Adopted: 12 March 2024

DOI: 10.2903/j.efsa.2024.8725

SCIENTIFIC OPINION

Assessment of the feed additive consisting of *Levilactobacillus brevis* DSM 21982 for all animal species for the renewal of its authorisation (Marigot Ltd T/A Celtic Sea Minerals)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) | **Vasileios Bampidis** | **Giovanna Azimonti** | **Maria de Lourdes Bastos** | **Henrik Christensen** | **Mojca Durjava** | **Birgit Dusemund** | **Maryline Kouba** | **Marta López-Alonso** | **Secundino López Puente** | **Francesca Marcon** | **Baltasar Mayo** | **Alena Pechová** | **Mariana Petkova** | **Fernando Ramos** | **Roberto Edoardo Villa** | **Ruud Woutersen** | **Montserrat Anguita** | **Rosella Brozzi** | **Yolanda García-Cazorla** | **Matteo Lorenzo Innocenti** | **Elisa Pettenati** | **Joana Revez** | **Jordi Tarrés-Call** | **Nicole Bozzi Cionci**

Correspondence: feedap@efsa.europa.eu

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of the application for renewal of *Levilactobacillus brevis* DSM 21982 as a technological feed additive, silage additive, for all animal species. The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation. The Panel concluded that the additive remains safe for all animal species, consumers and the environment. Regarding user safety, the additive should be considered as a skin and respiratory sensitiser. No conclusions can be drawn on the eye irritancy potential of the additive. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

KEYWORDS

Levilactobacillus brevis DSM 21982, QPS, renewal, safety, silage additives, technological additives

CONTENTS

Abstract 1
1. Introduction 3
   1.1. Background and Terms of Reference 3
   1.2. Additional information 3
2. Data and methodologies 3
   2.1. Data 3
   2.2. Methodologies 4
3. Assessment 4
   3.1. Characterisation 4
      3.1.1. Characterisation of the additive 4
      3.1.2. Characterisation of the active agent 4
      3.1.3. Conditions of use 5
   3.2. Safety 5
      3.2.1. Conclusions on safety 6
   3.3. Efficacy 6
4. Conclusions 6
Abbreviations 6
Acknowledgements 6
Conflict of interest 6
Requestor 6
Question numbers 6
Copyright for non-EFSA content 6
Panel members 6
Legal notice 6
References 7

1 | INTRODUCTION

1.1 | Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest 1 year before the expiry date of the authorisation.

The European Commission received a request from Marigot Ltd T/A Celtic Sea Minerals[2] for the renewal of the authorisation of the additive consisting of *Levilactobacillus brevis* DSM 21982,[3] when used as a feed additive for all animal species (category: technological additives; functional group: silage additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 14(1) (renewal of the authorisation). The dossier was received on 20 September 2022 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00581. The particulars and documents in support of the application were considered valid by EFSA as of 12 July 2023.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of *Levilactobacillus brevis* DSM 21982, when used under the proposed conditions of use (see Section 3.1.3).

1.2 | Additional information

The additive *Levilactobacillus brevis* (previously *Lactobacillus brevis*) DSM 21982 is currently authorised for use in feed for all animal species (1k20715).[4]

EFSA issued one opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2012).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[5] in support of the authorisation request for the use of *Levilactobacillus brevis* DSM 21982 as a feed additive.

In accordance with Article 38 of the Regulation (EC) No 178/2002[6] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[7] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 24 January to 14 February 2024 for which no comments were received.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 12 July 2023 to 12 October 2023 for which the received comments were considered for the assessment.

The European Union Reference Laboratory considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of *Levilactobacillus brevis* DSM 21982 in animal feed are valid and applicable for the current application.[8]

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2]Marigot Ltd t/a Celtic Sea Minerals, Strand Farm, Curragbinny, Carrigaline, Co. Cork, Ireland.

[3]Previously referred as *Lactobacillus brevis* DSMZ 21982.

[4]Commission Implementing Regulation (EU) No 838/2012 of 18 September 2012 concerning the authorisation of *Lactobacillus brevis* (DSMZ 21982) as a feed additive for all animal species. OJ L 252, 19.09.2012, p. 9.

[5]Dossier reference: FEED-2021-1891.

[6]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p.1–48.

[7]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

[8]Evaluation report received on 02 September 2011 is available on the EU Science Hub (https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en).

2.2 | Methodologies

The approach followed by the EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) to assess the safety and the efficacy of *Levilactobacillus brevis* DSM 21982 is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3 | ASSESSMENT

The additive *Levilactobacillus brevis* DSM 21982 is currently authorised for all animal species as a technological additive (functional group: silage additives). This assessment regards the renewal of the authorisation.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive currently authorised is a preparation containing *Levilactobacillus brevis* DSM 21982 at a minimum concentration of 8×10^{10} colony forming units (CFUs)/g.

The applicant declared that the manufacturing process and the composition have not been changed since the previous authorisation and that no antimicrobials are used during the manufacturing process.[10] The active agent is grown by fermentation and concentrated by centrifugation. Cryoprotectants ([redacted]) are added, and the cell concentrate is freeze dried.[11] The freeze-dried cells concentrate may be standardised with anhydrous dextrose or maltodextrin.[12]

Analytical data to confirm the specifications were provided for five batches of the additive showing an average value of *L. brevis* counts of 6.6×10^{11} CFU/g (range: 3.5×10^{11}–1.5×10^{12} CFU/g).[13]

Five batches of the additive were analysed to evaluate the microbiological quality.[14] The following values were obtained: 115 CFU/g for coliforms, 20 CFU/g for yeasts and 55 CFU/g for *Enterobacteriaceae* (for three batches, the values were < 10 CFU/g). The amounts of *Escherichia coli* and filamentous fungi were < 10 and < 20 CFU/g, respectively. *Salmonella* spp. was not detected in 25 g of sample.

Three batches of the additive were analysed for the presence of arsenic, lead, cadmium and mercury. The following values were obtained: 0.26–0.32 mg/kg for arsenic, 0.015–0.11 mg/kg for lead, 0.11–0.15 mg/kg for cadmium and 0.002–0.008 mg/kg for mercury.[15]

The analysis of mycotoxins, including total aflatoxins, fumonisins (B1, B2, B3), ochratoxin A, deoxynivalenol, zearalenone, and T-2 and HT-2 toxins showed values below the limit of detection (LOD) or of quantification (LOQ) of the analytical methods.[16]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

No new data were provided regarding the physico-chemical properties or stability of the additive under assessment. Since no changes were introduced in the manufacturing process, the data described in the previous opinion (EFSA FEEDAP Panel, 2012) are still valid.

3.1.2 | Characterisation of the active agent

The active agent was isolated from grass, and it is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) under the accession number DSM 21982.[17] It has not been genetically modified.

The taxonomical identification of the active agent was confirmed by Average Nucleotide Identity determination using the whole genome sequence (WGS) data. The value obtained [redacted] was 97.68% with the type strain *Levilactobacillus brevis* DSM 20054^T.[18] This was further confirmed by a phylogenomic analysis carried out with a gene set of 71 core genes

[9]Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[10]SECTION II_Identity-21982, manufacturing process and manufacturing dec 21982.

[11]Cryoprotectants.

[12]SECTION II_Identity-21,982 and manufacturing process.

[13]Five batches 2023 confidential.

[14]Five batches 2023 confidential.

[15]Heavy metals and mycotoxin determination of three batches of *Levilactobacillus brevis* DSM 21982 confidential.

[16]LOD: 3.5 µg/kg T2/HT2- Toxins; 2.8 µg/kg ochratoxin A; 25 µg/kg fumonisins (B1, B2, B3); 134 µg/kg deoxynivalenol. LOQ: 5.8 µg/kg total aflatoxins; 50 µg/kg zearalenone.

[17]Strain Deposit_DSM 16680_16627_21982.

[18]T1675R1840_2023.

from selected *Levilactobacillus* spp. genomes. The strain DSM 21982 clustered with the *L. brevis* strains, including the type strain *Levilactobacillus brevis* DSM 20054^T. From the analysis of the genome sequence, a plasmid was predicted.

The susceptibility of *L. brevis* DSM 21982 to antimicrobials was tested using a broth microdilution method and including the set of antimicrobials recommended by EFSA (EFSA FEEDAP Panel, 2018).[19] All minimum inhibitory concentration values were equal or below the corresponding cut-off values, except for ampicillin (4 vs. 2 mg/L), tetracycline (16 vs. 8 mg/L) and chloramphenicol (one of the three replicates: 8 vs. 4 mg/L), which were one dilution above the cut-off values. Exceeding the cut-off value by one dilution is considered to be within the normal range of variation and, thus, not a matter of concern. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The WGS data of the strain DSM 21982, including the plasmid, were interrogated for the presence of antimicrobial resistance genes by a search against the [redacted] databases.[20] No hits were identified exceeding the thresholds recommended by EFSA (EFSA, 2021).

3.1.3 | Conditions of use

The additive is currently authorised for use as a silage additive for all animal species. Under those provisions of the authorisation,[21] it is specified that:

- In the directions for use of the additive and premixture, indicate the storage temperature and storage life.
- Minimum dose of the additive when used not in combination with other microorganisms as silage additive: 1×10^8 CFU/kg of fresh material.
- For safety, it is recommended to use breathing protection and gloves during handling.

The applicant has requested to maintain the same conditions of use.[22]

3.2 | Safety

In its previous opinion, the Panel concluded that following the Qualified Presumption of Safety (QPS) approach to safety assessment, *L. brevis* DSM 21982 is safe for target species, consumers and the environment (EFSA FEEDAP Panel, 2012). Regarding user safety, the Panel concluded that: 'Dermal, eye irritation and skin sensitisation were not tested. Although users at the farm level are exposed to the additive only for a short period of time when preparing the aqueous suspension, given the lack of specific information and its proteinaceous nature, the active agent should be considered to have the potential to be a skin and respiratory sensitizer'.

The applicant declared that no incidents or safety issues have been documented or reported for target animals, consumers, users and environment since the approval of the additive.[23]

In the context of this application, in line with the requirements of the QPS approach for safety assessment (EFSA BIOHAZ Panel, 2023), the identity of the strain as *L. brevis* was confirmed, and evidence that the strain does not show acquired antimicrobial determinants for antibiotics of human and veterinary importance was provided. Consequently, the conclusions already reached are still valid, and the Panel considers that *L. brevis* DSM 21982 remains safe for the target species, consumers and the environment.

An extensive literature search was performed by the applicant to support the safety of the *L. brevis* species, including the *L. brevis* DSM 21982 strain. The search covered the period 2012–2021 and the terms used included the active agent at strain and species levels and commercial names containing the additive. Nine databases were searched. One publication was selected but it was not further considered since it did not regard any safety assessment related to the active agent under assessment.[24]

No specific data have been submitted on the effects of the additive on user safety. Considering the nature of the additive, the FEEDAP Panel concludes that the additive should be considered as a skin and respiratory sensitiser, and any exposure through skin and respiratory tract is considered a risk. In the absence of data, no conclusion can be reached on the eye irritation potential of the additive.

[19] T1675R1832_2023.

[20] 1675R1840_2023, T1675_diamond_all_with_subtype_Annex1A and T1675_blastn_Annex1B.

[21] Commission Implementing Regulation (EU) No 838/2012 of 18 September 2012 concerning the authorisation of *Lactobacillus brevis* (DSMZ 21982) as a feed additive for all animal species. OJ L 252, 19.09.2012, p. 9.

[22] DescriptAdd-updated.

[23] SECTION_III_Safety of use (workers).

[24] Annex_III_2_Extensive Literature Review for Lactobacillus Brevis DSM 21982 final.

3.2.1 | Conclusions on safety

The FEEDAP Panel concludes that *L. brevis* DSM 21982 remains safe for the target species, consumers and the environment. Considering the nature of the additive, the FEEDAP Panel concludes that the additive should be considered as a potential skin and respiratory sensitiser. The Panel cannot conclude on the eye irritation potential of the additive.

3.3 | Efficacy

The present application for the renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

4 | CONCLUSIONS

The applicant has provided evidence that the additive currently on the market complies with the existing terms of authorisation.

The Panel concludes that *Levilactobacillus brevis* DSM 21982 remains safe for all animal species, consumers and the environment. Regarding user safety, the additive should be considered as a skin and respiratory sensitiser, and any exposure through skin and respiratory tract is considered a risk. No conclusions can be drawn on the eye irritancy potential of the additive.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

ABBREVIATIONS

AMR	antimicrobial resistance
CFU	colony forming unit
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
WGS	whole genome sequence

ACKNOWLEDGEMENTS

The Panel wishes to thank Jaume Galobart and Fabiola Pizzo for the support provided to this scientific output.

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBERS

EFSA-Q-2022-00581

PANEL MEMBERS

Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Durjava, Birgit Dusemund, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Roberto Edoardo Villa and Ruud Woutersen.

REFERENCES

EFSA (European Food Safety Authority). (2021). EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA Journal*, *19*(7), 6506. https://doi.org/10.2903/j.efsa.2021.6506

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Álvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernández Escámez, P. S., Prieto Maradona, M., … Herman L, C. S. (2023). Scientific opinion on the 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 FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Aquilina, G., Bories, G., Chesson, A., Cocconcelli, P. S., de Knecht, J., Dierick, N. A., Gralak, M. A., Gropp, J., Halle, I., Hogstrand, C., Kroker, R., Leng, L., Puente, S. L., Haldorsen, A.-K. L., Mantovani, A., Martelli, G., Mézes, M., Renshaw, D., … Westendorf, J. (2012). Scientific opinion on the safety and efficacy of *Lactobacillus brevis* (DSMZ 21982) as a silage additive for all species. *EFSA Journal EFSA Journal*, *10*(3), 2617. https://doi.org/10.2903/j.efsa.2012.2617

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Galobart, J. (2018). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Fašmon Durjava, M., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Anguita, M., … Innocenti, M. L. (2021). Guidance on the renewal of the authorisation of feed additives. *EFSA Journal*, *19*(1), 6340. https://doi.org/10.2903/j.efsa.2021.6340

How to cite this article: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Durjava, M., Dusemund, B., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Anguita, M., Brozzi, R., … Bozzi Cionci, N. (2024). Assessment of the feed additive consisting of *Levilactobacillus brevis* DSM 21982 for all animal species for the renewal of its authorisation (Marigot Ltd T/A Celtic Sea Minerals). *EFSA Journal*, *22*(4), e8725. https://doi.org/10.2903/j.efsa.2024.8725