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SCIENTIFIC OPINION

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Assessment of the feed additive consisting of *Lentilactobacillus buchneri* ATCC PTA-2494 for all animal species for the renewal of its authorisation (Pioneer Hi-Bred International, Inc.)

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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 *Lentilactobacillus buchneri* ATCC PTA-2494 as a technological additive (functional group: silage additives) for all animal species. The applicant has provided evidence that the additive currently on the market complies with the existing terms of the authorisation. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive remains safe for all animal species, consumers and the environment. Regarding user safety, the Panel considers that any exposure through skin and respiratory tract is considered a risk. The Panel cannot conclude on the eye irritation potential of the additive due to the lack of data. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

KEYWORDS

Lentilactobacillus buchneri ATCC PTA-2494, QPS, renewal, safety, silage additives, technological additives

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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 one year before the expiry date of the authorisation.

The European Commission received a request from Pioneer Hi-Bred International, Inc.[2] for the renewal of the authorisation of the additive consisting of *Lentilactobacillus buchneri* ATCC PTA-2494,[3] when used as a feed additive for all animal species (category: technological additive; 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 7 November 2022 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00778. The particulars and documents in support of the application were considered valid by EFSA as of 2 May 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 *Lentilactobacillus buchneri* ATCC PTA-2494, when used under the proposed conditions of use (see Section 3.1.3).

1.2 | Additional information

The additive *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) ATCC PTA-2494 is currently authorised for use in feed for all animal species (1k20741).[4]

EFSA issued one opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2013).

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 *Lentilactobacillus buchneri* ATCC PTA-2494 as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 2 May 2023 to 2 August 2023; the comments received were considered for the assessment.

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 9 August to 30 August 2023 for which no comments were received.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active agent 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]Pioneer Hi-Bred International., Inc., 7100 NW 62nd Avenue, Johnston, IA 50131—United States of America, represented in the EU by Corteva AgriScience Belgium bv, Rye Montoyer 25, 1000, Brussels, Belgium.

[3]Previously referred as *Lactobacillus buchneri* LN 4637/ATCC PTA-2494.

[4]Commission implementing the regulation (EU) No 1113/2013 of 7 November 2013 concerning the authorisation of preparations of *Lactobacillus plantarum* NCIMB 40027, *Lactobacillus buchneri* DSM 22501, *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323, *Lactobacillus buchneri* LN 40177/ATCC PTA-6138 and *Lactobacillus buchneri* LN 4637/ATCC PTA-2494 as feed additives for all animal species. OJ L 298, 8.11.2013, p.29.

[5]Dossier reference: FEED-2022-8790.

[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 available on the EU Science Hub https://joint-research-centre.ec.europa.eu/publications/20-fad-dossiers_en.

2.2 | Methodologies

The approach followed by the FEEDAP to assess the safety of *Lentilactobacillus buchneri* ATCC PTA-2494 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 *Lentilactobacillus buchneri* ATCC PTA-2494 is currently authorised as a technological additive (functional group: silage additives) for use in fresh material for all animal species. 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 of *L. buchneri* ATCC PTA-2494 with a minimum concentration of 1×10^{10} colony forming unit (CFU)/g.

The applicant declared that the manufacturing process and the composition of the additive have not been modified since the previous authorisation.[10] The additive is a powder consisting of the freeze-dried cells concentrate and cryoprotectants ([redacted]). The freeze-dried cells concentrate may be standardised with carriers (maltodextrin or calcium carbonate) and anti-caking agents (e.g. sodium aluminosilicate).[11]

The analysis of six batches of the additive showed compliance with the specifications of the active agent (mean 8×10^{11} CFU/g and range $7.0–9.2 \times 10^{11}$ CFU/g).[12]

One batch of the additive with maltodextrin used as a carrier was analysed for microbial contamination.[13] No additional data on microbial contamination were made available for the additive under assessment as such, but for five batches of premixtures containing the additive and other active agents.[14] The levels of coliforms, yeasts and filamentous fungi were all < 10 CFU/g. *Salmonella* spp. was not detected in 25 g samples.[15] No information was made available for *Enterobacteriaceae*.

No data were submitted regarding the impurities of the additive.[16] The microbial contamination in the batches analysed does not raise safety concerns. However, the FEEDAP Panel notes that the data on microbial contamination regarding the premixtures may not represent the additive under assessment. Additionally, in the absence of sufficient evidence, the presence of impurities in the additive under assessment cannot be excluded.

Since no changes were introduced in the manufacturing process or in the composition of the additive since its first authorisation, the data on the physico-chemical properties and stability of the additive described in the previous opinion (EFSA FEEDAP Panel, 2013) are still considered valid. Some new data regarding the physico-chemical properties, dusting potential, particle size distribution and bulk density of a premixture containing the additive in combination with other active agents were provided.[17] However, these data are not considered relevant for the current assessment.

3.1.2 | Characterisation of the active agent

The strain was originally isolated from high-moisture maize. It is deposited at the American Type Culture Collection (ATCC) under the accession number ATCC PTA-2494.[18] It has not been genetically modified.

[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]2.3_Manufacturing_LN4637_updated_marked and 20240301_LN4637_Identification of the additive_A3.

[11]20240301_LN4637_Identification of the additive_A3.

[12]Annex II.2 Viability Counts for L. buchneri LN4637 marked and Annex II.2b Viability Counts for L. buchneri LN4637_marked.

[13]20240301_LN4637_Identification of the additive_A3.

[14]20240301_LN4637_Identification of the additive_A3.

[15]Annex II.3 CoA impurities marked_LN.

[16]2.1–2 Characterisation_LN4637 CC2.

[17]Annex II.11 Stability for L. buchneri Strains LN40177 and LN4637 in Water marked.

[18]Annex II.5 LN 4637 ATCC deposit certificate.

The taxonomic identification of the strain was confirmed by a bioinformatic analysis using the whole genome sequencing (WGS) data. The taxonomic assignment was based on average nucleotide identity (ANI) with an OrthoANI value of 97.4% when compared to the type strain *Lentilactobacillus buchneri* ATCC 4005^{T}.[19]

The susceptibility of *L. buchneri* ATCC PTA-2494 to antimicrobials was tested using a broth microdilution method and including the battery of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018). All the minimum inhibitory concentration values were equal to or fell below the corresponding cut-off values, except for chloramphenicol, which was one dilution above the cut-off value (8 vs. 4 mg/L). 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.[20]

The WGS data of the strain were interrogated for the presence of antimicrobial resistance (AMR) genes against the ARG-ANNOT,[21] ResFinder and NCBI Bacterial Antimicrobial Resistance Reference Gene databases.[22] 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 silage additive for all animal species. Under other provisions of the authorisation,[23] it is specified that:

- In the directions for use of the additive and premixture, indicate the storage temperature and storage life.
- Minimum content of the additive when used without combination with other microorganisms as silage additives: 1×10^8 CFU/kg fresh material.
- The additive shall be used in easy to ensile material.
- For safety: it is recommended to use breathing protection and gloves during handling.

The applicant did not request any change in the current conditions of the authorisation.[24]

3.2 | Safety

In its previous opinion the FEEDAP Panel concluded that, following the Qualified Presumption of Safety (QPS) approach, *L. buchneri* ATCC PTA-2494 is considered safe for the target species, consumer and the environment (EFSA FEDAP Panel, 2013). Regarding user safety, the Panel concluded that the additive may cause irritation to skin/eyes and should be considered as a skin and respiratory sensitiser.

The applicant states that no adverse effects, including accidents, for target animals, consumers, users and the environment have been reported since the first authorisation of the product.[25]

In the context of the current application, in line with the requirements of the QPS approach for safety assessment (EFSA BIOHAZ Panel, 2023), the identity of the strain as *L. buchneri* was confirmed, and evidence was provided that the strain does not show AMR for antibiotics of human and veterinary importance. Consequently, the conclusions previously reached are still valid, and the Panel concludes that *L. buchneri* ATCC PTA-2494 remains safe for the target species, consumers and the environment.

A literature search was performed to support the safety of *L. buchneri* ATCC PTA-2494.[26] The search covered the period 2013–2022 and involved a total of four databases: Web of Science, Core Collection, CAB Abstracts and MEDLINE. The literature search retrieved 56 hits. Of these, 25 publications were considered potentially relevant. None of the publications described safety concerns related to the use of *L. buchneri* ATCC PTA-2494.

No specific data have been submitted on the effects of the additive on user safety. The applicant submitted eye and skin irritation studies (according to the OECD guidelines 438 and 404, respectively), which tested a premixture containing the additive in combination with other active agents, maltodextrin and sodium aluminosilicate.[27] The results showed no evidence of irritation, but the product tested does not allow to conclude on the additive.

[19] Annex_II.6_WGS_LN_marked.

[20] Annex II.7 LN4637_MIC statement marked.

[21] This database is not maintained. The Panel only considered the results obtained from the databases that are maintained.

[22] Annex_II.6_WGS_LN_marked, 20240301_LN4637_Identification_of_the_additive_A3 and Annex II.6_raw data_AMR.

[23] Commission implementing the regulation (EU) No 1113/2013 of 7 November 2013 concerning the authorisation of preparations of *Lactobacillus plantarum* NCIMB 40027, *Lactobacillus buchneri* DSM 22501, *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323, *Lactobacillus buchneri* LN 40177/ATCC PTA-6138 and *Lactobacillus buchneri* LN 4637/ATCC PTA-2494 as feed additives for all animal species. OJ L 298, 8.11.2013, p. 29.

[24] 2.5_Conditions_of_use_LN4637 and LN_PubSum_LN_ATCC PTA-2494_CC2.

[25] Section_III_LN4637 and 20240301_Letter adverse effect_LN4637.

[26] Annex_III.1_LN_lit_search_2022_marked.

[27] Annex III.2a_Eye, Annex III.2b_Skin and 20240301_LN4637_Identification of the additive_A3.

Owing to the nature of the additive, the FEEDAP Panel concludes that the additive should be considered as a potential 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.

3.2.1 | Conclusions on safety

The FEEDAP Panel concludes that the active agent *L. buchneri* ATCC PTA-2494 remains safe for the target species, consumers and the environment. Considering the nature of the additive, the FEEDAP Panel concludes that *L. buchneri* ATCC PTA-2494 should be treated as a potential skin and respiratory sensitiser and any exposure through skin and respiratory tract is considered a risk. The Panel cannot conclude on the eye irritation potential of the additive.

3.3 | Efficacy

The present application for 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 conditions of authorisation. However, no additional data on microbial contamination and impurities on the additive under assessment, necessary to fully characterise the additive, were provided.

The Panel concludes that the active agent *Lentilactobacillus buchneri* ATCC PTA-2494 remains safe for the target 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 due to the lack of data.

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

ABBREVIATIONS

ANI	average nucleotide identity
AMR	antimicrobial resistance
CFU	colony forming unit
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
OECD	Organisation for Economic Co-operation and Development
WGS	whole genome sequencing

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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

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