority shall take into account in particular the information already received by the Authority in the technical dossiers for the safety evaluation of the corresponding food enzymes and any other relevant new scientific and technical information that has become available.

2 | DATA AND METHODOLOGIES

2.1 | Data

Scientific opinions on food enzymes published in the EFSA Journal between 2014 and January 2024 were searched to identify those using *B. licheniformis* as production strain. Raw data on the taxonomic identification of each production strain were retrieved from the related technical dossiers submitted by the applicants.

2.2 | Methodologies

The original applications and the corresponding scientific opinions were screened to confirm whether the taxonomic identity analysis of the production strains was conducted by a computational approach using WGS data, in accordance with the 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021c). The data in section 'Source of the Food enzymes' of the scientific opinions were verified with the analysis data provided in the corresponding dossiers. The identity of the production strains as *B. licheniformis* was considered unequivocally confirmed when the WGS of the production strain was compared with the genome of the *B. licheniformis* type strain and showed either an average nucleotide identity (ANI) of > 95% or a digital DNA–DNA hybridisation (dDDH) value of > 70%. Confirmation of the species identity by phylogenomic analysis based on WGS data was also considered sufficient. The outcome of this analysis was used to (i) confirm the taxonomic identification of formerly assessed *B. licheniformis* production strains and (ii) identify a list of applications for which an unambiguous identification of the production strain could not be achieved.

3 OUTCOME

EFSA published scientific opinions on food enzymes produced with *B. licheniformis* strains since August 2017. By January 2024, a total of 27 such scientific opinions were published in EFSA Journal. The information on the methodology used and the results of the identification of the production strain was extracted and collated in a tabulated form to facilitate its analysis (Table 1).

TABLE 1 Examination of the taxonomic identification of individual production strains.

B. licheniformis	EFSA question number	Reference to published opinion	Date of publication in EFSA journal	Basis of the taxonomic identification analysis		Unambiguous
production strain name				WGS	16S rRNA	identification as B. licheniformis
NZYM-CX	Q-2022-00545	EFSA CEP Panel, 2023b https://doi.org/10.2903/j.efsa.2023.8406	Nov-23	✓		Yes
NZYM-AC	Q-2013-00586	EFSA CEP Panel, 2023c https://doi.org/10.2903/j.efsa.2023.8393	Nov-23	✓		Yes
T74	Q-2022-00592	EFSA CEP Panel, 2023a https://doi.org/10.2903/j.efsa.2023.8160	Aug-23	✓		Yes
NZYM-DI	Q-2021-00225	EFSA CEP Panel, 2022h https://doi.org/10.2903/j.efsa.2022.7470	Aug-22	✓		Yes
NZYM-AY	Q-2021-00292	EFSA CEP Panel, 2022g https://doi.org/10.2903/j.efsa.2022.7467	Aug-22	✓		Yes
NZYM-BC	Q-2013-00685	EFSA CEP Panel, 2022e https://doi.org/10.2903/j.efsa.2022.7370	Jul-22	✓		Yes
NZYM-BT	Q-2015-00093	EFSA CEP Panel, 2022f https://doi.org/10.2903/j.efsa.2022.7358	Jul-22	✓		Yes
NZYM-CY	Q-2021-00137	EFSA CEP Panel, 2022d https://doi.org/10.2903/j.efsa.2022.7366	Jun-22	✓		Yes
NZYM-SD	Q-2021-00138	EFSA CEP Panel, 2022b https://doi.org/10.2903/j.efsa.2022.7368	Jun-22	✓		Yes
NZYM-FR	Q-2021-00142	EFSA CEP Panel, 2022c https://doi.org/10.2903/j.efsa.2022.7367	Jun-22	✓		Yes
NZYM-LU	Q-2021-00222	EFSA CEP Panel, 2022a https://doi.org/10.2903/j.efsa.2022.7359	Jun-22	✓		Yes
NZYM-KE	Q-2012-00898	EFSA CEP Panel, 2021a https://doi.org/10.2903/j.efsa.2021.6433	Mar-21	✓		Yes
DP-Dzb52	Q-2016-00093	EFSA CEP Panel, 2021b https://doi.org/10.2903/j.efsa.2021.6564	Apr-21		✓	No
DP-Dzb45	Q-2016-00143	EFSA CEP Panel, 2020c https://doi.org/10.2903/j.efsa.2020.6311	Nov-20		✓	No
NZYM-VR	Q-2019-00442	EFSA CEP Panel, 2020b https://doi.org/10.2903/j.efsa.2020.6184	Jul-20		✓	No
DP-Dzr50	Q-2016-00096	EFSA CEP Panel, 2020a https://doi.org/10.2903/j.efsa.2020.5972	Jan-20		✓	No
DP-Dzb25	Q-2016-00202	EFSA CEP Panel, 2019g https://doi.org/10.2903/j.efsa.2019.5900	Nov-19		✓	No
DP-Dzf24	Q-2015-00448	EFSA CEP Panel, 2019e https://doi.org/10.2903/j.efsa.2019.5739	Jun-19		✓	No
DP-Dzb44	Q-2015-00836	EFSA CEP Panel, 2019f https://doi.org/10.2903/j.efsa.2019.5738	Jun-19		✓	No
DP-Dzr46	Q-2016-00095	EFSA CEP Panel, 2019d https://doi.org/10.2903/j.efsa.2019.5684	May-19		✓	No
NZYM-CE	Q-2015-00064	EFSA CEP Panel, 2019c https://doi.org/10.2903/j.efsa.2019.5685	Apr-19		✓	No
DP-Dzp39	Q-2015-00667	EFSA CEP Panel, 2019b https://doi.org/10.2903/j.efsa.2019.5554	Jan-19		✓	No
DP-Dzb54	Q-2015-00666	EFSA CEP Panel, 2019a https://doi.org/10.2903/j.efsa.2019.5549	Jan-19		✓	No
NZYM-JB	Q-2016-00031	EFSA CEP Panel, 2018c https://doi.org/10.2903/j.efsa.2018.5476	Nov-18		✓	No
NZYM-AV	Q-2014-00794	EFSA CEP Panel, 2018b https://doi.org/10.2903/j.efsa.2018.5318	Jul-18		✓	No
NZYM-AN	Q-2015-00084	EFSA CEP Panel, 2018a https://doi.org/10.2903/j.efsa.2018.5317	Jul-18		✓	No
NZYM-JA	Q-2015-00275	EFSA CEP Panel, 2017 https://doi.org/10.2903/j.efsa.2017.4896	Aug-17		✓	No

As shown in Table 1, 12 production strains were identified by WGS analysis and 15 production strains by 16S rRNA gene analyses, as summarised in Table 2.

TABLE 2 Summary of the taxonomic identification of B. licheniformis production strains in published scientific opinions.

Taxonomic identification method used in former evaluation	Number of production strains are-examined	Production strain unambiguously identified as <i>B. licheniformis</i> by the present report	Production strain not confirmed as <i>B. licheniformis</i> by the present report
16S rRNA sequence analysis	15	0	15
Whole genome sequencing analysis	12	12	0
Total	27	12	15

^aEach production strain was reported in a single scientific opinion.

The retrospective screening revealed a significant reliance on 16S rRNA sequence analysis for taxonomic identification in applications for food enzymes declaring *B. licheniformis* as the production strain before October 2021. Upon enforcement of WGS-based taxonomic identification as requested by the latest EFSA guidance of 2021, a shift towards a more robust identification was taken. The strains analysed by WGS can be considered unequivocally identified as *B. licheniformis*. Timewise, the scientific risk assessment of all those 15 applications was concluded before the latest guidance for the submission of dossiers on food enzymes (EFSA CEP Panel, 2021c).

4 | CONCLUSIONS

The Panel examined the raw data and methods of 27 technical dossiers of food enzymes with *B. licheniformis* production strains. The taxonomic identification was established by 16S rRNA gene analyses for 15 strains and by WGS analysis for 12 strains. As a conclusion, only these 12 strains could be confirmed as *B. licheniformis*.

5 | DOCUMENTATION AS PROVIDED TO EFSA

- 1. Application for authorisation β -amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JA). EFSA-Q-2015-00275.
- 2. Application for authorisation of α -amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN). EFSA-Q-2015-00084.
- 3. Application for authorisation of α -amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AV)'. EFSA-Q-2014-00794.
- 4. Application for authorisation of acetolactate decarboxylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JB)'. EFSA-Q-2016-00031.
- 5. Application for authorisation of α -amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb54). EFSA-Q-2015-00666.
- 6. Application for authorisation of pullulanase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzp39). EFSA-Q-2015-00667.
- 7. Application for authorisation of endo-1,4- β -xylanase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CE). EFSA-Q-2015-00064.
- 8. Application for authorisation of glucan 1,4-alpha-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzr46). EFSA-Q-2016-00095.
- 9. Application for authorisation of from a genetically modified strain of Bacillus licheniformis (strain DP-Dzf24). EFSA-Q-2015-00448.
- 10. Application for authorisation of α -amylase from a genetically modified strain of Bacillus licheniformis (DP-Dzb44). EFSA-Q-2015-00836.
- 11. Application for authorisation of α -amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb25). EFSA-Q-2016-00202.
- 12. Application for authorisation of glucan 1,4- α -maltohydrolase from a genetically modified strain of *Bacillus licheniformis* DP-Dzr50. EFSA-Q-2016-00096.
- 13. Application for authorisation of phospholipase C produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-VR). EFSA-Q-2019-00442.
- 14. Application for authorisation of α -amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb45). EFSA-Q-2016-00143.
- 15. Application for authorisation of Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-KE). EFSA-Q-2012-00898.
- 16. Application for authorisation of α -amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb52). EFSA-Q-2016-00093.

- 17. Application for authorisation of pullulanase produced by a genetically modified strain of Bacillus licheniformis (strain NZYM LU). EFSA-Q-2021-00222.
- 18. Application for authorisation of maltogenic amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-SD). EFSA-Q-2021-00138.
- 19. Application for authorisation of maltogenic amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-FR). EFSA-Q-2021-00142.
- 20. Application for authorisation of maltogenic amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM CY). EFSA-Q-2021-00137.
- 21. Application for authorisation of Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BC). EFSA-Q-2013-00685.
- 22. Application for authorisation of β -Galactosidase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-BT). EFSA-Q-2015-00093.
- 23. Application for authorisation of Alpha-amylase produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AY). EFSA-Q-2021-00292.
- 24. Application for authorisation of phosphoinositide phospholipase C produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM DI). EFSA-Q-2021-00225.
- 25. Application for authorisation of Alpha-amylase produced by Bacillus licheniformis (strain T74). EFSA-Q-2022-00592.
- 26. Application for authorisation of Subtilisin produced by Bacillus licheniformis (strain NZYM-CX). EFSA-Q-2022-00545
- 27. Application for authorisation of Alpha-amylase from Bacillus licheniformis (strain NZYM-AC). EFSA-Q-2013-00586.

ABBREVIATIONS

WGS whole genome sequence
ANI Average nucleotide identity
dDDH digital DNA–DNA hybridisation

CONFLICT OF INTEREST

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REQUESTOR

European Commission

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EFSA-Q-2024-00013

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REFERENCES

Branquinho, R., Meirinhos-Soares, L., Carriço, J. A., Pintado, M., & Peixe, L. V. (2014). Phylogenetic and clonality analysis of *Bacillus pumilus* isolates uncovered a highly heterogeneous population of different closely related species and clones. *FEMS Microbiology Ecology*, *90*(3), 689–698. https://doi.org/10.1111/1574-6941.12426

Burgess, S. A., Lindsay, D., & Flint, S. H. (2010). Thermophilic bacilli and their importance in dairy processing. *International Journal of Food Microbiology*, 144(2), 215–225. https://doi.org/10.1016/j.ijfoodmicro.2010.09.027

Dunlap, C. A., Kwon, S.-W., Rooney, A. P., & Kim, S.-J. (2015). *Bacillus paralicheniformis* sp. nov., isolated from fermented soybean paste. *International Journal of Systematic and Evolutionary Microbiology*, 65, 3487–3492. https://doi.org/10.1099/ijsem.0.000441

EFSA (European Food Safety Authority). (2007). Introduction of a qualified presumption of safety (QPS) approach for assessment of selected microorganisms referred to EFSA - opinion of the scientific committee. EFSA Journal, 5(12), 587. https://doi.org/10.2903/j.efsa.2007.587

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Alvarez-Ordonez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernandez Escamez, P. S., ... Herman, L. (2022). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 15: Suitability of taxonomic units notified to EFSA until September 2021. EFSA Journal, 20(1), 7045. https://doi.org/10.2903/j.efsa. 2022.7045

- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), (2023). Update of the list of qualified presumption of safety (QPS) recommended microorganisms intentionally added to food or feed as notified to EFSA. EFSA Journal, 21(1), 7747. https://doi.org/10.2903/j.efsa.2023.7747
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano, V., Bolognesi, C., Castle, L., Cravedi, J.-P., Fowler, P., Franz, R., Grob, K., Gürtler, R., Husøy, T., Kärenlampi, S., Mennes, W., Milana, M. R., Penninks, A., Smith, A., Tavares Pocßas, M. F., Tlustos, C., Wölfle, D., Zorn, H., ... Engel, K.-H. (2017). Scientific opinion on the safety evaluation of the food enzyme β-amylase from genetically modified bacillus licheniformis strain NZYM-JA. *EFSA Journal*, *15*(8), 4896. https://doi.org/10.2903/j.efsa.2017.4896
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano, V., Bolognesi, C., Castle, L., Chipman, K., Cravedi, J.-P., Fowler, P., Franz, R., Grob, K., Gurtler, R., Husøy, T., Karenlampi, S., Mennes, W., Milana, M. R., Pfaff, K., Riviere, G., Srinivasan, J., Tavares Pocßas, M. F., Tlustos, C., ... Engel, K.-H. (2018a). Scientific opinion on the safety evaluation of the food enzyme alpha-amylase from a genetically modified bacillus licheniformis (strain NZYM-AN). EFSA Journal, 16(7), 5317. https://doi.org/10.2903/j.efsa.2018.5317
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano, V., Bolognesi, C., Castle, L., Chipman, K., Cravedi, J.-P., Fowler, P., Franz, R., Grob, K., Gurtler, R., Husøy, T., Karenlampi, S., Mennes, W., Milana, M. R., Pfaff, K., Riviere, G., Srinivasan, J., Tavares Pocas, M. F., Tlustos, C., ... Engel, K.-H. (2018b). Scientific opinion on the safety evaluation of the food enzyme a-amylase from a genetically modified bacillus licheniformis (strain NZYM-AV). EFSA Journal, 16(7), 5318. https://doi.org/10.2903/j.efsa.2018.5318
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Bruschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Riviere, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Herman, L., Karenlampi, S., ... Chesson, A. (2018c). Scientific opinion on the safety evaluation of the food enzyme acetolactate decarboxylase from a genetically modified bacillus licheniformis (strain NZYM-JB). EFSA Journal, 16(11), 5476. https://doi.org/10.2903/j.efsa.2018.5476
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids). (2019). Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal, 17(6), 5741. https://doi.org/10.2903/j.efsa.2019.5741
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Bruschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivère, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Aguilera, J., ... Chesson, A. (2019a). Scientific opinion on the safety evaluation of the food enzyme a-amylase from a genetically modified strain of bacillus licheniformis (DP-Dzb54). EFSA Journal, 17(1), 5549. https://doi.org/10.2903/j.efsa.2019.5549
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Bruschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Karenlampi, S., ... Chesson, A. (2019b). Scientific opinion on the safety evaluation of the food enzyme pullulanase from a genetically modified bacillus licheniformis (strain DP-Dzp39). EFSA Journal, 17(1), 5554. https://doi.org/10.2903/j.efsa.2019.5554
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Bruschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Engel, K.-H., Karenlampi, S., ... Chesson, A. (2019c). Scientific opinion on the safety evaluation of the food enzyme endo-1,4-b-xylanase from a genetically modified bacillus licheniformis (strain NZYM-CE). EFSA Journal, 17(4), 5685. https://doi.org/10.2903/j.efsa.2019.5685
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Bruschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., ... Chesson, A. (2019d). Scientific opinion on the safety evaluation of the food enzyme glucan 1,4-a-maltotetraohydrolase from bacillus licheniformis (strain DP-Dzr46). EFSA Journal, 17(5), 5684. https://doi.org/10.2903/j.efsa.2019.5684
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Bruschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Riviére, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Penninks, A., ... Chesson, A. (2019e). Scientific opinion on the safety evaluation of the food enzyme glucan 1,4-a-maltotetraohydrolase from bacillus licheniformis (strain DP-Dzf24). EFSA Journal, 17(6), 5739. https://doi.org/10.2903/j.efsa.2019.5739
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Bruschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Penninks, A., ... Chesson, A. (2019f). Scientific opinion on the safety evaluation of the food enzyme a-amylase from bacillus licheniformis (strain DP-Dzb44). EFSA Journal, 17(6), 5738. https://doi.org/10.2903/j.efsa.2019.5738
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Glandorf, B., Aguilera, J., ... Chesson, A. (2019g). Scientific opinion on the safety evaluation of the food enzyme a-amylase from a genetically modified strain of bacillus licheniformis (DP-Dzb25). EFSA Journal, 17(11), 5900. https://doi.org/10.2903/j.efsa.2019.5900
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Riviere, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., Andryszkiewicz, M., ... Chesson, A. (2020a). Scientific opinion on the safety evaluation of the food enzyme maltogenic amylase from the genetically modified bacillus licheniformis strain DP-Dzr50. *EFSA Journal*, *18*(1), 5972. https://doi.org/10.2903/j.efsa.2020.5972
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lambre, C., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., ... Chesson, A. (2020b). Scientific opinion on the safety evaluation of the food enzyme phospholipase C from the genetically modified bacillus licheniformis strain NZYM-VR. EFSA Journal, 18(7), 6184. https://doi.org/10.2903/j.efsa.2020.6184
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lambré, C., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., ... Maia Jand Chesson, A. (2020c). Scientific opinion on the safety evaluation of the food enzyme a-amylase from the genetically modified bacillus licheniformis strain DP-Dzb45. *EFSA Journal*, *18*(11), 6311. https://doi.org/10.2903/j.efsa.2020.6311
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano, V., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lambre, C., Lampi, E., Mengelers, M., Mortensen, A., Riviere, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., ... Chesson, A. (2021a). Scientific opinion on the safety evaluation of the food enzyme a-amylase from the genetically modified bacillus licheniformis strain NZYM-KE. EFSA Journal, 19(3), 6433. https://doi.org/10.2903/j.efsa.2021.6433
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambre, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Riviere, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Karenlampi, S., Penninks, A., ... Chesson, A. (2021b). Scientific opinion on the safety evaluation of the food enzyme a-amylase from the genetically modified bacillus licheniformis strain DP-Dzb52. EFSA Journal, 19(4), 6564. https://doi.org/10.2903/j.efsa.2021.6564
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambre, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Riviere, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., ... Chesson, A. (2021c). Scientific Guidance for the submission of dossiers on Food Enzymes. *EFSA Journal*, *19*(10), 6851. https://doi.org/10.2903/j.efsa.2021.6851

- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., GrobK, L. E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Roos, Y., Liu, Y., ... Chesson, A. (2022a). Scientific opinion on the safety evaluation of the food enzyme pullulanase from the genetically modified bacillus licheniformis strain NZYM-LU. *EFSA Journal*, 20(6), 7359. https://doi.org/10.2903/j.efsa.2022.7359
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., GrobK, L. E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Andryszkiewicz, M., Arcella, D., ... Chesson, A. (2022b). Scientific opinion on the safety evaluation of the food enzyme glucan 1,4-a-maltohydrolase from the genetically modified bacillus licheniformis strain NZYM-SD. EFSA Journal, 20(6), 7368. https://doi.org/10.2903/j.efsa.2022.7368
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Manuel Barat Baviera, J., Bolognesi, C., Sandro Cocconcelli, P., Crebelli, R., Michael Gott, D., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., VanLoveren, H., Vernis, L., Zorn, H., Herman, L., Andryszkiewicz, M., ... Chesson, A. (2022c). Scientific opinion on the safety evaluation of the food enzyme glucan 1,4-a-maltohydrolase from the genetically modified bacillus licheniformis strain NZYM-FR. *EFSA Journal*, 20(6), 7367. https://doi.org/10.2903/j.efsa. 2022.7367
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., GrobK, L. E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Andryszkiewicz, M., Arcella, D., ... Chesson, A. (2022d). Scientific opinion on the safety evaluation of the food enzyme glucan 1,4-a-maltohydrolase from the genetically modified bacillus licheniformis strain NZYM-CY. *EFSA Journal*, 20(6), 7366. https://doi.org/10.2903/j.efsa.2022.7366
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grobk, L. E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., Aguilera, J., ... Chesson, A. (2022e). Scientific opinion on the safety evaluation of the food enzyme a-amylase from the genetically modified bacillus licheniformis strain NZYM-BC. EFSA Journal, 20(7), 7370. https://doi.org/10.2903/j.efsa.2022.7370
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Andryszkiewicz, M., Arcella, D., ... Chesson, A. (2022f). Scientific opinion on the safety evaluation of the food enzyme b-galactosidasefrom the genetically modified bacillus licheniformis strain NZYM-BT. *EFSA Journal*, *20*(7), 7358. https://doi.org/10.2903/j.efsa.2022.7358
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grobk, L. E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Aguilera, J., Liu, Y., & Chesson, A. (2022g). Scientific opinion on the safety evaluation of the food enzyme a-amylase from the genetically modified bacillus licheniformis strain NZYM-AY. EFSA Journal, 20(8), 7467. https://doi.org/10.2903/j.efsa.2022.7467
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., GrobK, L. E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Gomes, A., Liu, Y., & Chesson, A. (2022h). Scientific opinion on the safety evaluation of the food enzyme phosphoinositide phospholipase C from the genetically modified bacillus licheniformis strain NZYM-DI. EFSA Journal, 20(8), 7470. https://doi.org/10.2903/j.efsa.2022.7470
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Aguilera, J., ... Chesson, A. (2023a). Safety evaluation of the food enzyme α -amylase from the non-genetically modified Bacillus licheniformis strain T74. EFSA Journal, 21(8), e8160. https://doi.org/10.2903/j.efsa.2023.8160
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Aguilera, J., ... Chesson, A. (2023b). Safety evaluation of the food enzyme subtilisin from the non-genetically modified Bacillus licheniformis strain NZYM-CX. EFSA Journal, 21(11), e8406. https://doi.org/10.2903/j.efsa.2023.8406
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Roos, Y., ... Chesson, A. (2023c). Safety evaluation of the food enzyme α-amylase from the genetically modified Bacillus licheniformis strain NZYM-AC. *EFSA Journal*, *21*(11), e8393. https://doi.org/10.2903/j.efsa.2023.8393
- Olajide, A. M., Chen, S., & LaPointe, G. (2021). Markers to rapidly distinguish bacillus paralicheniformis from the very close relative, bacillus licheniformis. Frontiers in Microbiology, 11, 596828. https://doi.org/10.3389/fmicb.2020.596828
- Xu, F., Zeng, X., Hinenoya, A., & Lin, J. (2018). MCR-1 confers cross-resistance to bacitracin, a widely used in-feed antibiotic. *mSphere*, 3, e00411–e00418. https://doi.org/10.1128/mSphere.00411-18

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