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## Safety and efficacy of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788 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* and of *Lactobacillus buchneri* when used as a technological additive intended to improve ensiling at a proposed application rate of  $3.0 \times 10^8$  colony forming units (CFU)/kg fresh material. The two bacterial species are considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment. In previous opinions the identity of the strains has been clearly established and no antibiotic resistance of concern was detected. Therefore, the use of the strains 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 a potential respiratory sensitiser. In a previous application, three studies involving both strains used in combination at the same concentration were made with samples of whole crop maize with varied dry matter content. The same studies have been submitted in this application. The Panel reiterates its previous conclusions that *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323 at  $1.5 \times 10^8$  CFU of each active agent per kg of fresh forage, equivalent to  $3 \times 10^8$  CFU total lactobacilli/kg of forage significantly improve the aerobic stability of the silage.

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**Keywords:** technological additive, silage additive, *Lactobacillus hilgardii* CNCM I-4785, *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788, safety, QPS, Efficacy

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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.<sup>2</sup> for the authorisation of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788, 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 22 May 2018.

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 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788, when used under the proposed conditions of use (see Section 3.1.1).

### 1.2. Additional information

The additive is a preparation containing viable cells of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788. EFSA issued an opinion on the safety and efficacy of *Lactobacillus hilgardii* CNCM I-4785 (EFSA FEEDAP Panel, 2017) and another on *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788 (EFSA FEEDAP Panel, 2013), both when used as a silage additive for all animal species.

The additive composed of the combination of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788 is not authorised as a feed additive in the European Union (EU). *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788 is currently authorised as a technological additive (silage additive) for all animal species. *Lactobacillus hilgardii* CNCM I-4785 is not authorised as a feed additive in the EU.

## 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<sup>3</sup> in support of the authorisation request for the use of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788 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<sup>4</sup> 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.<sup>5</sup>

<sup>1</sup> 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.

<sup>2</sup> Lallemand SAS, 19 rue des Briquetiers, BP59, 31702 Blagnac, France on behalf of Danstar Ferment AG, Switzerland.

<sup>3</sup> FEED dossier reference: FAD-2018-0015.

<sup>4</sup> 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.

<sup>5</sup> The full reports is available on the EURL website: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2018-0015?search&form-return>

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788 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).

## 3. Assessment

The additive is a preparation of viable cells of a single strain of *L. hilgardii* and of *L. buchneri* intended for use as a technological additive (silage additive) in forages for all animal species.

### 3.1. Characterisation of the additive

The *L. hilgardii* strain was isolated from sugar cane and is deposited in the Collection Nationale de Cultures de Micro-organismes (CNCM) with the accession number CNCM I-4785. The *L. buchneri* strain was isolated from maize silage and is deposited in the CNCM with the accession number CNCM I-4323 and in the National Collection of Industrial and Marine Bacteria (NCIMB) with the accession number NCIMB 40788. In this opinion, this strain is referred as *Lactobacillus buchneri* CNCM I-4323.

*Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323 have been identified by molecular methods and their susceptibility to all relevant antibiotics established according to the criteria set by the FEEDAP Panel (EFSA FEEDAP Panel, 2012b) in the previous opinions on the individual strains (EFSA FEEDAP Panel, 2013; EFSA FEEDAP Panel, 2017).

The final additive has a minimum declared content of total lactobacilli (LAB) of  $1.5 \times 10^{11}$  colony forming units (CFU)/g. Analysis of five batches of a preparation with sucrose showed a mean value of  $1.75 \times 10^{11}$  CFU/g (range  $1.72\text{--}1.85 \times 10^{11}$  CFU LAB/g).<sup>6</sup>

Microbial and chemical contamination are routinely monitored at various points in the manufacturing process and in the final product. Action levels 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 (limit of detection (LOD):  $1.0 \mu\text{g}/\text{kg}$ ), deoxynivalenol (LOD:  $0.05 \text{ mg}/\text{kg}$ ), ochratoxin A (LOD:  $0.2 \mu\text{g}/\text{kg}$ ) and zearalenone (LOD:  $5 \mu\text{g}/\text{kg}$ ). Microbial or chemical contaminants were below the respective limit levels in three production batches.<sup>7</sup>

Three batches of the additive were examined for particle size distribution by laser diffraction and for dusting potential using a Heubach dustometer.<sup>8</sup> In average, 6.2% (v/v) of the additive consists of particles below  $50 \mu\text{m}$  and 0.7% (v/v) of particles below  $10 \mu\text{m}$ . The mean dusting potential of the three batches is  $2.5 \text{ g}/\text{m}^3$ .

#### 3.1.1. Stability

Three batches of the additive were stored in sealed aluminium foil bags at  $4^\circ\text{C}$  or  $21^\circ\text{C}$  for 18 months.<sup>9</sup> Negligible losses ( $< 0.5$  log) were observed over this period.

As the additive is intended to be distributed by the spraying of an aqueous suspension, the short-term stability in water was measured on a preparation of each active agent with dextrose.<sup>10</sup> Results showed that bacterial numbers were maintained (losses  $< 0.5$  log) for at least 2 days under ambient conditions for both strains.

<sup>6</sup> Technical dossier/Section II/Annex\_II\_1a.

<sup>7</sup> Technical dossier/Section II/Annex\_II\_1b.

<sup>8</sup> Technical dossier/Section II/Annex\_II\_1c.

<sup>9</sup> Technical dossier/Section II/Annex\_II\_4a.

<sup>10</sup> Technical dossier/Section II/Annex\_II\_4b.

### 3.1.2. Conditions of use

The additive is intended for use with all forages and for all animal species at a proposed minimum concentration of  $3 \times 10^8$  CFU LAB/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

The species *L. hilgardii* and *L. buchneri* are considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel 2017). 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.

In the previous opinions (EFSA FEEDAP Panel, 2013; EFSA FEEDAP Panel, 2017), the antibiotic resistance qualification has been met and the identity of the strains established as *L. hilgardii* and *L. buchneri*. Consequently, *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323 are considered to be suitable for the QPS approach to safety assessment and consequently, 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, skin sensitisation or inhalation toxicity 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 agents, 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 agents are 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

In a previous application, three studies were conducted with whole crop maize with a dry matter and a water soluble carbohydrates contents representing material easy to ensile (studies 1<sup>11</sup> and 2<sup>12</sup>) and moderately difficult to ensile (study 3<sup>13</sup>), as specified by Regulation (EC) No 429/2008 (EFSA FEEDAP Panel, 2017). The studies involved both strains used in combination at  $1.5 \times 10^8$  CFU of each active agent per kg of fresh forage.

From the results obtained the Panel concluded that the addition of *Lactobacillus hilgardii* CNCM I-4785 at  $1.5 \times 10^8$  CFU/kg forage, when used in combination with an equal concentration of *Lactobacillus buchneri* CNCM I-4323, significantly improves the aerobic stability of the silage tested. This was shown in maize forage with a dry matter content ranging from 30% to 40%.

The same studies have been submitted in this application and the FEEDAP Panel confirms its previous conclusion.

## 4. Conclusions

*Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* NCIMB 40788 qualify for the QPS approach for safety assessment and consequently, their use 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 a potential respiratory sensitiser.

<sup>11</sup> Technical dossier/Section IV/Annexes IV.1.

<sup>12</sup> Technical dossier/Section IV/Annexes IV.2.

<sup>13</sup> Technical dossier/Section IV/Annexes IV.3.

The Panel concludes that *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323 at  $1.5 \times 10^8$  CFU of each active agent per kg of fresh forage, equivalent to  $3 \times 10^8$  CFU LAB/kg of forage, significantly improve the aerobic stability of the silage.

## Documentation provided to EFSA

- 1) *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323 as a silage additive for all animal species and categories. October 2015. Submitted by Danstar Ferment AG represented in the EU by Lallemand SAS.
- 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.

## Chronology

Date	Event
3/4/2018	Dossier received by EFSA
9/4/2018	Reception mandate from the European Commission
22/5/2018	Application validated by EFSA – Start of the scientific assessment
2/10/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

## References

- EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. <https://doi.org/10.2903/j.efsa.2007.587>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for technological additives. EFSA Journal 2012;10(1):2528, 23 pp. <https://doi.org/10.2903/j.efsa.2012.2528>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. <https://doi.org/10.2903/j.efsa.2012.2740>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of *Lactobacillus brevis* (DSM 23231), *Lactobacillus buchneri* (DSM 22501), *Lactobacillus buchneri* (NCIMB 40788—CNCM I-4323), *Lactobacillus buchneri* (ATCC PTA-6138) and *Lactobacillus buchneri* (ATCC PTA-2494) as silage additives for all species. EFSA Journal 2013;11(4):3168, 9 pp. <https://doi.org/10.2903/j.efsa.2013.3168>
- 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, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ and Wester P, 2017. Scientific output on the safety and efficacy of *Lactobacillus hilgardii* CNCM I-4785 as a silage additive for all animal species. EFSA Journal 2017;15(4):4758, 9 pp. <https://doi.org/10.2903/j.efsa.2017.4758>

## Abbreviations

CFU	colony forming unit
CNCM	Collection Nationale de Cultures de Micro-organismes
DM	dry matter
EURL	European Union Reference Laboratory
LAB	total lactobacilli
LOD	limit of detection
NCIMB	National Collection of Industrial and Marine Bacteria
PFGE	pulsed field gel electrophoresis
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 4785 and *Lactobacillus buchneri* CNCM I-4323/NCIMB 40788**

In the current application authorisation is sought under Article 4(1) for *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323 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 and categories.

According to the Applicant, the active substance in the *feed additive* consists in viable cells of the non-genetically modified strains *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323. The *feed additive* is to be marketed as a powder constituted by *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323 in a 1:1 ratio, containing a minimum concentration of total lactobacilli  $1.5 \times 10^{11}$  colony forming unit (CFU)/g. The *feed additive* is intended to be added to silage at a minimum dose of  $3 \times 10^8$  CFU/kg fresh silage.

For the enumeration of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323 in the *feed additive* and *premixtures* of silage additives, the Applicant submitted the ring-trial validated spread plate method EN 15787 which was already evaluated by EURL in the frame of previous *Lactobacillus* spp. dossiers. Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323 in the *feed additive* per se and *premixtures*.

Since the unambiguous determination of the content of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* initially added to silage is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control to determine the feed additive in silage.

For the identification of *Lactobacillus hilgardii* CNCM I-4785 and *Lactobacillus buchneri* CNCM I-4323, the EURL recommends for official control pulsed field gel electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.