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Assessment of the feed additive consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30084 for all animal species for the renewal of its authorisation (Chr. Hansen A/S)

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on assessment of the application for renewal of authorisation *Lactiplantibacillus plantarum* (previously *Lactobacillus*) NCIMB 30084 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. There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concludes that the additive remains safe for all animal species, consumers and the environment under the authorised conditions of use. Regarding user safety, the additive should be considered as a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation, and skin and 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: technological additives, silage, *Lactiplantibacillus plantarum* NCIMB 30084, safety, QPS, renewal

Requestor: European Commission

Question number: EFSA-Q-2022-00322

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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 1 year before the expiry date of the authorisation.

The European Commission received a request from Chr. Hansen A/S[2] for the renewal of the authorisation of the additive consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30084, 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 19 May 2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00322. The particulars and documents in support of the application were considered valid by EFSA as of 12 January 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 *L. plantarum* NCIMB 30084, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The additive is a preparation containing *Lactiplantibacillus plantarum* NCIMB 30084. EFSA issued an opinion on the safety and efficacy of this product when used in forages for all animal species (EFSA FEEDAP Panel, 2013).

The additive is currently authorised for use in feed for all animal species (1 k20737).[3]

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[4] in support of the authorisation request for the use of *L. plantarum* NCIMB 30084 as a feed additive. The dossier was received on 19/5/2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00322.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] 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,[6] 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,[6] EFSA carried out a public consultation on the non-confidential version of the technical dossier from 09 June to 30 June 2023 for which no comments were received.

[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] Chr. Hansen A/S, 10–12 Boege Allé, DK-2970 Hoersholm, Denmark.
[3] Commission Implementing Regulation (EU) No 308/2013 of 3 April 2013 in OJ L 94, 4.4.2013, p. 1.
[4] Dossier reference: FEED-2022-3272
[5] 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.
[6] Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

In addition, the confidential version of the technical dossier was subject to a target consultation of the interested Member States from 12 January 2023 to 12 April 2023, for which the received comments were considered for the assessment.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' (elicitation) knowledge, to deliver the present output.

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.[7]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *L. plantarum* NCIMB 30084 is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3. Assessment

The product consisting of viable cells of *L*. (previously *Lactobacillus*) *plantarum* NCIMB 30084 is currently authorised as a technological additive (functional group: silage additive) for use in silage material for all animal species. This assessment regards the renewal of the authorisation of *L. plantarum* NCIMB 30084 as a silage additive.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive is currently authorised with a minimum content of the active agent (*L. plantarum* NCIMB 30084) of 5×10^{10} colony forming units (CFU)/g of additive. 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.[9] The active agent is grown by fermentation and concentrated by centrifugation and filtration. Cryoprotectants (17–42%) are added and the cell concentrate is freeze-dried. The final product is a powder containing the freeze-dried cell concentrate (which include a maximum of 6% of fermentation medium, a maximum of 3% of water and 17–42% of cryoprotectants), synthetic amorphous silica as anticaking agent (8%)[10] and maltodextrin as carrier (50–75%), to reach the minimum concentration specified for *L. plantarum* NCIMB 30084.[11]

Analytical data to confirm the specification was provided for nine samples of the additive, showing an average value of 4.69×10^{11} CFU/g additive, (range 2.3×10^{11}–6.7×10^{11} CFU/g additive[12]).[13] However, the Panel notes that these samples were obtained from only four independent fermentation batches.[14]

Five samples from three recent and independent batches of the additive were tested for aflatoxin B1, mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) concentrations. Results showed the following mean values: 0.006 mg Hg/kg (0.003–0.014 mg/kg), < 0.01 mg Pb/kg, 0.014 mg Cd/kg (0.01–0.015 mg/kg) and 0.031 mg As/kg (0.014–0.047 mg/kg). Aflatoxin B1 in all batches was below the limit of quantification (LOQ < 0.46 μg aflatoxin B1/kg).[15]

[7] Evaluation available on the EU Science Hub https://joint-research-centre.ec.europa.eu/system/files/2013-02/FinRep-FAD-uorg3.pdf

[8] 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.

[9] Statement_NCIMB30084_2023.

[10] Currently under re-evaluation by the FEEDAP Panel.

[11] Sect_II_Identity_L.plantarum_NCIMB30084_ID+Charact.

[12] Samples from common batches were considered as unique batches and their averages were used to calculate the final average of the nine samples.

[13] Annex_II_1.3a_New_CoAs_L.plan_NCIMB30084.

[14] Five samples from one fermentation batch, two samples from another fermentation batch, and one sample from two independent batches (one each).

[15] Annex_II_1.4.2_Undes_subs_NCIMB30084.

Specifications are set for coliforms (< 1,000 CFU/g), *Escherichia coli* (< 10 CFU/g), *Salmonella* spp. (no detection in 25 g of frozen product or 5 g of freeze-dried bulk) and yeasts and filamentous fungi (< 1,000 CFU/g). Nine samples from four independent fermentation batches of the additive showed compliance with the respective specifications. The Panel notes that the specification for *Salmonella* spp. detection has been modified and that data from analyses of 5 g instead of 25 g were provided for four out of nine samples (four independent). In addition, the applicant provided data on Enterobacteriaceae for three samples from two independent batches, with results below the LOQ (< 10 CFU/g).[16]

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

No new data have been provided regarding the physico-chemical properties or stability of the additive. Since no changes were introduced in the additive manufacturing process, the data described in the previous opinion still apply (EFSA FEEDAP Panel, 2013).

3.1.2. Characterisation of the active agent

L. plantarum NCIMB 30084 was isolated from silage, and it is deposited in the National Collections of Industrial, Food and Marine Bacteria (NCIMB) under the accession number NCIMB 30084.[17] It has not been genetically modified.

The taxonomical identification was confirmed by average nucleotide identity (ANI) based on the whole genome sequence (WGS). The results of this analysis showed an ANI of 99.9% with the type strain *L. plantarum* DSM 20174^T.[18]

The antimicrobial susceptibility profile of *L. plantarum* was tested using a broth microdilution method and including the set of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018). All the minimum inhibitory concentration (MIC) values were equal to or fell below the corresponding EFSA cut-off values, except for kanamycin, which was one dilution higher. Exceeding the cut-off by one dilution is considered within the normal range of variation and therefore, the strain is considered to be susceptible to all the relevant antibiotics.[19]

The WGS data of the strain were interrogated for the presence of antimicrobial resistance (AMR) genes against the ResFinder database and the NCBI Bacterial Antimicrobial Resistance Reference Gene Database. The search in ResFinder was performed at nucleotide level using BLASTn, while the second database was queried at amino acid level using BLASTX. Hits with [redacted] identity and [redacted] of coverage were further screened.[20] No hits of concern were identified.

3.1.3. Conditions of use

The additive is currently authorised for use in silage material for all animal species. Under other provisions of the authorisation, it is specified that:

- In the directions for the use of the additive, indicate the storage temperature and storage life.
- Minimum dose of the additive when it is not used in combination with other microorganisms as silage additive: 1×10^8 CFU/kg of fresh material.
- The additive shall be used in easy and moderately difficult to ensile 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.

3.2. Safety

The applicant states that no adverse effects or incidents/accidents have been reported from the use of the feed additive since the first authorisation of the product.[9]

3.2.1. Safety for the target species, consumers and environment

In its previous opinion, the FEEDAP Panel concluded that following the qualified presumption of safety (QPS) approach to safety assessment, the use of this strain in the production of silage was

[16] Annex_II_1.3a_New_CoAs_L.plan_NCIMB30084.
[17] Annex_II_2.1.2a_Deposit_statement_NCIMB30084.
[18] Annex_II_2.1.2b_ID_Certificate_NCIMB30084.
[19] Annex_II_2.2.2c_MIC statement_NCIMB 30084.
[20] Annex_II_2.2.2b_Genome_AMR_analysis_NCIMB 30084.

considered safe for target species, consumers and the environment (EFSA FEEDAP Panel, 2013). In the context of the current application, the identity of the strain as *L. plantarum* was confirmed, and evidence was provided that the strain does not show antimicrobial resistance for antibiotics of human and veterinary importance (EFSA BIOHAZ Panel, 2023). Consequently, the conclusions previously reached are still valid, and the Panel considers that *L. plantarum* NCIMB 30084 remains safe for the target species, consumers and the environment.

An extensive literature search, however, has been performed to support the safety of the *L. plantarum* species and the *L. plantarum* NCIMB 30084 strain. The literature search presented several methodological limitations and, therefore, was not further considered for the assessment.

3.2.2. Safety for the user

In the previous assessment (EFSA FEEDAP Panel, 2013), the FEEDAP Panel concluded regarding user safety: 'in the absence of data their potential to be irritants and/or to act as skin sensitisers cannot be totally excluded.[...] Given the proteinaceous nature of the active agent, the additive should be considered to have the potential to be a skin/respiratory sensitiser and treated accordingly'.

No specific data have been submitted for the renewal of the authorisation regarding the effects of the additive under assessment on the skin and eyes. Therefore, the FEEDAP Panel cannot conclude on the skin and eye irritancy potential of the additive. The FEEDAP Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of microorganisms are available. Therefore, no conclusion can be drawn on the skin sensitisation potential of the additive. Owing to the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser.

3.2.2.1. Conclusions on safety

The FEEDAP Panel concludes that there is no new evidence that would lead to reconsider the previous conclusions that *L. plantarum* NCIMB 30084 is safe for the target species, consumers and the environment under the authorised conditions of use. No conclusions can be drawn on the skin sensitisation, and skin and eye irritancy potential of the additive. The additive should be considered as a respiratory sensitiser.

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.

The FEEDAP Panel concludes that *L. plantarum* NCIMB 30084 remains safe for all animal species, consumers and the environment under the authorised conditions of use. Regarding user safety, the additive should be considered as a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation, and skin and 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.

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Abbreviations

CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety