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Safety and efficacy of *Lactobacillus hilgardii* CNCM I-4785 as a silage additive for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed was asked to deliver a scientific opinion on the safety and efficacy of a strain of *Lactobacillus hilgardii* when used as a technological additive intended to improve ensiling at a proposed application rate of 1.5×10^8 colony-forming units (CFU)/kg fresh material. The bacterial species *L. hilgardii* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment. As the identity of the strain has been clearly established and as no antibiotic resistance of concern was detected, the use of the strain as a silage additive is considered safe for livestock species, for consumers of products from animals fed the treated silage and for the environment. In the absence of data, no conclusion can be drawn on the skin and eye irritancy or skin sensitisation of the additive. The additive should be considered as a potential respiratory sensitiser. Three studies with laboratory-scale silos were made using samples of easy and moderately difficult to ensile forage. In each case, replicate silos containing untreated forage were compared with identical silos containing the same forage treated with the combination of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* NCIMB 40788 each at an intended concentration of 1.5×10^8 CFU/kg fresh matter each. The results showed that the addition of *Lactobacillus hilgardii* CNCM I-4785 at the proposed dose, when used in combination with an equal concentration of *Lactobacillus buchneri* NCIMB 40788, improves significantly the aerobic stability of the silage tested. This was shown in maize forage with a dry matter content ranging from 30% to 40%.

Keywords: technological additive, silage additive, *Lactobacillus hilgardii* CNCM I-4785, safety, QPS, efficacy

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Amendment: This scientific opinion has been amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Lallemand S.A.S.[2] for the authorisation of *Lactobacillus hilgardii* CNCM I-4785, when used as a feed additive for all animal species (category: Technological additive; functional group: Silage additive).

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 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 2 December 2016.

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 product *Lactobacillus hilgardii* CNCM I-4785, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The additive is a preparation containing viable cells of *Lactobacillus hilgardii* CNCM I-4785. It has not been previously authorised as a feed additive in the European Union.

The species *L. hilgardii* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, EFSA BIOHAZ Panel 2015). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show resistance to antibiotics of human and veterinary importance.

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[3] in support of the authorisation request for the use of *Lactobacillus hilgardii* CNCM I-4785 as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[4] and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lactobacillus hilgardii* CNCM I-4785 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Lallemand S.A.S. 19 rue des Briquetiers, BP59, 31702 Blagnac, France.
[3] FEED dossier reference: FAD-2016-0050.
[4] 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.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports?title=FAD-2016-0050&combine=&field_eurl_date_of_report_value%5Bvalue%5D%5Byear%5D=&field_eurl_date_of_report_value_1%5Bvalue%5D%5Byear%5D=

Panel, 2012b),and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c).

3. Assessment

The additive is a preparation of viable cells of *Lactobacillus hilgardii* CNCM I-4785 intended for use as a technological additive (silage additive) for all animal species in combination with *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The strain was isolated from sugar cane. It is deposited in the Collection Nationale de Cultures de Micro-organismes (CNCM) with the accession number CNCM I-4785.[6] It has not been genetically modified.[7]

Taxonomical identification of the product strain as *L. hilgardii* was established by analysing the complete sequence of the 16S rRNA gene and by phenotypical tests. Strain-specific identification and genetic stability analysis was achieved by pulsed-field gel electrophoresis.[8]

The bacterial strain was tested for antibiotic susceptibility using broth microdilution techniques. The battery of antibiotics used included those recommended by EFSA (EFSA FEEDAP Panel, 2012c).[9] As all the minimum inhibitory concentration (MIC) values were below the corresponding EFSA cut-off values for obligate heterofermentative lactobacilli, no further investigation is required and the strain is considered susceptible to all relevant antibiotics.

3.1.2. Manufacturing process and characterisation of the product[10]

The manufacturing process is detailed in the dossier. The additive has a minimum declared content of 5.5×10^{11} colony-forming units (CFU)/g.

Analysis of five batches showed a mean value of 5.5×10^{11} CFU/g (range 3.5–6.5 $\times$ 10^{11} CFU/g).[11]

Microbial and chemical contamination is routinely monitored at various points in the manufacturing process and in the final product. Limits are set for coliforms (10^2 CFU/g), *Escherichia coli* (10 CFU/g), staphylococci (< 10 CFU/g), yeasts and filamentous fungi (10^2 CFU/g), *Salmonella* spp. (absent in 25 g), aflatoxins (B1, B2, G1 and G2: 1.0 μg/kg), deoxynivalenol (0.05 mg/kg), ochratoxin A (0.2 μg/kg) and zearalenone (5 μg/kg). No microbial or chemical contaminants were detected in three production batches.[12,13]

Three batches of the additive were examined for particle size distribution by laser diffraction[14] and for dusting potential using a Heubach dustometer.[15] In average, 7.1% (v/v) of the additive consists of particles below 50 μm and 3.1% (v/v) of particles below 10 μm. The mean dusting potential of the three batches is 13.4 g/m^3.

3.1.3. Stability[10]

The dossier contains information describing the shelf-life of the additive and its stability in water.[16,17]

[6] Technical dossier/Section II/ Annex_II_3b.
[7] Technical dossier/Section II/ Annex_II_3a.
[8] Tehcnical dossier/Section II/Annex_II_3c.
[9] Technical dossier/Section II/ Annex_II_3h.
[10] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[11] Technical dossier/Section II/ Annex_II_2a.
[12] Technical dossier/Section II/ Annex_II_2b.
[13] Limit of quantification: *Escherichia coli* (< 10 CFU/g), coliforms (< 10 CFU/g), staphylococci (< 10 CFU/g), filamentous fungi (< 10 CFU/g), yeasts (< 40 CFU/g), *Salmonella* spp. (absence in 25 g), aflatoxins (B1, B2, G1 and G2: < 1.0 μg/kg), deoxynivalenol (< 0.05 mg/kg), ochratoxin A (< 0.2 μg/kg) and zearalenone (< 5 μg/kg).
[14] Technical dossier/Section II/ Annex_II_2d.
[15] Technical dossier/Section II/ Annex_II_2e.
[16] Technical dossier/Section II/ Annex_II_5a.
[17] Technical dossier/Section II/ Annex_II_5b.

3.1.4. Conditions of use

The additive is intended for use with all forages and for all animal species at a proposed minimum concentration of 1.5×10^8 CFU/kg forage in combination with *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323 at the same concentration (1.5×10^8 CFU/kg fresh material). It is to be applied as such or as an aqueous suspension.

3.2. Safety

3.2.1. Safety for the target species, consumers and environment

In the view of the FEEDAP Panel, the antibiotic resistance qualification has been met and the identity of the strain established as *L. hilgardii*. Consequently, *Lactobacillus hilgardii* CNCM I-4785 is considered to be suitable for the QPS approach to safety assessment and consequently is presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

3.2.2. Safety for the user

No specific data on skin/eye irritation or skin sensitisation were provided for the additive under application. Therefore, no conclusions can be drawn on the skin/eye irritancy or skin sensitisation of the additive of the additive. Given the proteinaceous nature of the active agent, the additive should be considered to be a potential respiratory sensitiser.

Once an active agent has been authorised as a silage additive, different formulations can be placed on the market with reference to that authorisation. The applicant listed several cryoprotectants and carriers which would allow multiple formulations of the additive to be produced, and consequently, not all forms can be directly tested for user safety. However, for assessing the safety for the user of the additive, the active agent is the principal concern provided that other components do not introduce safety issues. For this specific product, the excipients used in the preparation of the final formulation do not introduce additional risks.

3.3. Efficacy[10]

Three studies with laboratory-scale silos were made using samples of easy to ensile (studies 1 and 2) and moderately difficult to ensile (study 3), as specified by Regulation (EC) No 429/2008.[18] In each case, replicate silos containing untreated forage were compared with identical silos containing the same forage treated with the combination of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* NCIMB 40788 at an intended concentration of 5×10^7 CFU/kg fresh matter each. The results showed that the addition of *Lactobacillus hilgardii* CNCM I-4785 at 1×10^8 CFU/kg forage, when used in combination with an equal concentration of *Lactobacillus buchneri* NCIMB 40788, improves significantly the aerobic stability of the silage tested. This was shown in maize forage with a dry matter content ranging from 30 to 40%.

4. Conclusions

As the identity of the strain has been established as *Lactobacillus hilgardii* CNCM I-4785 and no antibiotic resistance of concern has been detected, following the QPS approach to safety assessment, the use of this strain as a silage additive is considered safe for the target species, consumers of products from animals fed treated silage and the environment.

In the absence of data, no conclusion can be drawn on the skin and eye irritancy or skin sensitisation of the additive. The additive should be considered to have the potential to be a respiratory sensitiser.

The addition of *Lactobacillus hilgardii* CNCM I-4785 at 1.5×10^8 CFU/kg forage, when used in combination with an equal concentration of *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323, improves significantly the aerobic stability of the silage tested. This was shown in maize forage with a dry matter content ranging from 30% to 40%.

[18] Technical dossier/Section IV/Annexes IV.1, IV.2 and IV.3.

Documentation provided to EFSA

1) *Lactobacillus hilgardii* CNCM I-4785. August 2016. Submitted by Lallemand.
2) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for *Lactobacillus hilgardii* CNCM I-4785.
3) Comments from Member States.

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EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2015. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 3: Suitability of taxonomic units notified to EFSA until September 2015. EFSA Journal 2015;13(12):4331, 25 pp. doi:10.2903/j.efsa.2015.4331

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539

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Abbreviations

CFU	colony-forming unit
CNCM	Collection Nationale de Cultures de Micro-organismes
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
MIC	minimum inhibitory concentration
QPS	qualified presumption of safety

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Lactobacillus hilgardii* CNCM I-4785

In the current application authorisation is sought under Article 4(1) for *Lactobacillus hilgardii CNCM I-4785* under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species.

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the non-genetically modified strain *Lactobacillus hilgardii* CNCM I-4785. The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus hilgardii* CNCM I-4785 content of 3×10^{10} Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of 1.5×10^{8} CFU/kg fresh *silage*.

For the identification of *Lactobacillus hilgardii* CNCM I-4785, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of *Lactobacillus hilgardii* CNCM I-4785 in the *feed additive per se*, the Applicant submitted the ring-trial validated spread plate method EN 15787. Based on the performance characteristics available, the EURL recommends this method for official control.

Since the enumeration of initially added *Lactobacillus hilgardii* CNCM I-4785 in silage is not achievable by analysis, the EURL cannot recommend any method for official control.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.